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EDITORIAL

TIME-OUT

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Amidst the ongoing COVID-19 Pandemic, we wonder when everything will settle down. Lives have been lost… one too many, livelihoods disrupted, events cancelled, travel plans placed on the back burner and education halted. Our country has the highest number of cases in our part of the world. We had the longest lockdown too. When the lockdown was getting too long, calls from various sectors to ease the lockdown was pressing. A surge of cases was seen thereafter prompting the medical community to call for a time-out. The call for time-out was interpreted in various ways. Some agreed, some voiced-out dissent and many were passive. For the non-medical sector, the clamor was not to give in as the economy has been badly hit and prolonging the lockdown will beat up people's finances more. For some, they thought that the medical community was too lame to call for a time-out when it was what we signed-up for when we took the Hippocratic oath.

Time-out means a brief cessation or suspension of activity, for non-medical personnel or those not involved in any way with the ongoings of the pandemic, it came as though the call was because we are tired and we want to rest. For the medical group, the call was to suspend easing of the lockdown as it caused surge in cases and there isn't enough facilities to accommodate and handle them. For in reality, can the entire medical community be on a time-out? It's probably one of the fewest professions where if we did go on a time-out, lives lost would be countless. Food production can stop for a day, transportation groups can call for a strike, wars can have ceasefires, classes can be suspended and even places of worship can be closed down. But can we stop a mother from giving birth, an appendix from rupturing, an aneurysm from bursting … many medical events can't be helped and they need immediate action for a life to be saved. Indeed, that is what we signed-up for when we chose our profession.

The same thing goes in our commitment to continue to educate. The pandemic doesn't give us an excuse to stop publishing, as important findings in science is paramount to stopping this pandemic, researches are ongoing and that is one of our only hope to get back into pre-COVID days. Continuing education, now available in many ways doesn't stop with a pandemic. Read on our latest issue as a way of continuously learning during this unprecedented time of our lives.

ORIGINAL ARTICLE

Optimized Tube Dilution Technique and Sole Carbon Utilization Assay for Anti-leptospiral In Vitro Screening of Plant Extracts

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Introduction: Leptospirosis is one of the neglected re-emerging zoonoses that is of public health concern globally. The need to discover novel therapeutic alternatives for leptospirosis through screening for and elucidating the mechanism/s of the anti-leptospiral activity of plant extracts is therefore necessary. This study analyzes the optimized tube dilution technique and the Biolog™ sole carbon utilization phenotype microarray as screening tool for anti-leptospiral activity of plant extracts.

Methods: The suitability of the optimized tube dilution technique was evaluated by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and motility inhibition property of a plant extract and an antimicrobial control (pen G) against 4 dominantly circulating *Leptospira* serovars/serogroup in the Philippines. Likewise, the suitability of the Biolog™ sole carbon utilization assay was evaluated using a plant extract and selected antimicrobials against *L. interrogans* serovar Manilae strain K64 *and L. interrogans* serovar Losbanos strain K37.

Results: The MIC, MBC, and motility inhibition property of a plant extract and the antibiotic controls as well as its effect on the carbon utilization phenome of the *Leptospira* serovars gave consistent results, within and between several runs. With standard deviation = 0 for all serovars. The MIC and MBC of the antimicrobial control (pen G), the positive control, was 10 ug/ml. The growth control (leptospires without treatment), the negative control, showed presence of motile leptospires. The MIC and the MBC of the test plant extract was 250 ug/ml - 500 ug/ml. Results of the carbon utilization phenome or pattern of carbon utilization were consistent within the 3 replicates and between two runs.

Conclusion: The optimized tube dilution technique and the Biolog™ sole carbon utilization assay is a potential in vitro screening tool for determining anti-leptospiral activity of plant extracts.

KEYWORDS: *anti-leptospiral, tube dilution technique, phenotype microarray*

INTRODUCTION

Leptospirosis, an acute febrile disease caused by genus *Leptospira,* is a preventable and treatable disease in animals and humans. It is now considered an emerging global disease due to re-emergence of the disease in non-endemic areas and becoming an urban problem in highly endemic areas.[1,2] In most of the developing countries in the Asia Pacific region, leptospirosis is largely a water-borne disease and in the Philippines, poor sanitation and increase in urban slums along with frequent typhoons, contribute to the risk of infection.[3] Currently, studies are focused on the need for continued monitoring of the prevailing serovars in a given geographical area and improvement of diagnostic capabilities to elucidate the current disease burden of leptospirosis.[4-13] However, there are limited studies on the in vitro and in vivo screening to find alternative treatments (i.e. use of herbal plants) for leptospirosis. The Leptospirosis Task Force issued the Philippine Clinical Practice Guidelines on the Diagnosis, Management and Prevention of Leptospirosis (2010) on antibiotic use and it states that for mild cases of leptospirosis, doxycycline (hydrochloride or hyclate) is the drug of choice. Alternative drugs include amoxicillin and azithromycin dihydrate. Although there is still no problem on the antimicrobial resistance on the current recommended antimicrobials, the "One Health" Operational Concept of the Global Leptospirosis Environmental Action Network (GLEAN), has drawn the global and local efforts on research, development and innovation on genomics, diagnosis, vaccines and on therapeutic alternatives in addressing leptospirosis, as an emerging global disease.

Plants have been used for health and medical purposes for several centuries. Numerous studies have been conducted on the antibacterial properties of plants on various pathogenic and multi-drug resistant organisms.[14-16] However, screening for anti-leptospiral activity of plants is limited. Since leptospires have surface structures that share features of both gram-positive and gram-negative bacteria, evaluating plants with known antibacterial property in terms of their ability to either inhibit the growth or directly kill the said bacteria can be an initial approach in order to find alternative means for treating leptospirosis.

Traditional methodologies for evaluating antimicrobials, such as the tube and agar-diffusion based assays, to determine the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) had been utilized in the screening for anti-leptospiral properties of some plants.[17-21] However, plant extracts have distinct properties that need to be considered when being tested using the tube and agar-diffusion based assays such as the interference of the leaf pigments. The Biolog™ sole carbon utilization phenotype microarray is a unique cellular analysis tool that offers a comprehensive approach to identify how a natural product such as plant extracts prevents microbial growth.[22-24] To the knowledge of the authors, there has not been any published paper yet reporting the use of this technology to test for antimicrobial activity of plant extracts against *Leptospira.* Moreover, since suitability of the growth requirements of the microorganism (test system) as well as the inherent properties of a plant ethanolic leaf extract (test item) is crucial to the accuracy and precision of the tube or agar-diffusion based assays and the Biolog™ sole carbon utilization phenotype microarray, therefore, optimizing the standard tube dilution technique and the Biolog™ phenotype microarray intended to screen for anti-leptospiral activity of plant extracts may contribute to the initial efforts for discovering therapeutic alternatives for leptospirosis.

MATERIALS AND METHODS

Equipment and Reagents

Dark field microscope (Olympus BX43) was used to observe the presence or absence of viable leptospires in the in-vitro screening assays for anti-leptospiral property. The Biolog™ GEN III MicroStation System (Biolog™ Inc., Hayward, CA, USA) was used in the sole carbon source utilization phenotype microarray assay. The GEN III Microplate™ and the inoculating fluid (IF-C) used in the sole carbon source utilization assay were all obtained from Biolog™. The USP-grade antimicrobial standards, penicillin (Pen G), doxycycline (Doxy), and polymixin B (PolB), were purchased from Sigma-Aldrich Pte. Ltd. Singapore. The ethanol, phosphate buffered saline (PBS), and *Leptospira* culture medium (i.e., Korthof's medium) were of analytical grade.

Plant material, quality control testing, and extraction procedure

To optimize the tube dilution technique and the Biolog™ Gen III sole carbon source utilization phenotype

microarray for its suitability as in vitro screening tool for anti-leptospiral activity of plant extracts, a matrix-specific test item i.e. plant material known to have an antibacterial property to various pathogenic organisms and with reported MIC to *L. interrogans* serovar Manilae strain K6 was used. The test plant material (coded as "PELE" to protect its patent potential) was collected from the agricultural unit of the National Integrated Research Program on Medicinal Plants (NIRPROMP) in UPLB, Laguna. Particularly, pesticides were not used during cultivation and the farm where the plant material was grown was located far from highways and industrial areas. Standard methods were used in preparing the leaves and processing into powder form. Only the leaves from the 1st through 3rd apical shoots of the plant were collected. Inclusion criteria for the tops include absence of insects and absence of wilting and dark spots. The leaves were then washed with distilled water, air dried and ground into powdered form through a blender and packed properly until further use. In addition to the test plant material, reference antibiotic control/s and a growth control (leptospire without treatment) served as the positive and negative control, respectively during the optimization of the methods.

Subsequently, as required for plants with potential for registration as traditionally-used herbal products in the Philippines, based on FDA Issuance AO 184 Series of 2004, quality control testing of the plant material was done.[25] A standard procedure for plant extraction was adapted. The plant ethanolic leaf extract was prepared by soaking 100 g of the dried powdered leaves in 300 ml absolute ethanol for 24 hours, with occasional stirring. Afterwards, it was filtered using Whatman filter No. 1 and the remaining residue was re-extracted with additional 300 ml of absolute ethanol. The final ethanolic leaf extract was collected and placed in a rotary evaporator at 50^{0}C for 2-3 hours. The residue was then evaporated to dryness in a dish placed in a water bath at 50^{0}C. The leaf extract powder was stored in a sealed amber bottle and kept in the freezer (-20^{0}C) until use.

Leptospira *strains*

*S*tock cultures of *L. interrogans* serovar Manilae strain K64, *L. interrogans* serovar Losbanos strain K37, *L. interrogans* serovar Ratnapura strain K5, and *L. borgpetersennii* serogroup Javanica strain K6, were obtained from the Leptospirosis Prevention and Control Laboratory (LepCon), Department of Medical Microbiology, College of Public Health, University of the Philippines Manila. These strains represented the groups of *Leptospira* that were previously reported to be predominantly circulating in the Philippines.[5,6] All organisms were maintained by continuous culture in Korthof's medium. The inoculum was prepared by adding the stock strain of leptospires into Korthof's medium (1:16) and incubated at 30^{0}C for 4-7 days to a density of approximately 1 x 10^8 cells/ml verified by dark field microscopy and enumerated using the Thoma counting chamber.

Antimicrobial and plant extract stock preparation

Penicillin G, doxycycline and polyymixin B were used as antimicrobial control/ positive controls, depending on the assay. Pen G was used as the reference antimicrobial control in the tube dilution technique. Pen G, doxycycline and polymixin B were used as the reference antimicrobial controls in Biolog™ Gen III sole carbon source utilization phenotype microarray. Stock antimicrobial solutions (2,500 ug/ml) were prepared using the USP-grade powders of penicillin G, doxycycline, and polymixin B diluted in sterile distilled water and stored in one-time-use aliquots at −80^{0}C. Similarly, stock plant leaf extract solution (125,000 ug/ml) was freshly prepared daily and vortex-mixed to ensure homogeneity. The concentrations of the stock solutions were prepared so that at least a 1:10 ratio of the antimicrobial control to Korthof's medium can be achieved to ensure that enough growth media is available for the leptospires to grow.

MIC and MBC by Tube Dilution Technique

In this study, the minimum inhibitory concentration (MIC) is defined as the lowest concentration of plant extract or antimicrobial agent that showed inhibition of growth as indicated by the absence of motile leptospires after incubation for 7 days at 30°C. The minimum bactericidal concentration (MBC) is defined as the lowest concentration of plant extract or antimicrobial agent that showed absence of motile leptospires, when an inoculum from the tubes without motile leptospires from an MIC set up was transferred into fresh Korthof's medium without test agent and further incubated for 7 days at 30°C. The presence or

absence of motile leptospires was checked using the dark field microscopy.

The optimized tube dilution technique in this study was a modification of the broth microdilution technique.[17,26] The optimized tube dilution technique used the Korthof's growth medium in tubes (macrodilution) and dark field microscopy to check for the presence or absence of motile leptospires. While the broth microdilution used the Ellinghausen-McCullough-Johnson-Harris (EMJH) growth medium in microwells and spectrophotometry with alamar dye to determine the viability of leptospires. Based on the MICs of the test plant material at 250 ug/ml (Yabes et al., *unpublished*) and pen G (10 ug/mL) against *L. interrogans* serovar Manilae strain K64,[27,28] stock solutions were prepared and added to Korthof's medium (1:10) to achieve the desired highest concentration. Subsequent concentrations of the plant extract and pen G were prepared in tubes by serial dilution. Antimicrobial-containing tubes included final concentrations of pen G (i.e., 0.5, 5, and 10 ug/ml). Plant extract-containing tubes had final concentrations ranging from 250 - 10,000 ug/ml.

To determine the MIC, one part of the *Leptospir*a inoculum (1 x 10^8 cells/ml) was added to 9 parts of Korthof's medium containing treatments (plant extract and antimicrobial control) at desired concentrations. The growth control or negative control (leptospires in Korthof's without treatment) was prepared similarly and included in every run. The tubes were incubated for 7 days at 30°C and observed for motility of the leptospires using dark field microscopy daily from days 4-7. The lowest concentration that showed absence of motile leptospires on the 7th day was reported as the MIC.

To determine the MBC, inoculum was taken from the tubes in the MIC set up with the lowest concentration of plant extract or antimicrobial agent that showed absence of motile leptospires verified by dark field microscopy. The inoculum was transferred to freshly prepared Korthof's medium (1:10) and subsequently incubated for another 7 days. The lowest concentration that showed absence of motile leptospires on the 7th day was reported as the MBC.

Test for Motility Inhibition Property

In this study, positive for motility inhibition is defined as the lowest concentration of plant extract or antimicrobial agent that showed the reduction in the percentage of motile leptospires to approximately 50% on the 24th hour as compared to the growth control. To determine the motility inhibition property of the extract, a method described previously was adapted.[17,29] Briefly, 10 ul sample from each tube in the MIC set up was placed on the slide and observed under dark field microscope at 0, 3, 5 and 24 hr incubation. Results were scored as follows; a score of "100%" indicated that the leptospires were motile and comparable with the growth control (leptospires without treatment). A score of "0%" indicated absence of motile leptospires. While a score of "50%" indicated that the number of motile leptospires was reduced to approximately 50% as compared with the growth control. Thus, the reduction in the percentage of motile leptospires to approximately 50% on the 24th hour was reported as motility inhibition. Images of leptospires under the dark field microscope were electronically captured and the "percentage and motility" of leptospires were evaluated by at least 2 researchers.

Biolog^{TM} phenotype microarray (PM) sole carbon utilization technology

The Biolog^{TM} Gen III microplate was used to analyze the ability of the leptospires to metabolize 71 major classes of biochemicals belonging to sugars, hexose phosphates, amino acids, hexose acids, esters, carboxylic acids, and fatty acids. Significant respiration in the wells was observed when the carbon source is utilized. The increased respiration caused the reduction of the tetrazolium redox dye, forming an irreversible purple color, which was then used to colorimetrically indicate utilization of the carbon sources or resistance to inhibitory chemicals. These carbon sources are necessary for the growth of mostly gram negative and gram-positive bacteria.[22-24,30-33]

The inoculum i.e., PBS-washed *Leptospira* cells in IF-C, was prepared using *Leptospira* grown in 5 ml Korthof's at 30^{o}C for 4-7 days with bacterial density of approximately 1 x 10^8 cells/ml. The bacterial solution was centrifuged for 10 minutes at 1500 x g. The supernatant was discarded carefully to remove the Korthof's medium. The *Leptospira* cells were then washed 2 times with 5 ml of PBS and centrifuged for 10 minutes at 1500 x g. After discarding the supernatant, the *Leptospira* cells were re-suspended in 5 ml of IF-C. Simultaneously, to prepare the IF-C spiked with

treatments containing sub-MIC (0.25x and 0.5x the MIC) of the plant extract or antimicrobial controls (penG, doxy and pol B), stock solutions were added to the IF-C (10 ml) to achieve the desired concentration of the treatments. Subsequently, 1.5 ml of the *Leptospira* cells in IFC was added to the IF-C spiked with treatments. The inoculum (100ul) was aseptically dispensed to each well of the Biolog™ Gen III microplates and incubated at 30°C. The microplates were read from day 0 to 7 using the Biolog™ Microstation 2 set at 590 and 750 nm. Based on the Biolog Gen III kit protocol, results may be interpreted in three ways, visual inspection of color formation in the wells, as well as the graphic result and the optical densities (OD) produced by the Biolog™ Microstation. OD readings at 590 nm were encoded in Microsoft Excel to generate the corrected ODs and subsequent data analysis. Corrected ODs were calculated by subtracting the OD of the negative control well from the OD of each test well in the Biolog™ Gen III microplate. The use of corrected OD ($\geq$0.100) as basis for setting the threshold for "positive for carbon source utilization" was adapted from previous studies.[30-33] Readings of the negative control wells (n=60), positive control wells (n=60) and the tetrazolium wells (n=120) in this study were also considered in setting up the threshold. Those wells with corrected OD of $\geq$0.100 were considered well utilized and interpreted as positive for carbon source utilization while those wells with corrected ODs <0.100, although above the corrected OD of the negative control, were considered negative or borderline and interpreted as negative for carbon source utilization.

Quality Control and Internal Validity

Quality control samples (i.e., growth control/negative control, positive/antimicrobial control, and plant extract control) were used in the pilot run and during the actual experiments. Freshly prepared plant extract and suspension of actively growing leptospires were used in each run. The suitability of the tube dilution technique for its intended use as anti-leptospiral screening tool of plant extracts was evaluated using the plant extract and the antimicrobial controls (pen G) against the 4 *Leptospira* serovars/serogroups. The determination of MIC, MBC and motility inhibition using the tube dilution technique were performed, in different runs over several days (3 runs in triplicates using strain K64 and 1 run in triplicates using the other 3 *Leptospira* serovars/serogroups). Similarly, the Biolog™ Gen III sole carbon utilization phenotype microarray was also performed in 2 runs in triplicates using strain K64 and strain K37. The suitability of the Biolog™ Gen III for its intended use as anti-leptospiral screening tool of plant extracts was evaluated by the consistency of the results of the carbon source utilization phenome and the inhibition of the carbon source utilization of the three replicates using the 2 *Leptospira* serovars, as well the results of the negative control wells, positive control wells and the tetrazolium wells. Only three replicates per serovar was used since the manufacturer's claim for reproducibility in the package insert of the Biolog™ Gen III test kit is excellent due to controlled conditions required in the whole procedure. Similarly, at least three replicates for each serovar was also used in the tube dilution technique since pilot study of the optimized tube dilution method also showed consistent MIC when used in plant extracts.

Ethical Statement

This study was performed in adherence to Good Laboratory Practice (GLP) standards. All experiments using *Leptospira* cultures were performed in a university-based BSL-II facility. Strict compliance to the regulations of the UP Manila Institutional Biosafety and Biosecurity Committee (IBBC), as well as technical review and research registration procedures with UPM REB (Research Ethics Review Board) and UP Manila RGAO (Registration Grants Administration Office) were observed. The data on the optimization of the anti-leptospiral in vitro screening methods for plant extracts described in this paper was the preliminary data of the two subsequent researches funded by the UP National Institutes of Health and the Philippine Institute of Traditional and Alternative Medicine (PITAHC), Department of Health that used these optimized methods in screening for anti-leptospiral activity of plant extracts.

RESULTS

The optimized tube dilution technique described in this study gave consistent results of MIC, MBC, and motility inhibition property of the antimicrobial control and the plant extract, within runs and between runs over several days, with standard deviation = 0 for all serovars (Table 1). The MIC and MBC of the antimicrobial control

(pen G), which also served as the positive control, was 10 ug/ml against *L. interrogans* serovar Manilae strain K64*, L. interrogans* serovar Losbanos strain K37*, L. interrogans* serovar Ratnapura strain K5, and *L. borgpetersennii* serogroup Javanica strain K6. The growth control (leptospires without treatment) which served as the negative control showed presence of motile leptospires after incubation for 7 days at 30°C. The MIC and the MBC of the test plant extract was 250 ug/ml against *L. interrogans* serovar Manilae strain K64*, L. interrogans* serovar Losbanos strain K37, and *L. interrogans* serovar Ratnapura strain K5*,* while the MIC against *L. borgpetersennii* serovar Javanica strain K6 was 500 ug/ml (Yabes et al., *unpublished*). In this study, the MIC is equivalent to its MBC, thus the antibacterial activity against the four serovars/serogroup is bactericidal.

Table 1. MIC, MBC and motility inhibition property of the plant extract (PELE) and antimicrobial control against four (4) dominantly circulating Leptospira serovars/serogroup in the Philippines.

***Leptospira* serovars/serogroup**	**Plant extract (PELE)**		**Pen G**	
	MIC and MBC (ug/ml)	**Motility inhibition property (ug/ml)**	**MIC and MBC (ug/ml)**	**Motility inhibition property (ug/ml)**
L. interrogans serovar Manilae strain K64 (n=9)	250	5,000	10	>10
L. interrogans serovar Losbanos strain K37 (n=3)	250	1,250	10	>10
L. interrogans serovar Ratnapura strain K5 (n=3)	250	>10,000	10	>10
L. borgpetersennii serogroup Javanica strain K6 (n=3)	500	>10,000	10	>10

Results of the carbon utilization phenome or pattern of carbon utilization of *L. interrogans* serovar Manilae strain K64 and *L. interrogans* serovar Losbanos strain K37 using the optimized Biolog™ Gen III sole carbon utilization phenotype microarray were consistent within the 3 replicates and between two runs. Likewise, when the two *Leptospira* serovars were treated with sub-MICs of the plant extract and antimicrobial controls (pen G, doxycycline and polymixin B), consistent pattern of inhibitions, between and within runs, were also obtained. In addition, the internal validity of the microplates showed that all the negative control wells (n=60) gave an average corrected OD of 0.040, which was also consistent with visual inspection, that no purple color was observed in all the negative control wells. All the positive control wells showed corrected ODs $\geq$0.100 (n=60). Similarly, all the wells containing tetrazolium blue and tetrazolium violet, which are indicators of cell respiration, showed corrected ODs $\geq$0.100 (n=120). Comprehensive results and discussion of the carbon utilization phenome of *L. interrogans* serovar Manilae strain K64 and *L. interrogans* serovar Losbanos strain K37 and the effect on the inhibition pattern of the plant extract and the 3 antimicrobial controls on the 2 *Leptospira* serovars using the optimized Biolog™ Gen III sole carbon utilization phenotype microarray will be discussed in a separate paper.

DISCUSSION

The optimized tube dilution technique described in this study is suitable in vitro screening tool for anti-leptospiral activity of plant extracts. Based on the results of this study, both the optimized tube dilution technique and the Biolog™ sole carbon utilization assay phenotype microarray have the potential of being used as an in vitro screening tool for anti-leptospiral activity of plant extracts. It showed consistent results of MIC, MBC, and motility inhibition property of the antimicrobial control

and plant extract when tested against 4 *Leptospira* serovars/serogroup that are dominantly circulating in the Philippines. Moreover, the obtained MIC of the antimicrobial control (pen G) was within the reported MIC of aminobenzyl penicillin (ampicillin) ranging from 6.25 - 12.5 ug/ml against *L. interrogans* serovar icterohaemorrhagiae using the microdilution technique.[26] Similarly, the obtained MIC of the plant extract was within the range of the MICs of other plant extracts evaluated for anti-leptospiral activity.[17-21] The result was also comparable with the study of another plant with anti-leptospiral property such as the *Andrographis paniculata* Nees (Acanthaceae), which showed an MIC of 200-600 ug/ml against *L. interrogans* serovar australis and *L. interrogans* serovar icterohaemorrhogica.[21] In addition, it has the advantage of circumventing the interference of plant leaf pigments in spectrophotometric assays by using the dark field microscopy as an alternative way of determining the viability of leptospires through the MIC, MBC, and motility inhibition assays.

Although the Biolog™ Gen III sole carbon utilization phenotype microarray was introduced in the 90's, to the knowledge of the authors, there has not been any published paper yet reporting the use of this technology for *Leptospira*. The closest so far was its use to a related anaerobic spirochete, *Brachyspira pilosicoli* 95/100, B2904 and WesB.[34] Its use for Genus *Leptospira* could have been limited because the Biolog™ standard protocol for inoculum preparation was for microorganisms grown on solid media, in which the bacterial density estimation uses a turbidimeter. This however was not applicable for leptospires, since the means of estimating the leptospire's density is via direct enumeration using a counting chamber under a dark-field microscope. Thus, the Biolog™ standard protocol of inoculum preparation appropriate for organisms grown in solid media was completely modified. To satisfy the required actively growing cell inoculum, the use of the *Leptospira* grown in Korthof's medium for 4-7 days was used. To mimic the 40% transmittance used in *B. pilosicoli* study,[34] PBS-washed *Leptospira* was added to the inoculating fluid to obtain an estimated density of 1.3 x 10^7 cells/ml. Interestingly, this was the same bacterial density that was found to be also optimum in the tube dilution technique. Similarly, PBS-washed *Leptospira* suspended in the inoculating fluid was used in order to have a media-free inoculum. This medium-free condition was done to avoid confounders brought about by the *Leptospira* culture media and to allow the leptospires to solely depend and utilize the carbon source in the wells for bacterial cell respiration. Lastly, monitoring the carbon source utilization up to 4th day was also noted to be optimum for leptospires using the Biolog™ sole carbon utilization technology. It considered the slowing of the metabolism of cells and the decreased availability of carbon sources needed to support later growth phases. In addition, the inclusion of growth control (i.e., leptospires without treatment) ensured that the *Leptospira* inoculum used in the runs were actively growing and that the readings were not due to contamination with other bacteria. Likewise, inclusion of plant extract control (i.e., plant extract in Korthof's medium without any treatment) was noted to be a good means of ruling out bacterial contamination and abiotic dye reduction in the IF-C coming from the plant extract since these may potentially interfere in the assay leading to false positive results. The modifications done on the methods, to suit with the growth requirements of *Leptospira* and the inherent properties of plant extract, showed that it can be a standard screening tool for compounds with anti-leptospiral property. Moreover, results from this pioneering study on the use of the Biolog™ Gen III in *Leptospira* suggest that this technology may be used for these bacteria and may possibly serve as basis for other microorganisms grown on liquid media.

CONCLUSION

Traditional methodologies for evaluating antimicrobials are being used in the screening for anti-leptospiral properties. However, crucial to the accuracy and precision of any procedure is its suitability with the growth requirements of the leptospires as well as the inherent properties of a plant extract. This study showed that both the optimized tube dilution technique and the Biolog™ Gen III sole carbon utilization phenotype microarray technology have acceptable reproducibility, within and between runs. The modified tube dilution technique described in this study is a simple, reliable technique, comparable to the commonly used broth microdilution technique. Moreover, although this is the first study reporting the use of Biolog™ sole carbon source utilization phenotype microarray technology for *Leptospira*, this in vitro assay was found to be a

promising tool in evaluating plants with anti-leptospiral property. Lastly, due to the promising results obtained from these methods, these were subsequently used in similar researches that aim to screen for anti-leptospiral properties of a plant extract known to have anti-bacterial property with the aim of discovering novel therapeutic alternatives for leptospirosis.

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ORIGINAL ARTICLE

Exclusive Breastfeeding from Birth to 6 Months for Reducing Community Acquired Pneumonia in Children Up to 5 Years of Age: A Systematic Review and Meta-Analysis

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background: Exclusive breastfeeding up to 6 months of age is the global recommendation of the World Health Organization because of its established benefits. Previous studies show that exclusive breastfeeding can protect infants during infancy but effects of breastfeeding beyond infancy are inconclusive. This study aims to identify if exclusive breastfeeding up to 6 months of age is protective for pneumonia up to 5 years of age.

Methods: Systematic literature search was conducted on the following electronic databases: Pubmed, MEDLINE, EMBASE, CINAHL, SciHub, Herdin, Google Scholar, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews to identify all relevant studies assessing the effect of exclusive breastfeeding on development of pneumonia in children from birth to 5 years of age. Fixed effects meta-analysis was performed to generate pooled effect estimates (odds ratio) on the probability of developing pneumonia up to 5 years of age in exclusively breastfed compared to non-exclusively breastfed infants.

Results: Five studies were included in the analysis. Exclusive breastfeeding from birth to 6 months has a protective effect against pneumonia in children up to 5 years of age. The probability of developing pneumonia in children until 5 years of age was significantly lower in those who were exclusively breastfed compared to those who were not exclusively breastfed *(OR=0.86; 95%CI=0.77-0.95, p-value=0.003*) by 23%. Systematic review showed benefit of exclusive breastfeeding and continued breastfeeding for longer protection against developing pneumonia.

Conclusion: Exclusive breastfeeding from birth to 6 months is associated with statistically significant reduction in the incidence of pneumonia up to 5 years of age. Results highlighted the importance of exclusive breastfeeding up to 6 months of age as an intervention in reducing pneumonia morbidity up to 5 years of age, thereby supporting the global recommendation of breastfeeding.

KEYWORDS: *exclusive breastfeeding, pneumonia, breastmilk*

INTRODUCTION

Pneumonia is the leading cause of child mortality, a major cause of global morbidity, and one of the most common reasons for hospitalizations among the poorest children. It accounts for 15% of all deaths in children under 5 years old, with an estimated 808,694 children in 2017.[1] The disease affects children and families everywhere but is most prevalent in South Asia and sub-Saharan Africa. Worldwide, public health interventions are facilitated to prevent pneumonia that include immunization, adequate nutrition, exclusive breastfeeding and zinc supplementation.[2]

Exclusive breastfeeding for the first 6 months of life is key to improving children's natural defenses. Human milk contains a wealth of immunologic factors that fight against infections during infancy, and breast milk has evolved to provide the best nutrition, immune protection, regulation of growth, development and metabolism for the human infant.[3] The predominant antibody in breast milk-secretory IgA, confers its immune protection by inhibiting the adherence to or penetration of the gastrointestinal tract by pathogens and by phagocytosis of pathogens. Secretory IgA also provide immunological protection to the infant, whilst its own immune system matures.[4] Exclusive breastfeeding in early infancy significantly reduces the risk of in-patient admission for suspected pneumonia in the first 6 months of life. Breast-fed infants are better protected against infections like otitis media, diarrhea, respiratory infections, infection-induced wheezing and invasive H. influenza infections for several years after the termination of breastfeeding.[5] This is presumably the result of a number of potentially immune stimulatory factors in the milk like antibodies, lymphocytes, cytokines and certain hormones like leptin which may specifically stimulate TH1 lymphocytes.[6]

Protective effects of breastfeeding on infectious diseases are well known, but whether or not such effects last after infancy are rarely investigated. Studies done in United Kingdom reported that protective associations of breastfeeding with respiratory tract infection wore off soon after cessation of breastfeeding.[7] Moreover, protective effect of breastfeeding lasted up to the age of 2 years only.[8] The mechanisms by which human milk confers protective effects that last beyond infancy and after breastfeeding ends are unclear. Few studies have begun to investigate on the protective effect up to 5 years of age of exclusive breastfeeding and have shown promising outcomes. It has been speculated that immunologic factors in breast milk influence the development of the infant's immune system such that they influence the pathogenesis of illness later in life.[9]

To date, there is scarcity of Philippine studies on the effect of breastfeeding beyond the breastfeeding years on childhood pneumonia. However due to the theoretical possibilities and the presence of conflicting studies, this study was performed to systematically review and analyze available studies and evaluate if exclusive breastfeeding is protective against pneumonia up to the age of 5 years. This study aims to contribute more evidence in upholding exclusive breastfeeding as a measure of preventive health care. It also aims to generate robust data to support policy making measures on breastfeeding that will aid in achieving the Millennium Development Goals and therefore mitigate inequities in the access to health services. Breastfeeding is natural, safe and sustainable source of nutrition and protection for children. It is recognized by the International Convention on the Rights of the Child as a key component of every child's human right. It is therefore important that every Filipino child, benefit from the results of this study and therefore impact the society as well. Information to be gathered in this study can empower mothers, health workers and administrators in reinforcing the advantages of breastfeeding to achieve best health outcomes.

Operational Definition of Terms and Variables

Exclusive Breastfeeding – practice of giving infants breast milk from mother or wet nurse or expressed breast milk with no other liquids or solids except vitamin drops or syrups, mineral supplements, prescribed medicines or oral rehydration solution from birth to 6 months of age.

Non-exclusive Breastfeeding – practice of giving infants milk other than breast milk or those who did not fit the definition of exclusively breastfed that include those who never breastfed, partially breastfed or are formula-fed. In this study, infants who were exclusively breastfed for less than 6 months were included in this group.

Community Acquired Pneumonia – an acute disease that is marked by inflammation of lung tissue accompanied by infiltration of alveoli and often bronchioles with white blood and fibrinous exudate, is

characterized by fever, chills, cough, difficulty in breathing, fatigue, chest pain, and reduced lung expansion and is typically caused by an infectious agent.

METHODOLOGY

Search Strategy and Study Identification

Literature search was conducted on the following electronic databases: Pubmed, MEDLINE, EMBASE, CINAHL, SciHub, Herdin, Google Scholar, the Cochrane Central Register of Controlled Trials and the Cochrane Database of Systematic Reviews to identify all relevant trials (randomized controlled trials, cohort, cross-sectional, or case-control studies) between 1980 and December 2017. The literature search used the following terms or combination of keywords: exclusive breastfeeding, breast milk, human milk, pneumonia, community acquired pneumonia. No language and publication restrictions were applied. Excluded were trials wherein respiratory infections were not fully defined as pneumonia such as bronchiolitis, bronchitis, tuberculosis, asthma or included only upper respiratory tract infection such as colds or otitis media. If respiratory infections were not defined or upper respiratory tract infections and lower respiratory tract infections were combined, the researcher included studies where such cases were hospitalized, assuming illness is due to acute LRTI. Studies including infants born to HIV positive mothers were excluded because of the possibility of altered immune status of infants.

Study Selection

Observational studies comparing exclusive breastfeeding and non-exclusive breastfeeding and incidence of pneumonia from birth to 5 years of age were included. Studies that did not define infection as pneumonia were excluded. Systematic reviews and previous meta-analyses were excluded but were reviewed to identify potential studies. The author used titles and abstracts to exclude studies which clearly did not meet the set inclusion criteria. The common reasons for exclusion of articles from the electronic search were age group more than 5 years and missing data on non-exclusive breastfeeding. Full articles were retrieved for further assessment if the abstracts indicated that there was a possibility that the study fulfilled the inclusion criteria. Two authors independently screened the studies for study eligibility. For this meta-analysis, those studies that compared exclusive breastfeeding from non-exclusive breastfeeding and pneumonia incidence from birth to 5 years were considered. The investigator and another peer independently reviewed and assessed inclusion criteria. Five potentially eligible papers were identified and reviewed. The primary outcome measure was the prevention of community acquired pneumonia up to 5 years of age.

Data Collection and Processing

The journals were screened and peer reviewed by another reviewer to assess study eligibility. The investigator and another peer independently reviewed and assessed inclusion criteria, extracted the data, assessed risk of bias and resolved disagreements. Data concerning details of the study population, intervention and outcomes were extracted independently using the data extraction form from the Cochrane Library. From each paper, the researcher extracted information related to general information (title, authors, year of publication and number of patients from the study), study characteristics (method of randomization and blinding), intervention (duration of exclusive breastfeeding), participant characteristics (inclusion and exclusion criteria, age group, number of patients in each intervention), outcomes (incidence of developing pneumonia after exclusive breastfeeding compared to non-exclusive breastfeeding) and results (data were expressed as weighted mean differences, odds ratio).

Each included study was assessed based on the following indicators of risk of bias namely random sequence generation, allocation concealment, blinding of participants and outcome assessment, incomplete outcome data and selective outcome reporting. A verdict of Low Risk meant low risk of bias, High Risk meant high risk of bias and Unclear Risk for unknown risk of bias were used criteria for judging. See Appendix.

Data Analysis

Meta-analysis using fixed effects was conducted using Review Manager 5.3. Generic Inverse Variance method was used to include studies with only Odds Ratio as given data. In studies where the researcher was unable to extract all the information needed from the study, the plan was to contact the authors. Fortunately, all numerical data needed were adequate for analysis. Both positive and negative results were reported among

the studies. Subgroup analysis at 6-12 months of age was done because previous studies showed that the benefit of exclusive breastfeeding only lasted during time of breastfeeding. Publication bias was assessed by a funnel plot using occurrence of pneumonia at 5 years as endpoint.

Ethical Considerations

The study protocol was reviewed and approved by the hospital's ethics review committee. Personal information from any of the study will not be released without the consent of the stakeholders. The investigator and all key personnel have completed the Good Clinical Practice (GCP) training on the responsible conduct of research with human data. All gathered information was reviewed and kept transparent throughout the study.

RESULTS

The search retrieved a total of 233 references. After screening the studies against the inclusion criteria five studies were included in the meta-analysis. A flowchart diagram for the studies evaluated and the reasons for exclusion are shown in **Figure 1.**

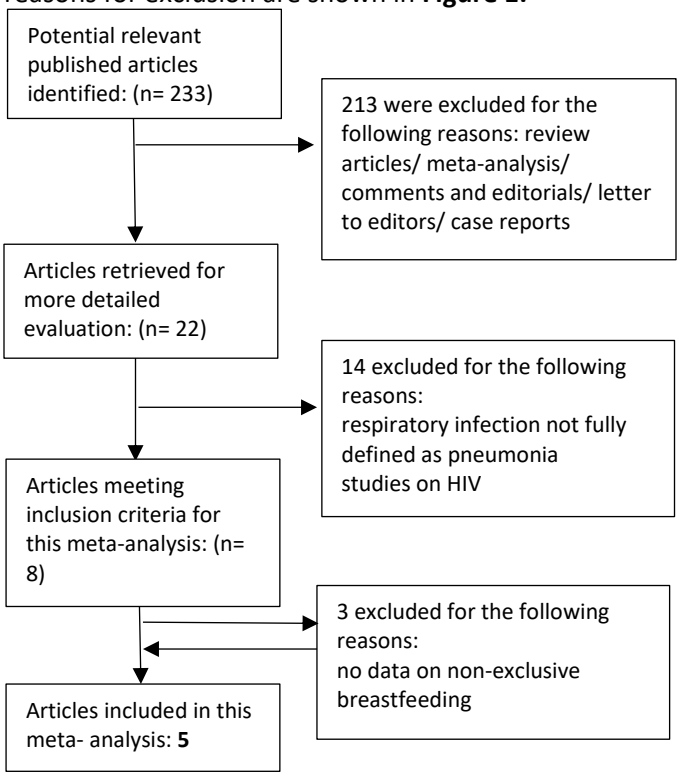

Figure 1. Flow Chart of Study Selection.

Study Characteristics

The study characteristics are summarized in **Table 1.** A total of 45,776 patients with pneumonia were included. The total sample population for each study were 39531 (Yamanaka et al), 5322 (Tromp et al.), 1281 (Li et al), 120 (Svivastava et al) and 512 (Tazinya et al). All studies were published from 2014 to 2018 and were conducted in different countries. They were composed of three cohort studies, one case-control and one cross-sectional study. By WHO region, the included studies were conducted in America (n=1), European (n=1), Western Pacific (n=1), South East Asia (n=1) and Africa (n=1). All the cohort studies and the case control study included recruited subjects since birth and followed up until 5 years of age, while the cross-sectional study recruited subjects from 2 months of age to 5 years of age. In all studies, data were collected from interviews, survey questionnaire and hospital records. The included studies showed high risk for selection bias, performance bias and detection bias. The quality assessment of these studies is summarized in the appendix section. The results of the meta-analyses that investigated the effect of exclusive breastfeeding on pneumonia are shown in **Table 2**.

Table 1. Characteristics of study population

Study, Year	Study Design	Population	Intervention	Control	Outcome
Yamakawa et al., 2015	Cohort (Nationwide Longitudinal Study)	All singleton children who were born after 37 gestational week, born between 10th and 17th January or 10th and 17th July 2001	Exclusive breastfeeding for 6 months from birth	Formula milk, Partial breastfeeding and exclusive breastfeeding of less than 4 months	Hospitalization for Pneumonia until 5 years of age
Tromp et al., 2017	Cohort (Population Based Prospective Study)	Mothers delivered from April 2002 through January 2006	Exclusive breastfeeding 6 months and 6 months and beyond	Never breastfed and breastfeeding for less than 3 months	Pneumonia incidence until 5 years of age
Li et al., 2014	Cohort (Prospective Longitudinal Study)	Mothers on late pregnancy until 1 year after birth from 2005-2012	Exclusive breastfeeding until 6 months and 6 months and beyond	Formula feeding	Pneumonia incidence until 5 years of age
Srivastava et al., 2015	Prospective Case- Control Study	Children aged 1 month to 5 years	Exclusive breastfeeding up to 6 months	Non- exclusive breastfeeding	Pneumonia incidence up to 5 years of age
Tazinya et al., 2018	Cross-Sectional Study	Children under 5 years of age	Exclusive Breastfeeding up to 6 months	Non-exclusive breastfeeding	Pneumonia up to 5 years of age

Table 2. Outcome data of all trials included in the meta-analysis

Study	Incidence of Pneumonia (n/N)	
	Exclusive Breastfeeding	*Non-exclusive Breastfeeding*
Yamakawa et al., 2015	8862/39531	30651/39531
Tromp et al., 2017	2827/5322	2495/5322
Li et al., 2014	1106/1281	175/1281
Svivastava et al., 2015	81/120	39/120
Tazinya et al., 2018	438/512	74/512

Exclusive Breastfeeding and Pneumonia up to 5 years

Pooled estimate of Odds Ratio showed that exclusive breastfeeding was significantly associated with lower odds of pneumonia at age 5 years old *(OR=0.86; 95%CI=0.77-0.95, p-value=0.003).* Only the studies of Tromp et al (2017) and Yamakawa et al (2015) showed significantly lower odds for pneumonia among children who were exclusively breastfed. The rest of the studies did not show significant differences between exclusively and non-exclusively breastfed children. See figure 2. Studies are homogenous *(I^2=0%, p-value=0.41).*

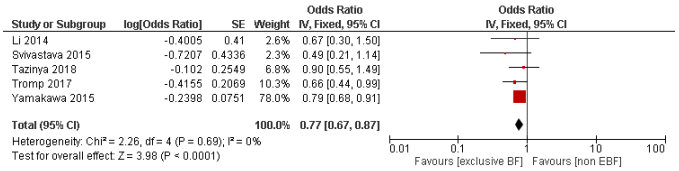

Figure 2. Forest plot comparing the odds of pneumonia up to 5 years between exclusively breastfed compared to non-exclusively breastfed.

Exclusive Breastfeeding compared to Non-Exclusive Breastfeeding from 6 to 12 months

Results from the analyses were available to examine the effect of exclusive breastfeeding compared to non-exclusive breastfeeding in the incidence of pneumonia from 6 to 12 months. The studies are homogenous. *(I^2=30%, p-value=0.22).* Pooled estimate of Odds Ratio showed no significant differences in odds for pneumonia between the two groups *(OR=0.95; 95%CI=0.84-1.01, p-value=0.07).* Only the study of Tromp et al (2017) showed significantly lower odds for pneumonia among children who were exclusively breastfed. The rest of the studies did not show significant differences between exclusively and non-exclusively breastfed children. **See Figure 3.**

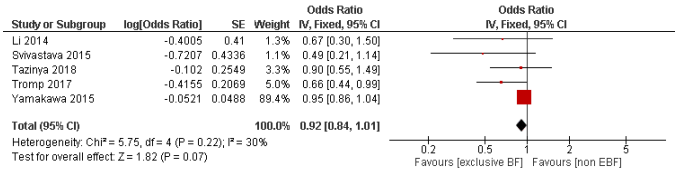

Figure 3. Forest plot showing the odds of pneumonia at 6-12 months old between exclusively breastfed compared to non-exclusively breastfed.

DISCUSSION

The study highlighted the protective effect of exclusive breastfeeding against pneumonia up to five years of age. Findings of the study showed that exclusive breastfeeding from birth to 6 months reduced the probability of pneumonia up to 5 years old by 23%. These results are congruent to the findings of a cohort study done in 1998 by Wilson et. al which concluded that exclusive breastfeeding was associated with reduced risk of respiratory illness even after cessation of breastfeeding.[10] The results were also consistent with the systematic review done by Horta et al.[11] wherein findings showed that breastfeeding reduces the risk for respiratory infection and this protective effect did not change with age. The protective effects as shown by the results were observed on ages 36 to 48 months and not 6-12 months. These findings may be explained by the fact that breastfeeding may truly have long-term protective effects on respiratory infections and such protective effects may become apparent as the children becomes older. Breastmilk contains immune and non-immune compounds which are likely to contribute to long term benefits to infants by facilitating immune development and maturation.[12] Similar to the findings of the study, a prospective longitudinal study found that breastfeeding duration, including breastfeeding longer than 6 months, was not associated with pneumonia or lung infection in 6-year-old children.[13] The results are in contrast to another study wherein findings showed protective effect

of breastfeeding was said to be strongest in the first 6 months of life.[14]

In this study, exclusive breastfeeding showed no significant difference in the odds of pneumonia for the immediate period of 6-12 months. This result is consistent with a study by Quigley et al, which suggests that the protective effect of breastfeeding might wear off after breastfeeding cessation after introduction of solid food that may prevent children from maintaining their immune properties within a sufficient level.[7] It may also be affected by the differences in the population for infectious diseases during the first year of life are strongly related to the gestational age and birth weight, and the present study did not exclude them as subjects.

These results, altogether, support the WHO recommendation for exclusive breastfeeding during the first six months of life at least and beyond. Breastfeeding mothers should be encouraged and supported in making decisions to initiate breastfeeding and maintain exclusive breastfeeding for the first 6 months. Although there is a lack of literature assessing the effect of exclusive breastfeeding and beyond, the studies in this review consistently showed positive effect of breastfeeding even after 6 months. A study in Japan by Yamakawa et al.,[9] observed that the protective effect on the risk of hospitalization for respiratory infections was evident between 18 to 30 months and 30 to 42 months. It suggests that longer duration of breastfeeding was associated with reduced risk of hospitalization for respiratory tract infections and that the protective effect was stronger if breastfeeding was done beyond 6 months. The study of Tromp et al.[15] found breastfeeding for 6 months or longer to be associated with a reduced risk for lower respiratory tract infections after infancy till the age of 4 years. These are consistent with a cross-sectional study done by Chantry[16] in 2006, that showed exclusive breastfeeding for more that 6 months provides more protection against respiratory diseases than does exclusive breastfeeding in less than 4 or 6 months. Protection was also found in the age group of 6 to 72 months in whom the odds of pneumonia were more than fourfold lower if full breastfeeding was continued through 6 months. The results therefore can be beneficial in improving breastfeeding practices given the potentially harmful cost of infection. These findings are compatible with the hypothesis that the protective effect of the duration of breastfeeding for respiratory tract infections persist after infancy.

Similar to these findings, a prospective longitudinal study by Li et al.[13] found that exclusive breastfeeding longer than 6 months was not associated with pneumonia or lung infection in 6 years old children. However, the odds of 6 years old children having greater than 2 sick visits in a year for ear, throat and sinus infection where found to be associated with duration and exclusivity of breastfeeding, the timing of supplementing breastfeeding with formula and breast milk intensity. Previous studies also stated that lack of exclusive breastfeeding wasn't significantly associated with pneumonia. The study of Srivastava[14] concluded the lack of exclusive breastfeeding was not significantly associated with pneumonia when all the children between 1 month to 5 years were considered. Nevertheless, considering only children less than 1 year old among subjects, it was observed that lack of exclusive breastfeeding was significantly associated with pneumonia. In contrast, the study by Tazinya[17] established that children who were inadequately breastfed and did not have significant different proportion of respiratory infections when compared those who were exclusively breastfed.

This review confirms and expands the evidence of the recommendation of the Department of Health on exclusive breastfeeding campaign dubbed "Breastfeeding TSEK: Tama, Sapat at Exclusibo" targeting the new and expectant mothers in urban areas, which aims to reduce child mortality and improve child survival. These data should help convince more mothers to exclusively breastfeed their infants up to 6 months of age.

All of the studies included in this review did not utilize methods to reduce reverse causality bias. Since all studies included were observational, the possibility of confounding is high. However, the effect sizes of the studies involved were large and consistent among age groups and outcomes. The analyses were limited by inclusion of measures calculated with raw data for potential confounders such as age of mothers, socio-economic status, educational background, vaccination status and other acute illnesses.

Finally, although the study yielded the benefit of exclusive breastfeeding on childhood pneumonia up to 5 years of age, the results did not aim to estimate the risk

of breastfeeding children born with congenital infections, malformations or preterm. In addition, there is also a dearth of local data for analysis and data on breastfeeding beyond 6 months of age. In 2004, infant and young child feeding practices were assessed using the WHO assessment protocol and the Philippines rated poor to fair. Findings showed four out of ten newborns were initiated to breastfeeding within an hour after birth, three out of ten infants less than six months were exclusively breastfed and the median duration of breastfeeding was only thirteen months.[18] These are the areas that are yet to be ventured into when it comes to producing cohort studies on breastfeeding and pneumonia. A review on the impact of breastfeeding promotion is also suggested given the low coverage of exclusive and continued breastfeeding in the Philippines.

CONCLUSIONS AND RECOMMENDATIONS

Exclusive breastfeeding from birth to 6 months is associated with statistically significant reduction in the incidence of pneumonia up to 5 years of age. These findings are compatible with the protective effect of breastfeeding for respiratory tract infection that persists after infancy and supports the WHO recommendation for exclusive breastfeeding up to 6 months of age. More observational studies on exclusive breastfeeding and pneumonia with local data, diverse population of children and on continued breastfeeding are needed.

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APPENDIX

Yamanaka, 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	All babies born between 10th and 17th January or 10th and 17th July 2001 all over Japan were targeted
Allocation concealment (selection bias)	High risk	None
Blinding of participants and personnel (performance bias)	High risk	Longitudinal Survey was used
Blinding of outcome assessment (detection bias)	High risk	None
Incomplete outcome data (attrition bias)	Low risk	88% response rate from participants
Selective reporting (reporting bias)	High risk	Longitudinal Survey was used
Other bias	Unclear risk	

Tromp, 2017

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	None
Allocation concealment (selection bias)	High risk	None
Blinding of participants and personnel (performance bias)	High risk	No
Blinding of outcome assessment (detection bias)	High risk	Postal parent-reported questionnaires were used
Incomplete outcome data (attrition bias)	Low risk	Consent for postnatal follow-up was provided by 7893 participants from 9778 mothers
Selective reporting (reporting bias)	High risk	Questionnaire form
Other bias	Unclear risk	

Li et al., 2014

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	None
Allocation concealment (selection bias)	High risk	None
Blinding of participants and personnel (performance bias)	Unclear risk	None
Blinding of outcome assessment (detection bias)	High risk	None
Incomplete outcome data (attrition bias)	Low risk	1542 mother-child pairs
Selective reporting (reporting bias)	Unclear risk	Survey Form for 6 years follow up study
Other bias	Unclear risk	

Svivastava et al., 2015

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	All cases and controls were selected from the age group of 1 month to 5 years if they fulfilled the inclusion criteria
Allocation concealment (selection bias)	Unclear risk	None
Blinding of participants and personnel (performance bias)	High risk	Questionnaire forms were utilized
Blinding of outcome assessment (detection bias)	High risk	None
Incomplete outcome data (attrition bias)	Low risk	120 participants with 60 control subjects and 60 cases subjects
Selective reporting (reporting bias)	High risk	Questionnaire and Interviews
Other bias	Unclear risk	

Tazinya et al., 2018

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Participants were enrolled by a consecutive convenient sampling method
Allocation concealment (selection bias)	High risk	Participants were enrolled by a consecutive convenient sampling method
Blinding of participants and personnel (performance bias)	High risk	None
Blinding of outcome assessment (detection bias)	High risk	Structured Questionnaire
Incomplete outcome data (attrition bias)	High risk	Out of 620 children screened, 512 completed the study
Selective reporting (reporting bias)	High risk	Structured Questionnaire
Other bias	Unclear risk	

ORIGINAL ARTICLE

Antimicrobial Stewardship (AMS) Program in Private Hospitals in the Philippines: Its Acceptability, Barriers, and Enablers

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background: Antimicrobials are drugs that are often misused and inappropriate antimicrobial prescribing often results in poor clinical outcome and drug resistance. Monitoring and regulation of antimicrobial use is currently being done by the Department of Health through the Antimicrobial Stewardship (AMS) Program. There is a need to determine the factors that affect successful implementation of an AMS program in private hospitals in the Philippines. This study was conducted to identify the enablers and potential barriers in implementing an AMS program in nine (9) private hospitals.

Methodology: A concurrent mixed methods design was used to assess various stakeholders' (physicians, administrators, other AMS members) perceptions of existing or proposed AMS programs, and to identify barriers and enablers in their implementation. Quantitative data were collected using self-administered survey questionnaire to assess clinician's acceptance of AMS programs. Qualitative data were collected through semi-structured one-on-one interviews of clinicians and other AMS personnel and focus group discussions (FGD) of selected clinician groups. Data were gathered from October 2018 to October 2019.

Results: 409 clinicians were surveyed, 52 were interviewed and 46 sat for 13 sessions of FGDs. Overall, the survey established that physicians were well aware of antimicrobial resistance problem. Majority of the clinicians indicated general agreement with the currently practiced antimicrobial protocols in their hospitals and with the AMS program. However, there were disagreements in perceptions with how antimicrobial restrictions impair prescribing practices and overuse of the same. These responses were strong points of discussion during the Key Informant Interviews (KII) and FGDs. All respondents were amenable with the institutionalization of an AMS program in their hospitals. The hospital leadership's commitment was determined to be the key enabler of a successful AMS program's implementation. Barriers identified for hospitals with existing AMS programs were: lack of dedicated staff, resistance and/or non-cooperation of physicians, lack of support from non-medical departments, and inadequate cooperation between hospital

personnel. Barriers identified, regardless of the status of the AMS programs were: deficiency in knowledge with developing and implementing an AMS program, inadequate information dissemination, unavailability of an IT-based monitoring for antibiotic use, and the influence of pharmaceutical companies on stakeholders with regards to antimicrobial use.

Conclusions: Similar enablers and barriers to a successful implementation of an AMS program were seen in the different hospitals. A hospital leadership's commitment was determined to be the key enabler. The success or failure of any AMS program appears to depend on physician understanding, commitment and support for such a program. By involving the main players in an AMS program- the hospital administrators, clinicians and other key members, perceived barriers will be better identified and overcome, and enablers will help allow a successful implementation of an AMS program.

This multi-center study was funded by Philippine Council on Health Research and Development (PCHRD) and Pediatric Infectious Disease Society of the Philippines (PIDSP) and was conducted by the PIDSP Research Committee.

BACKGROUND

Antimicrobials are commonly prescribed drugs in all age groups, in many situations they are either misused or overused. This inappropriate prescribing habit contributes to increased cost of medical care, prolonged course of an illness, and increased rates of antibiotic resistance.

Antimicrobial resistance is recognized as one of the greatest threats to human health worldwide. One of the manageable causes of antimicrobial resistance is the overuse and misuse of antimicrobial agents in humans, animals, agriculture, and consumer products.[1] Antimicrobial resistance is expected to develop over time, as a consequence of any antimicrobial use, whether appropriate or not, as microorganisms mutate and acquire resistance to a drug when exposed to it. To counteract this process, antimicrobials should be used responsibly and appropriately, in order to preserve their usefulness, recognizing that the use of these drugs is accompanied by a myriad of individual and societal effects.

In the Philippines, data from the Antimicrobial Resistance Surveillance Program (ARSP) of the Department of Health (DOH) has shown an alarmingly high resistance of various pathogens to first-line antimicrobials. Multi-drug resistance (MDR) among bacterial organisms-*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii,* is a public health concern because of the limited treatment options, high cost of care, and infection control challenges. MDR and extensive drug resistance (XDR) rates are increasing.[2] These disturbing data reinforce the importance of promoting the rational use of antibiotics. This concern is the main goal of creating a program that institutes antimicrobial stewardship (AMS).

The ARSP data is a wake-up call to both government and private sectors to prioritize the implementation of an AMS program in the clinical setting. The DOH, for its part, has started to roll-out and implement the AMS program in all government and private hospitals. In a controlled clinical environment, such as a tertiary government hospital, the implementation of an AMS program may be relatively straightforward. This may not be the case in most private hospitals and small government hospitals.

While it is equally important to implement an AMS program in private hospitals, doing so can be challenging, given the marked variations in the prescribing habits of private clinicians. The current DOH AMS Manual highlights the need for the support and cooperation of hospital administrators, for the program to become successful.

As a society whose members are stewards of rational antimicrobial use, the Pediatric Infectious Disease Society of the Philippines (PIDSP) looked into the challenges of implementing an AMS program in private hospitals. More importantly, physician perceptions regarding restricted antimicrobial prescribing and an institutionalized AMS program, in general, were sought. In so doing, enablers and potential barriers to the implementation of an AMS program would be identified.

METHODS

Data were gathered to assess clinicians' perceptions and to identify barriers and enablers in the implementation of a hospital-based AMS program, using qualitative and quantitative methods. Convenience sampling was employed. Medical directors, pediatricians, internists, medical technologists, pharmacists and infection control nurses from nine privately-owned hospitals, from different parts of the country, were asked to participate in this descriptive, cross-sectional study. For ethical reasons, the names of these hospitals are withheld, and the participants anonymized. The protocol was reviewed and approved by the Institutional Review Board (IRB) and ethics committee of each hospital.

The hospitals were stratified as follows: three NCR Hospitals with an existing AMS program; three NCR hospitals with no, or had just started an AMS program; and one hospital each, from Luzon, Visayas and Mindanao with no AMS program. Data were gathered from October 1, 2018 to October 31, of 2019.

Quantitative data was collected using a 26-item opinion survey administered to determine the clinicians' acceptability of an AMS program. The instrument was adapted from a validated tool from the Greater New York Hospital Association (GNYHA), with minor revisions made. The survey questionnaire was revised using Cronbach's alpha, which showed a reliability of 0.762, thus validating the revisions' entry into the final version

of the questionnaire. Likert scale and frequency distribution and measures of central tendency (mean, mode and range) were determined.

Qualitative data were generated from interviews and FGDs to ascertain the participants' positions regarding an AMS program, including the struggles and setbacks they faced, their strategies, successes and suggestions, in the implementation of the said program. A set of guide questions were used. The responses were recorded and transcribed, but were not read back to the participants. A Qualitative Descriptive Study (QDS), an approach commonly used in health research, and loosely grounded on more conventional qualitative approaches was used to process the data (Kim, 2017). Themes were determined through axial coding.

Two research assistants distributed and retrieved the 26-item survey questionnaire from selected clinicians in the target hospitals. The questionnaire has 4 sub-parts: a. Antimicrobial Resistance: Scope of The Problem and Key Contributors; b. Antibiotic Prescribing Practices; c. Antimicrobial Stewardship Programs; and d. Acceptability of an Antimicrobial Stewardship Program. Collected data were processed manually and rechecked before they were statistically processed in Microsoft Excel. Results were reviewed for consistency and accuracy and frequencies, means and modes were determined.

RESULTS AND ANALYSIS

The demographic data and type of participation of study participants from the 9 hospitals were as follows: 409 clinicians (Pediatricians, n= 282, Internists, n= 127) were surveyed, 52 were interviewed individually as Key Informant Interviewee (KII) with 13 Focus Group Discussion (FGD) sessions and an average of 2-6 respondents per session (a total of 46 participants) were conducted. Summary in Tables 1 and 2.

Table 1. AMS Survey Respondents (N=409)

HOSPITAL	PEDIA	IM	TOTAL
I A	29	13	42
II A	55	2	57
III A	51	11	62
IV B	19	0	19
V B	23	0	23
VI B	20	1	21
NCR Sub Total	***197***	***27***	***224***
VII B	35	41	76
VIII B	35	41	76
IX B	15	18	33
Provincial Sub Total	***85***	***100***	***185***
TOTAL	**282**	**127**	**409**

Legend: A- Hospital with AMS program **B-** with no or which had just started an AMS program

Table 2.1. Participants of Key Informant Interviews. (n=52)

POSITION	Number of Respondents
Medical Director	7
Department Head (Internal Medicine)	4
Department Head (Pediatrics)	8
Infection Control Head / Pharmacist Head	7
AMS Physician	6
AMS / ICC Nurse	7
Clinical Pharmacist	9
Microbiology Head	2
ICS Department Manager	2
TOTAL (n)	**52**

Table 2.2. Participants in Focused Group Discussions, 13 sessions. (n=46)

Department	Number of Respondents
Adult Department (Metro Manila)	2
Adult Department (Provincial)	10
Pediatrics Department (Metro Manila)	15
Pediatrics Department (Provincial)	19
TOTAL (n)	**46**

A. AMS: Acceptability –

Antimicrobial Resistance: Scope of The Problem and Key Contributors

The first part of the questionnaire was on Antimicrobial Resistance: Scope of The Problem and Key Contributors. The respondents were asked to respond to 6 items about antibiotic resistance, surveillance and related protocols. Table 3 consolidates the responses from the different hospitals.

Table 3. Antimicrobial Resistance: Scope of The Problem and Key Contributors

Questionnaire Items	Hospitals										
	I	II	III	IV	V	VI	VII	VIII	IX	Mode	RANGE
1. Antibiotic resistance is a significant problem in this institution	3	4	4	4	4	2	4	4	4	4	(2, 4)
2. A MDRO patient's room is cleaned according to hospital cleaning protocol after discharge	4	4	5	5	4	4	4	4	4	4	(4,5)
3. Adherence to hand-hygiene protocols is efficient in this institution.	4	4	5	5	4	4	4	4	4	4	(4,5)
4. The institution does enough to control the development of resistant organisms through surveillance.	4	4	5	5	4	5	4	4	4	4	(4,5)
5. This institution provides an adequate MDRO education program for its staff.	4	4	4	4	4	4	4	4	4	4	4
6. A patient is likely to develop an MDRO infection during their stay (while admitted) in this institution.	2	2	3	3	2	3	3	2	4	2	(2, 4)
Legend: 1 - Strongly Disagree, 2 - Disagree, 3 - Neither, 4-Agree, and 5 - Strongly Agree											

Table 3 shows that the participants generally agreed that antibiotic resistance is a significant problem in their institutions, with a mode of 4. The responses were not unanimous, however, with a range of 2 to 4. A case in point is hospital VI, wherein the collective response to this item no.1 was 2, indicating that antibiotic resistance is not a significant problem in that hospital. Hospital I registered a 3, suggesting that doctors in said hospital are evenly divided on the matter. When mean scores (data not shown) for Hospital VI were obtained, compared to mode, the result was closer to a 3, suggesting a considerable diversity of opinion within the hospital.

For items nos. 2 to 5, all the respondents agreed that their institutions ensure that hygienic protocols are in place to mitigate the development of drug resistance, such as the cleaning of an MDRO-patient's room, according to hospital cleaning protocol after discharge; there is adherence to hand-hygiene protocols; enough efforts are being done to control the development of resistant organisms through surveillance; and adequate MDRO education for its staff is being provided.

For item no. 6, there was a wide variation of responses, with a mode of 2, but a mean of 2.8. This implies a significant variation in perception with regards to acquiring an MDRO infection during a hospital stay. The 6 questions established that hospitals were very much aware of the antimicrobial resistance problem, that they agreed that necessary protocols were being followed, but there was uncertainty whether such protocols were effective enough.

Antibiotic Prescribing Practices

The respondents were asked about their antibiotic prescribing practices.

Table 4. Antibiotic Prescribing Practices

Questionnaire Items	Hospitals										
	I	II	III	IV	V	VI	VII	VIII	IX	MODE	RANGE
7. Microbiology lab results are efficiently communicated to the attending physician.	5	4	5	5	4	4	4	4	4	4	(4,5)
8. I regularly consider the antibiotic susceptibility patterns at this institution (e.g. the institutional antibiogram) when empirically prescribing antibiotics.	4	4	5	5	4	4	4	4	4	4	(4, 5)
9. If medically appropriate, intravenous antibiotics should be stepped down to an oral alternative after three days.	4	4	4	4	4	4	4	4	4	4	4
10. Restrictions on antibiotics impair my ability to provide good patient care.	2	2	3	3	4	4	3*	2	2	2	(2, 4)
11. Antibiotics are overused in this institution.	4	2	2	2	2	2	4	3	4	2	(2, 4)
12. More judicious use of antibiotics would decrease antimicrobial resistance.	5	5	5	5	5	5	5	5	5	5	5
13.Antimicrobial stewardship programs improve patient care.	5	5	5	5	5	4	5	5	5	5	5

Legend: 1 - Strongly Disagree, 2 - Disagree, 3 - Neither, 4-Agree, and 5 - Strongly Agree

* with opposite responses from two departments. The responses were2 and 4.

The questions in part 2 of the questionnaire (Table 4) center on protocols for antibiotic dispensation. The respondents opined that microbiology results were efficiently communicated to them, with responses of 4 and 5. The respondents also similarly agreed with the item no. 8, on considering the hospital antibiogram when writing antibiotic orders, and item no. 9, stepping down from intravenous to oral antimicrobials in three days, when appropriate. Despite the agreement on the need for antibiotic restrictions, responses to item no.10 showed some variability in responses on whether such restrictions affect patient care, with 2 (22%) hospitals (V and VI, NCR-no AMS) responding that such policies impair the ability to give good care, and a plurality of 6 (66%) disagreeing that it does so. These polarized opinions were expressed even in the same hospital (VII, Provincial), wherein one department answered 2 (agree), and the other 4 (disagree). When mean (2.77) was compared to mode (2), the former points to the general sentiment being closer to "neither," rather than to "disagree," which is the mode response. This indicates that between hospitals, and between departments within a hospital, opinions varied.

Item no. 11, on whether or not antibiotics are overused in the institution had similar variability of answers for item no. 10; median answer was 2, but mean was 2.7 (which is closer to a "neither" answer). But hospitals I, VII, IX, all big hospitals with 2 provincial hospitals but no AMS yet, agreed mean (4) that antibiotics are already overused in their institution.

Items 12 (judicious use of antimicrobials would decrease resistance) and 13 (AMS improves patient care) showed a uniform and almost unanimous "strongly agree" response in all hospitals, except for one "agree" for item no. 13.

Antimicrobial Stewardship Program

Part 3 (Table 5) are questions on opinions on the antimicrobial stewardship program.

Table 5. Antimicrobial Stewardship Program

Questionnaire Items	Hospitals										
	I	II	III	IV	V	VI	VII	VIII	IX	Mode	RANGE
14. Antimicrobial stewardship programs reduce the problem of antimicrobial resistance.	5	5	5	5	5	5	5	5	5	5	5
15. Antimicrobial stewardship programs decrease this institution's infection rates.	4	5	5	5	5	4	5	5	5	5	(4,5)
16. This institution has a functional antimicrobial stewardship program.	4	4	5	5	4	4	5	4	4	4	(4,5)
17. Personal and individual efforts regarding antimicrobial stewardship improves this institution's resistance problem.	4	4	5	5	4	4	5	4	4	4	(4,5)
18. This institution provides adequate training on antimicrobial prescribing and use.	4	4	5	5	4	3	4	4	4	4	(3, 5)
19. Additional staff education is needed on antimicrobial prescribing and use.	4	4	4	4	4	4	4	4	4	4	4
20. Prescribing physicians are the only disciplines who need to understand antimicrobial stewardship.	4	2	1	1	2	2	2	2	2	2	(1, 4)
Legend: 1 - Strongly Disagree, 2 - Disagree, 3 - Neither, 4-Agree, and 5 - Strongly Agree											

Strong undisputed agreements strongly agree were expressed concerning the effectiveness of antimicrobial stewardship programs in reducing the problem of antimicrobial resistance (item no. 14) and decreasing infection rates (item no.15). All agreed that their institutions have functioning AMS programs (item no. 16) and that individual contributions towards the same help their institution in improving antimicrobial resistance (item no. 17).

The respondents agreed that their hospital provides adequate training for antimicrobial dispensation, except for hospital VI which replied "neither" (item no. 18), and all agreed on the need for additional staff education (item no.19). These responses were strong points of conversations during the Key Informant Interviews (KII) and FGDs.

All disagreed that only physicians are the only ones who need to understand antimicrobial stewardship, except for Hospital I, that answered, "Agree."

Acceptability of Antimicrobial Stewardship Program

The final segment of the questionnaire is on the acceptability of the AMS in private hospitals (Table 6).

Table 6. Acceptability of Antimicrobial Stewardship Program

Questionnaire Items	Hospitals										
	I	II	III	IV	V	VI	VII	VIII	IX	Mode	Range
21.I am amenable to having an AMS program in our institution	5	5	5	4	5	4	5	4	5	5	(4, 5)
22.I support the programs of the AMS committee in our institution	4	4	5	5	4	4	5	5	5	5	(4, 5)
23.I am willing to attend the educational sessions conducted by the AMS committee	4	4	5	5	4	4	5	4	5	4	(4, 5)
24.I am willing to be subjected to antibiotic audit when you prescribe restricted antibiotics	4	4	5	5	4	3	4	4	5	4	(3, 5)
25.I agree with the 7th day automatic stop order policy of the AMS committee	4	4	4	4	4	4	4	4	5	4	(4, 5)
26.I agree with the antibiotic restriction policies of the AMS program in our institution	4	4	5	5	4	4	4	4	5	4	(4, 5)
Legend: 1 - Strongly Disagree, 2 - Disagree, 3 - Neither, 4-Agree, and 5 - Strongly Agree											

All of the respondents are amenable with the institutionalization of an AMS program (item no. 21); hospitals I, II and III already have an existing AMS program prior to the survey. All expressed support for an AMS in their hospitals (item no. 22) and expressed willingness to be subjected to antibiotic audit), except hospital VI, which had a neutral stand on this last item (item no. 24). The respondents agreed with a 7-day automatic stop order, and with the antibiotic restriction policies of the AMS program in their institutions.

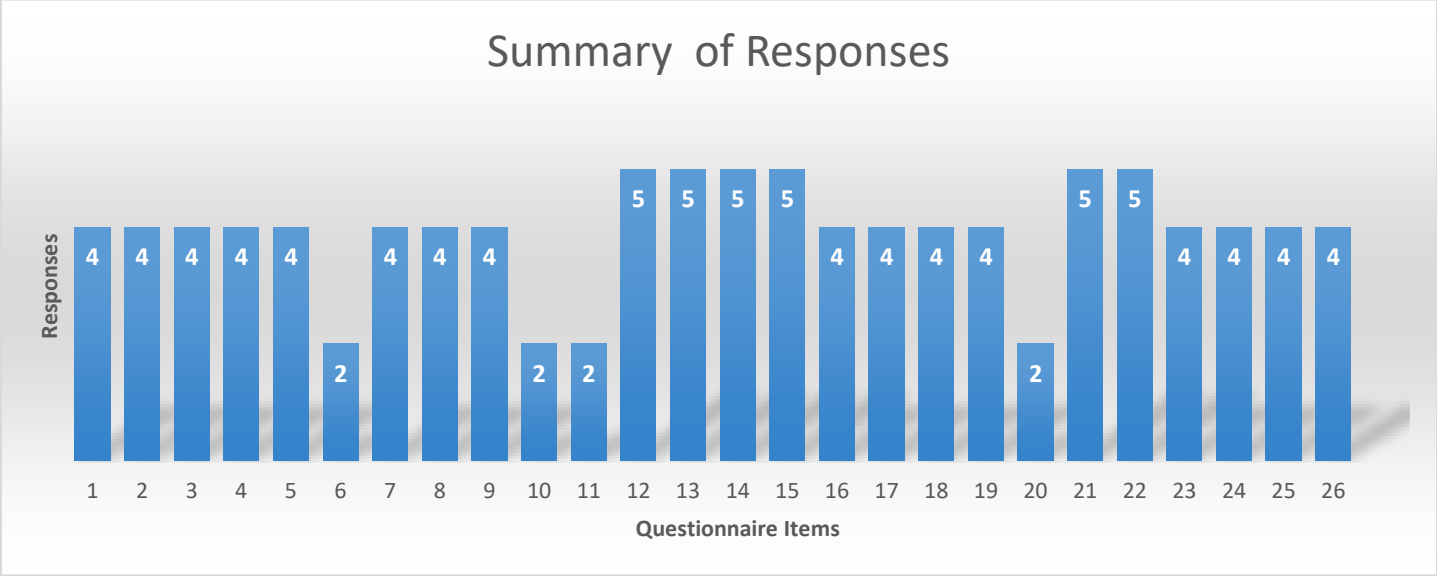

Figure 1. Summary of Responses to the 26-Item Opinion Survey

Figure 1 summarizes the responses to the survey using mode to highlight the prominent/dominant responses.

The statements questionnaire solicited opinions of agreement, disagreement and neutrality. Items # 6, 10, 11, and 20 were controversial statements framed as such to provoke disagreements or neutral responses. The rest of the items are positive statements constructed to elicit agreements or neutral responses. The opinion survey managed to gather diverse opinions in many of items especially for items # 6, 10, 11, and 20 which responses ranging from 1-5. Collectively, however, as shown in Figure 1, the trend of responses is within the framework of the questionnaire and is geared towards the acceptability of an AMS program as a viable means to curtail the emergence and progression of problems with antimicrobial resistance.

B. AMS: Enablers and Barriers

The following section centers on the enablers and barriers of implementing an AMS program. It looks into the experiences of physicians, administrators, microbiology heads and infection control nurses in hospitals with and without AMS programs, through FGDs and KIIs.

Enablers to AMS Implementation

Hospitals I, II, and III have organized and functioning AMS programs. The participants from these hospitals said that an AMS program is already an integral part of their operating procedures and they attributed the successful implementation to a few conditions. The respondents shared that the full commitment of the hospital's leadership is the key enabler for success. The leadership's commitment to comply with DOH and international accreditation (such as JCI) standards regarding AMS, fully enabled these hospitals to establish an AMS protocol. The respondents acknowledged the problem of MDROs and they have realized that a good AMS program is necessary to combat it. Such impressions were gauged from the FGDs and KIIs, during which the functions, policies and workings of the hospitals' AMS program were discussed. Prior to the formal implementation of their AMS programs, the three hospitals already had an existing program as part of the infection control committee. Such programs monitored their own infection control indicators and it became incumbent upon the infectious disease group to raise alarms" (KII 5). For hospitals without an AMS, the Infection Control Committee (ICC) is the *de facto* antimicrobial steward.

The discussions brought out the perception that it is the hospital administrator who decides on the guidelines, policies and educational programs of the AMS program (KII 1), thus, enhancing his or her role as a key enabler in the implementation of a successful program. Policies strengthen the program (KII 1), while education- for both consultants and patients can change perceptions and influence appropriate changes in antibiotic use (KII 1). An AMS program is not solely a government program, it is also necessary for international accreditation (KII 1; KII 6). Thus, this lead to the hospital management taking the lead in the formation of an AMS program. When a strong political will from within the hospital allowed the creation, establishment and functioning of a program, the workings of the AMS program became a routine process. Regular orientation and collaboration with stakeholders within the hospitals were also underscored as enablers for the AMS program's implementation.

Some suggestions provided for a successful AMS program were: creating a professionalized approach to medical treatment, providing and disseminating periodic infection rate updates to stakeholders, and conducting regular audits of antibiotic usage among clinicians.

Hospitals that are still in the process of establishing their AMS program were asked how an AMS program can be successfully established in their hospitals. The following responses were obtained from the FDGs and KIIs:

1. Leadership Commitment
2. Adherence to DOH AMS Guidelines
3. Educational Awareness Program: regular orientation and Information dissemination (posters and infographics)
4. Cooperation and collaboration among clinicians
5. Regular antibiogram review
6. Additional staff for the AMS team (e.g. clinical pharmacist)
7. Mutual respect among health professionals
8. Better communication on case referrals
9. Pilot AMS with initial evaluation

Box 1. Suggested Enablers for a successful AMS program

Some of the enablers, already mentioned earlier, like leadership commitment and adherence to DOH AMS Guidelines; regular orientation, as well as cooperation and collaboration also emerged as themes for the hospitals without AMS.

Of these themes-additional staff, educational programs and piloting the AMS warrant short elaboration. Many respondents mentioned the need for additional staff, especially clinical pharmacists, to routinely monitor antibiotic use. It was suggested that, to enable a functional AMS, more clinical pharmacists should be hired and be assigned to specific areas or floors in the hospitals, to allow better monitoring of antimicrobial use.

Educational program was also identified as an important enabler. The educational program may come in many forms, ranging from promoting information dissemination, carrying out awareness campaigns, encouraging open and regular communication with stakeholders (KII 1, IX), providing technical know-how on AMS (KII 2, V), and more importantly, conducting periodic updates on AMS outcomes to show that the program works (KII 5, I).

The respondents also suggested to pilot test the AMS program prior to its implementation. In hospital XI, a six-month pilot period was conducted to discover missing links, which were addressed accordingly (KII 4, XI). The result of the pilot testing can be used to recalibrate the prospective AMS program before its formal start of operation (hospital XI has recently launched its AMS program successfully).

1. Staff-related concerns
2. Need for additional manpower (like clinical pharmacists and microbiologists)
3. More workload for nurses
4. Resistance/ Non-cooperation from other doctors
5. Resistance/Lack of acceptance from some clinicians, some consultants, mostly from senior consultant
6. Lack of support from other departments/ lack of cooperation between hospital personnel
7. IT Resources
8. Unavailability of IT-based monitoring scheme for antibiotic use
9. Funding
10. Additional cost to patients (on additional AMS protocol for antibiotic use)
11. Lack of knowledge in developing and implementing the AMS program
12. Influence of pharmaceutical companies

Box 2. Barriers to AMS implementation (Hospitals with an AMS program)

Below were the barriers echoed by the participants from hospitals with no AMS or those hospitals on the process of putting up their respective AMS programs. Although these barriers are only perceived or anticipated they are similar to the actual barriers in Box 2.

1. Lack of manpower: Clinical pharmacists, Microbiologists, Nurses, Infectious Disease physicians
2. Lack of acceptance of AMS by clinicians
3. Additional expenses
4. Additional financial cost on the patients in relation to doing specimen culture and professional fee
5. Unavailability of medicines in the hospital's pharmacy
6. Drug sponsorships /Conflicts of interest
7. Unstable IT system
8. Lack of information dissemination

Box 2. Barriers to Implementation (Hospitals without an AMS)

DISCUSSION

This descriptive, cross-sectional study of perceptions and observations on antimicrobial stewardship in nine private hospitals acquired important data that may be useful in crafting AMS programs in the future, to aid hospitals that do not have existing AMS programs. There was unanimous agreement among clinicians, in as far as their individual hospitals were concerned, that: rooms from which patients had MDROs should be cleaned well upon a patient's discharge; hand hygiene protocols are being done efficiently; enough organism resistance surveillance is being done; adequate MDRO education is being provided; microbiology results are efficiently transmitted to the doctor; antibiotic susceptibility patterns are regularly considered when prescribing antibiotics; step-down from intravenous to oral antibiotic should be considered after three days, if appropriate; judicious antibiotic use decrease organism resistance rates; AMS programs improve patient care, reduce antimicrobial resistance, and decreases infection rates; their hospital has a functional AMS program, but additional staff education is needed on proper antimicrobial use; individual physician efforts to aid the AMS program help reduce antibiotic resistance; doctors support an AMS program, and are willing to attend educational sessions provided by such; doctors agree to a 7-day antibiotic automatic-stop order, and with antibiotic restriction policies of the AMS program. On the other hand, there was non-unanimity on the following items: antibiotic resistance is not a significant problem in 78%; 44% reported that a patient is unlikely to develop an MDRO infection during the hospital stay, with 44% being neutral; 44% disagreed that antibiotic restriction impairs a doctor's ability to give good patient care, but 33% were neutral. Fifty-five percent disagreed that antibiotics are overused in their hospital, but 33% agreed of their overuse. Eighty-nine percent agreed that their hospital provides adequate training on antibiotic use, but 11% (hospital VI) disagreed. Eighty-nine percent disagreed that only doctors should be educated on AMS programs, but 11% (Hospital I) agreed. Eighty-nine percent are willing to be subjected to antibiotic audit when using such, but 11% disagreed (Hospital VI). From FGDs and KIIs, enablers and barriers to a good AMS program were identified.

In this study, the three hospitals with an existing AMS program and another three hospitals without an AMS program in the provinces, are big private hospitals with teaching programs. The other three hospitals without an AMS program in NCR are also big private, but non-teaching hospitals. The institution of hospital based AMS programs are an offshoot of the DOH's creation of the National Antibiotic Guidelines in 2017, which was aimed at "optimizing antimicrobial use and helping to improve the quality of patient care and patient safety." Although the program is already in place in government hospitals, quite a few private hospitals already have an institutionalized AMS program. Many private hospitals are still in the process of complying with the DOH guidelines. An AMS program essentially promotes good antimicrobial stewardship, which is one of two major principles which impact on the problem of antimicrobial resistance. Several terms are used to refer to antimicrobial stewardship programs: antibiotic policies, antibiotic management programs, and antibiotic control programs, are some of these, which may be used interchangeably. The terms refer to programs intended to change antimicrobial use in health care institutions. This may employ any of the following individual strategies: 1) education through the creation of guidelines for antimicrobial use, 2) restriction in dispensing targeted antimicrobials only for their approved indications, 3) review and feedback of targeted antimicrobials for appropriateness, 4) computer assistance and use of information technology to implement strategies and use of expert systems to provide patient-specific recommendations at the point of care, and 5) antimicrobial cycling through scheduled rotation of antimicrobials used in hospitals or units within a hospital like the intensive care unit.[3,4] AMS programs elsewhere have become more common; a survey of 502 physician members of the Infectious Diseases Society of America's Emerging Infections Network, reported that 50% of the respondents indicated that their hospital had an antimicrobial restriction program in place. Teaching hospitals were significantly more likely to have such a program than non-teaching hospitals, 60% versus 17% [4,7].

Of the 26 items in this study's questionnaire, there was unanimous agreement among the respondent hospitals in 19 items, all of which sought reinforcement

on desired attitudes of physicians towards: identifying the problem of antimicrobial resistance and the factors that contribute to this, appropriate antimicrobial usage practices, the value of an AMS program, and the acceptability of an AMS program. Clinicians agreed on practices like adherence to hand hygiene protocols, educational programs, and considering their institutional antibiogram when empirically prescribing antibiotics. They showed strong agreements on the principle that more judicious use of antibiotics would decrease antimicrobial resistance and improves patient care. These results are similar to previous studies that reflect the view that the most favored interventions are those that provide information and education rather than restrict prescribing behaviour.[12]

Respondents indicated that antibiotic resistance is not a significant problem in 78%. Hospital I had a mean answer of 3 (neither) which may be because they have been implementing their AMS program already and may have seen improvements in their hospital antibiotic resistance while Hospital VI which has not started their AMS program yet, disagreed to the statement. This may reflect that hospitals may see improvements in their antibiotic resistance rates once an AMS program is in place.

Forty-four per cent reported that a patient is unlikely to develop an MDRO infection during the hospital stay, with 44% being neutral but 22% (Hospital VIII and IX) reported that patients are likely to develop an MDRO infection. These 2 provincial hospitals have just started their AMS program. Furthermore, there are more adult internists who answered the questionnaires in these 2 hospitals which may have affected their response. The development of MDRO has many factors to be considered and should be further investigated using individual hospital clinical data, antibiotic usage and different population (pediatric and adult).

This study tried to capture the respondents' opinions regarding the institutionalization of AMS in their hospitals of affiliation. Although there were some differences in opinions, the trend of responses gravitated towards an acknowledgement that MDROs are a major health problem and that antibiotics can be overused, for which reason, an AMS program is necessary. The acceptability of an AMS program, however is just one facet of a long process. The greater challenge is how to organize and establish it in each unique hospital.

Fifty-five per cent disagreed that antibiotics are overused in their hospital, but 33% agreed at their overuse. Although the term overuse may have been interpreted differently and should have been followed by a scale to further characterize the usage of antibiotics, the diversity of the answers indicates that the perception of clinicians may vary depending on their years of clinical experience and subspecialties.

Eighty-nine per cent agreed that their hospital provides adequate training on antibiotic use, but 11% (hospital VI) disagreed. For example, in the FGDs, respondents said that there is a need to "overhaul" the mindset in terms of antibiotic use by going "back to the literature" regarding a shorter seven-day course as being sufficient for most infections compared to fourteen days. Hospital VI have no AMS program structure yet and may have no activities for AMS program stated. The clinicians' answers may also indicate their satisfaction on the activities being done in their hospitals that support education and training.

Eighty-nine per cent are willing to be subjected to antibiotic audit when using such, but 11% disagreed (Hospital VI). Possible reasons for resistance to antibiotic audit are: a clinician may feel that he or she is competent to decide which antimicrobial is appropriate, and an external audit is unnecessary; another reason may be the additional cost to the patient, should there be one, by an audit. In some institutions, the AMS program can incur additional costs to patients when the restricted antibiotics ordered may need approval by the infectious disease specialists. In a study by MacDougall et al, 28% of participating institutions required prior approval by an infectious diseases clinician, before certain antimicrobials are dispensed, while 21% required approval by a clinical pharmacist.[4] Of note was that larger hospitals were more likely to have antimicrobial restriction programs compared to smaller ones.[5] In the Philippines, it can be argued that the AMS is still in its infancy, especially in privately-owned hospitals. All the participating large hospitals in this study have infectious disease clinicians who give approval for the certain prescribed restricted antimicrobials. However, to date, there are few private hospitals with in-house AMS programs and infectious disease specialists. Locally,

clinical pharmacists are not allowed to approve the use of restricted drugs. In most of these hospitals, the challenges and impact of ongoing AMS activities have yet to be clearly defined and evaluated.

This study found that the top enabler for an AMS program is a committed hospital leadership. Best practices shared by the hospitals with working AMS program identified hospital leadership's commitment and adherence to DOH AMS Guidelines as the main drivers for starting its implementation. This was followed by: regular educational awareness programs, such as providing regular orientations for all the staff, information dissemination (posters and infographics) and providing and disseminating a hospital antibiogram review. Other identified enablers are additional staff especially, clinical pharmacists, promoting mutual respect among health professionals, and better communication when a case is referred to multiple services. The findings in this study suggest that hospitals undergo similar experiences as AMS programs are implemented.

Barriers identified for hospitals with, and without, AMS programs, were: a deficiency in knowledge with developing and implementing the AMS program, inadequate information dissemination, unavailability of an IT-based monitoring scheme for antibiotic use, and the Influence of pharmaceutical companies upon stakeholders with regards to antimicrobial use. The low level of experience with AMS suggests a degree of unfamiliarity with data and interventions. These barriers, however, as one respondent said, are temporal: *"I don't think there's going to be permanent barriers. I mean it's always the independence of the doctors that's going to be a big problem" (KII4 VIIB)*. During the FGDs and KIIs, a recurring theme was that, the success or failure of any AMS program largely hinges on physician, the major player in the AMS program. The role of the physician in the AMS program can not be underestimated. In the hierarchy of health care delivery, the physician is at the pinnacle, and his/her decisions dictate the course of medical therapy. The importance of physician understanding and acceptance of the AMS program emerged as an important enabler for a successful program implementation. One of the common items that came out was the apparent superiority complex and the "*ako ang doctor"* (I am the doctor) mentality. For the respondents (who were mainly doctors), this mentality can be counter-productive to an AMS program, especially when antibiotic prescriptions are concerned. This problem can be exacerbated when different antibiotic treatment modalities are considered or offered, according to various specialists involved in a single case. This brought about calls from some respondents to suggest for the DOH to dialogue with specialty societies.

The AMS programs of hospitals I, II and III were not created overnight. The successful implementation of their AMS programs did not come without barriers. Prior to their fruition, the programs hurdled several obstacles, such as staff-related concerns (hiring additional clinical pharmacists and microbiologists; adding workload to nurses), and overcoming resistance from non-cooperating, mostly senior physicians. Several infrastructures were made operational, like information technology (I.T.) resources for medical records and improved monitoring systems. Compared with hospitals from countries like Singapore, local hospitals still need to develop a more sophisticated I.T.-based antibiotic monitoring scheme.

Lastly, it was identified that the influence of pharmaceutical companies over private hospitals and clinicians on drug use may affect antibiotic prescribing practices. It is common knowledge that drug companies can influence physicians who *"tend to prescribe more of the medicines that are being promoted by those companies that support them".* The prescription of broad spectrum antibiotics for illnesses that call for "basic" antibacterial and with a strong lobby from drug companies, is a reality and adds to antibiotic misuse. Drug companies and their transactions with physicians are "real barriers". For the AMS program to work, physicians will have to be educated and convinced about the ill-effects of inappropriate antibiotic use and not be swayed by pharmaceutical companies promotions.

LIMITATIONS

Limitations to this study were due to the convenience sampling selection bias which may affect the results particularly among the adult clinicians where the response rate on the questionnaires in the NCR was low and the hospitals selected were all large hospitals with an infectious disease specialist . No demographic

information was collected to test for bias between responders and non-responders, thus the investigators can only speculate on whether there were any important differences between these groups.

CONCLUSION

In this study, the identified enablers and barriers to a successful implementation of an AMS program, and an assessment of the perceptions of clinicians and hospital administrators, showed that in hospitals with an existing AMS program-the relevant themes and issues to program implementation, are similar as for hospitals without an AMS program. The common challenge was in finding qualified personnel willing and able to direct such programs and manage the team at each institution. The team members should also be fairly compensated for the additional time and effort thus funding is needed to implement the program.

The findings gathered from this research may be used to draft guidelines for the institutionalization of an effective and feasible AMS program for private hospitals, as mandated by the Department of Health. Hospitals administrators recognize that they need help from outside sources, including the DOH, to develop and implement an AMS program. A well-designed AMS program will be more acceptable and easier to roll-out for hospitals which have yet to start an AMS program and will enhance ongoing programs in hospitals with existing AMS-related activities.

This study found that physicians and hospital administrators agree that antimicrobial protocols need to be in place. Stakeholders need to continuously be educated on the complexity of the multifaceted problem of antimicrobial resistance, to appreciate the need for an AMS program. The success or failure of any AMS program appears to depend on the firm resolve and commitment of the hospital's leadership and physician understanding, commitment and support. By involving these main players in the AMS program and with a knowledge of other enablers and barriers, a successful development and implementation of an AMS program can be achieved.

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ORIGINAL ARTICLE

Clinical and Bacteriologic Profile of Neonatal Sepsis in a Tertiary Care Hospital: A 5-Year Review

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ABSTRACT

Background: Neonatal sepsis, a clinical syndrome characterized by non-specific signs and symptoms, is the most common cause of neonatal mortality and morbidity. It is classified into early or late-onset depending on the onset of symptoms, if within the first 72 hours or later. Early onset sepsis (EOS) occurs due to ascending infection following rupture of membranes or during passage through an infected birth canal. Late onset sepsis (LOS) can be nosocomial or community-acquired. A high index of suspicion and timely and judicious use of antibiotics are needed to achieve good outcomes.

Objective: This study looked into the clinical and bacteriologic profile of neonatal sepsis in a tertiary care hospital from January 2013 to December 2017.

Methodology: This was a retrospective observational study. Data on maternal risk factors, mode of delivery, gestational age, birth weight, birth setting, clinical manifestations, and blood culture and sensitivity were gathered. Descriptive statistics was used to analyze the data.

Results: Majority of cases were late onset sepsis with an equal distribution in those born via normal spontaneous delivery (NSD) and cesarean section (CS). There were more culture- positives in low birth weight (LBW) infants and those not delivered within a hospital. The most common maternal risk factor was UTI. Majority of culture-positive newborns presented with respiratory distress, poor feeding, fever, and irritability with respiratory distress being the most common manifestation for both EOS and LOS. Predominant isolates were CONS, *E. coli* and *Klebsiella sp.* Both *E. coli* and *Klebsiella* were resistant to both first-line empiric antibiotics - ampicillin and gentamicin but highly sensitive to piperacillin-tazobactam and imipenem.

Conclusion: Clinical signs and symptoms of neonatal sepsis are non-specific. The presence of respiratory distress, fever, poor feeding, and irritability together with other risk factors should raise suspicion for sepsis and prompt investigation and treatment. Predominant isolates seen were CONS, *E. coli* and *Klebsiella sp.* with resistance to first-line empiric antibiotics.

KEYWORDS: *neonatal sepsis, early-onset, late-onset, risk factors, clinical signs and symptoms, antibiotic sensitivity*

INTRODUCTION

Sepsis is the most common cause of neonatal morbidity and mortality. Neonatal sepsis is a generalized bacterial infection documented by a positive blood culture in the first four weeks of life, along with a clinical syndrome characterized by systemic signs of infection.[1] Although remarkable developments have been made in recent years to reduce the number of deaths globally, too many newborns continue to die. In 1990, both the United Nations (UN) and World Health Organization (WHO), prioritized the Millennium Development Goals (MDG), that targeted a 2/3 reduction in child mortality rate by 2015.[2] In 2013 however, 44% of deaths in children under five occurred during the neonatal period (up from 37% in 1990). It was found that the three major causes of neonatal deaths worldwide were infection from neonatal sepsis/pneumonia (36%), pre-term birth (28%), and birth asphyxia (23%).[2,5] Despite major advances and increasing research in neonatal care in developed countries, 40% of neonates with sepsis die or experience a major disability such as neurodevelopmental impairment.[3] In 2015, MDG data showed that neonatal deaths as a share of under-five deaths declined far more slowly than other under-five deaths during the same period (1990 to 2015).[2] In 2017, newborn deaths further increased and accounted for 45% of under-five deaths globally.[4] In the Philippines, report from the UNICEF showed that neonatal sepsis accounts for 13% of neonatal deaths and 10-12% of all causes of deaths in the country.[5]

Neonatal sepsis can be categorized into early or late-onset depending on the timing of occurrence of symptoms – whether within the first 72 hours of life or later. Early onset sepsis usually occurs due to ascending infection or passage through an infected maternal genital tract. Late onset sepsis on the other hand, is either nosocomial or community-acquired.[1] Each newborn should be evaluated for the presence of the following maternal and neonatal factors that are associated with an increased risk for sepsis: premature rupture of membranes (PROM) $\geq$ 18 hours, maternal fever or active maternal infection within days of delivery (such as urinary tract infection, septicemia, pneumonia/respiratory tract infection, chorioamnionitis), prematurity, fetal distress, low birth weight, meconium-staining and low APGAR score.[6,7,8] Common clinical signs and symptoms are non-specific and may involve different organ systems at one time. These include temperature instability (fever/ hypothermia), jaundice, respiratory distress (grunting, tachypnea, presence of retractions), apnea, hepatomegaly, poor feeding, vomiting, lethargy/poor activity, cyanosis, abdominal distension, irritability and diarrhea.[9]

Common laboratory tests such as complete blood count, C-reactive protein, and erythrocyte sedimentation rate are used to aid in diagnosis, even if they have limited diagnostic accuracy for neonatal sepsis.[10,11] The gold standard for diagnosing neonatal sepsis is still blood culture despite its low sensitivity - majority of sepsis cases are diagnosed in the presence of concerning clinical signs with negative culture results.[12,13] When culture for blood and/or other sterile sites are negative but the neonate continues to show signs consistent with infection, this is classified as clinical sepsis.[14] The limitations of ancillary tests and the low sensitivity of blood culture combined with non-specific clinical signs constrain pediatricians to treat patients based on a high level of suspicion. This necessitates initiation of empiric antibiotic therapy until sepsis is ruled out. Group B streptococcus (GBS) and *Escherichia coli* (*E. coli*) are the most common pathogens of EOS. Trends in the epidemiology of early-onset sepsis show a decreasing incidence of GBS disease. This is attributed to the implementation of prenatal screening and treatment for GBS.[15,16] For LOS, the most common organisms are coagulase-negative *Staphylococcus* (CONS), *Staphylococcus aureus (S. aureus) and Klebsiella*.[17]

In 2011, An international review was done by Waters, D et. al. to determine the most common etiology of community-acquired neonatal sepsis in low- and middle-income countries. The study, where the Philippines was included, showed that the most prevalent pathogens overall were *S. aureus* (14.9%), *E. coli* (12.2%), and *Klebsiella sp.* (11.6%). However, variations were observed between global regions and age-of-onset categories. *Klebsiella* was highly prevalent in Southeast Asia and showed the highest antimicrobial resistance.[18] Locally, a multi-center retrospective study done by Lazarte, C et. al. in 2006, showed that majority of admitted neonates had EOS. They also found that gram-negative organisms comprised the majority of neonatal infections, with *Pseudomonas* and *Burkholderia*

being the most prevalent.[19] Another retrospective study done by Baltazar et. al in 2014 in the NICU of a tertiary government hospital showed that the most common isolates were *Klebsiella* followed by CONS and *Enterobacter cloacae.* Most isolates were resistant to first-line antibiotics - ampicillin, penicillin and cefuroxime.[20] The diversity of organisms causing neonatal sepsis varies from one hospital to another and can change over time in the same location. Although most isolates remain sensitive to newer antibiotics, emergence of resistant strains is a potential problem due to changing patterns of antibiotic use.

The subtle and non-specific symptomatology of sepsis causes difficulty in early detection and timely treatment. The pathogens causing sepsis in the newborn and the antibiotic susceptibility varies from one hospital to another. Prompt treatment based on a rational protocol according to antibiotic susceptibility will greatly help in reducing morbidity and mortality from neonatal sepsis.

METHODOLOGY

A. Study Design and Setting

This is a retrospective observational study of neonatal sepsis cases admitted under the Department of Pediatrics, Hospital of the Infant Jesus Medical Center from January 2013 to December 2017.

B. Inclusion and Exclusion Criteria

All neonates admitted during the study period who fulfilled the sepsis case definition set by the Philippine Society of Newborn Medicine (Standards of Newborn Care 4th ed) and who had blood culture and sensitivity done before antibiotics were started were included. Patients who received antibiotics prior to hospital admission were excluded.

C. Data Collection

In-patient records of neonates who were admitted and who fulfilled the sepsis case definition were reviewed and analyzed. Details in the history including maternal risk factors, mode of delivery, gestational age, birth weight, birth setting, and clinical manifestations were recorded. Data on blood culture and sensitivity (done by conventional method) were also obtained. Descriptive statistics was used in the data analysis.

RESULTS

A total of 251 neonates, born within and outside of the hospital, and admitted because of suspected sepsis were included in the study. In both suspected and culture-positive cases, majority of neonates were males with a male to female ratio of 1.5:1. Culture positive cases were higher in females than in males (35%).

Table 1. Gender Distribution

Distribution	Suspected Case (%)	Culture Positive (%)	Culture Negative (%)
Total Number	251	78 (31.1)	173 (68.9)
Males	151 (60.2)	43 (28.5)	108 (71.5)
Females	100 (39.8)	35 (35)	65 (65)

The incidence of suspected sepsis was slightly higher in babies delivered by cesarean section (CS). However, there was an equal number of CS and normal spontaneous vaginal delivery (NSD) cases in those who were culture-positive.

Table 2. Distribution According To Mode of Delivery

Type of Delivery	Suspected Case (%)	Culture Positive (%)	Culture Negative (%)
Total number	251	78 (31.1)	173 (68.9%)
Normal Spontaneous Vaginal Delivery	119 (47.4)	39 (32.8)	80 (67.2)
Cesarean Section	132 (52.6)	39 (29.5)	93 (70.5)

Majority of suspected sepsis were delivered within a hospital (in-hospital or other hospitals). There were 455 deliveries in our hospital (in-hospital birth), and 63 were admitted due to suspected sepsis. Of these, 48 (76.2 %) were suspected EOS and 12 (25%) were culture-positive. Most of the cases born in other hospitals (n=145) were suspected LOS (86.2%) and 35 (28%) were culture-positive. It was observed that those born in a lying-in and at home had positive culture results (50% for both EOS and LOS), but this group comprised a significant minority in relation to the study population.

Table 3. Distribution According to Birth Setting and Age of Onset

Birth Setting	EOS			LOS		
	Suspected case (%)	Culture-Positive (%)	Culture Negative (%)	Suspected Case (%)	Culture Positive (%)	Culture Negative (%)
Total number (n = 251)	76 (30.3)	21 (27.6)	55 (72.4)	175 (69.7)	57 (32.6)	118 (67.4)
In-Hospital (n = 63)	48 (76.2)	12 (25)	36 (75)	15 (23.8)	4 (26.7)	11 (73.3)
Other Hospitals (n = 145)	20 (13.8)	5 (25)	15 (75)	125 (86.2)	35 (28)	90 (72)
Lying-in (n = 42)	8 (19)	4 (50)	4 (50)	34 (81)	17 (50)	17 (50)
Home (n = 1)	-	-	-	1 (100)	1 (100)	-

Based on gestational age, majority of neonates were born term and preterms comprised only 13.9% of cases. There was no significant difference in the number of culture positive cases among term and preterm neonates.

Table 4. Distribution According to Gestational Age

Gestational Age (in weeks)	Suspected Case (%)	Culture Positive (%)	Culture Negative (%)
Total number	251	78 (31.1)	173 (68.9)
≥ 37 weeks (term)	216 (86.1)	69 (31.9)	147 (68.1)
< 37 weeks (preterm)	35 (13.9)	9 (25.7)	26 (74.3)

Majority of subjects had a normal birthweight. We had 41 suspected sepsis cases with low birthweights (LBW) and 41.5% of these had positive cultures.

Table 5: Distribution of Neonates According to Birth Weight

Birth weight (in grams)	Suspected Case (%)	Culture Positive (%)	Culture Negative (%)
Total number	251	78 (31.1)	173 (68.9)
≥ 2 500 grams	210 (83.7)	61 (29.1)	149 (70.9)
< 2 500 grams (LBW)	41 (16.3)	17 (41.5)	24 (58.5)

Of the 251 suspected cases, 101 neonates (40.2%) had maternal risk factors. Of these, 23.8% were culture-positive. Urinary tract infection was the leading maternal risk factor noted.

Table 6. Distribution According to Maternal Risk Factors

Risk Factor	Suspected Case	Culture Positive (%)	Culture Negative (%)
PROM ≥ 18 hrs	16	3 (18.8)	13 (81.2)
Maternal fever	5	-	5 (100)
Urinary Tract Infection (UTI)	55	16 (29.1)	39 (70.1)
Upper Respiratory Tract Infection (URTI)	16	4 (25)	12 (75)
Bacterial vaginosis/vaginitis	7	1 (14.3)	6 (85.7)
Septicemia	2	-	2 (100)
Total	101 (40.2)	24 (23.8)	77 (76.2)

In the cases evaluated, the neonates presented with fever, poor suck/feeding, poor activity/lethargy, irritability, respiratory distress, cyanosis, seizures, jaundice, abdominal distention, vomiting, diarrhea, apnea and hypoglycemia. Most common manifestations were respiratory distress, poor feeding, fever, jaundice, and irritability with respiratory distress being the most common presentation for both EOS and LOS. Culture positive cases were highest in newborns who presented with irritability, followed by fever, respiratory distress, and poor suck (Tables 7, 8).

Table 7: Distribution Based On Presenting Clinical Signs

Clinical feature	Suspected Case	Culture Positive (%)	Culture Negative (%)
Respiratory distress	153	50 (32.7)	103 (67.3)
Poor feeding	95	26 (27.4)	69 (72.6)
Fever	65	27 (41.5)	38 (58.5)
Jaundice	50	12 (24)	38 (76)
Irritability	29	14 (48.3)	15 (51.7)
Poor activity/Lethargy	20	4 (20)	16 (80)
Vomiting	31	8 (25.8)	23 (74.2)
Diarrhea	28	7 (25)	21 (75)
Cyanosis	17	4 (23.5)	13 (76.5)
Seizures	4	1 (25)	3 (75)
Abdominal distension	4	1 (25)	3 (75)
Apnea	3	-	3 (100)
Hypoglycemia	2	-	2 (100)

Table 8: Comparison of Demographic and Clinical Features of EOS and LOS

	Early Onset Sepsis	Late Onset Sepsis
Male:Female	47:29 (1.6:1)	104:71 (1.5:1)
Term:Preterm	61:15 (4:1)	144:20 (7.2:1)
Normal:LBW	57:19 (3:1)	153:22 (6.9:1)
NSD:CS	27:49 (0.6:1)	93:82 (1.1:1)
Respiratory distress	36	117
Fever	14	51
Poor feeding	22	73
Irritability	2	27
Jaundice	25	25
Vomiting	10	21
Poor activity/Lethargy	9	11
Diarrhea	3	25
Cyanosis	7	10
Abdominal distension	3	1
Apnea	2	1
Seizures	2	2
Hypoglycemia	2	-

Of the 251 cases reviewed, 78 (31.1%) were culture-positive and 21 (27%) were among EOS and 57 (32%) among LOS. In both EOS and LOS, the predominant isolates were Coagulase Negative Staphylococcus, *E. coli* and *Klebsiella sp.* (Table 9).

Table 9: Frequency of Bacterial Isolates in EOS and LOS

Bacteria	EOS	LOS	Total
Gram-positive			
CONS	9 (42.8)	25 (44)	34 (43.6)
Staphylococcus aureus	2 (9.5)	1 (1.7)	3 (3.8)
Group B streptococci	-	3 (5.3)	3 (3.8)
Gram-negative			
Klebsiella	3 (14.3)	9 (15.8)	12 (15.4)
Pseudomonas	1 (4.8)	1 (1.7)	2 (2.6)
Escherichia coli	5 (23.8)	10 (17.5)	15 (19.2)
Acinetobacter baumanii	-	1 (1.7)	1 (1.3)
Enterobacter cloacae	1 (4.8)	7 (12.3)	8 (10.3)
Total	21 (27)	57 (73)	78 (100)_

Both gram-positive and gram-negative bacterial isolates showed low sensitivity to first-line empiric antibiotics (ampicillin and gentamicin). *Staphylococcus aureus* isolates showed intermediate sensitivity to vancomycin and clindamycin. Among gram-negative isolates, *Klebsiella sp.*, *E. coli* and *Enterobacter cloacae* were noted to have low sensitivity to most first and second-line empiric antibiotics. *Klebsiella sp.* isolates were sensitive to piperacillin-tazobactam, imipenem, and amikacin. *E. coli* was sensitive to piperacillin-tazobactam, cefotaxime, ceftriaxone, and imipenem. *Enterobacter cloacae* was sensitive to piperacillin-tazobactam, and ampicillin but was intermediate to gentamicin. The single isolate of *Acinetobacter baumanii* was sensitive to all tested antibiotics. *Pseudomonas sp.* was sensitive to piperacillin-tazobactam. In general, both gram-positive and gram-negative bacterial isolates were sensitive to both piperacillin-tazobactam and imipenem in vitro. It is important to note that methods to screen for extended-spectrum β –lactamase (ESBL) producing strains were not performed in this study.

Table 10: Antibiotic Sensitivity Pattern of Gram-Positive Organisms

Antibiotics	Organisms (%)		
	CONS n = 34	***S. aureus*** n = 3	***GBS*** n = 3
Oxacillin	8 (23)	2 (67)	2 (67)
Clindamycin	22 (65)	2 (67)	1 (33)
Vancomycin	32 (94)	2 (67)	3 (100)

Table 11: Antibiotic Sensitivity Pattern of Gram-Negative Organisms

Antibiotics	Organisms (%)				
	Klebsiella sp. n = 12	***Escherichia coli*** n = 15	***Pseudomonas sp.*** n = 2	***Acinetobacter baumanii*** n = 1	***Enterobacter cloacae*** n = 8
Ampicillin	3 (25)	6 (40)	-	1 (100)	8 (100)
Cefuroxime	3 (25)	10 (67)	-	1 (100)	3 (38)
Cefotaxime	3 (25)	15 (100)	-	1 (100)	3 (38)
Ceftriaxone	3 (25)	15 (100)	-	1 (100)	4 (50)
Piperacillin-Tazobactam	12 (100)	15 (100)	2 (100)	1 (100)	8 (100)
Imipenem	12 (100)	14 (93)	2 (100)	1 (100)	7 (88)
Ciprofloxacin	12 (100)	2 (13)	2 (100)	1 (100)	8 (100)
Gentamicin	6 (50)	2 (13)	-	1 (100)	5 (62)
Amikacin	12 (100)	8 (53)	2 (100)	1 (100)	2 (25)

Of the 251 suspected sepsis cases, 242 (96%) were discharged improved. Among 78 culture-positive neonates, 76 (97%) were sent home, while 2 were discharged against medical advice (DAMA). There were 7 deaths, and all were EOS cases. The most common risk factors in those who died were prematurity, maternal UTI and PROM. All deaths presented with respiratory distress on admission. None of those who died had a positive blood culture.

Table 12: Outcome of Patients

	EOS (n=76)		**LOS** (n=175)	
Total (n=251)	**Culture Positive (%)** 21 (27.6)	**Culture Negative (%)** 55 (72.4)	**Culture Positive (%)** 57 (32.6)	**Culture Negative (%)** 118 (67.4)
Died (n=7)	-	7 (100)	-	-
Improved (n=242)	19 (7.9)	48 (19.7)	57 (23.6)	118 (48.8)
DAMA (n=2)	2 (9.5)	-	-	-

DISCUSSION

Neonatal sepsis is one of the major health concerns in developing countries, including the Philippines. In this retrospective study, the incidence of suspected or clinical sepsis was higher among males compared to females. Similar male preponderance has been reported in several studies [5,6,9] but our study showed a higher rate of culture confirmed sepsis in the female population. Most studies present a higher incidence of sepsis among preterm and low birth weight babies as they are more susceptible to infection due to their inherent weak immune system.[21,22] However, our study showed a higher percentage of sepsis among term than preterm neonates. This probably reflects differences in population characteristics and the occurrence of other predisposing factors among the study participants. Although we have more subjects that have normal birth weights, the percentage of culture positives among suspected cases was higher in low birth weight infants.

As to mode of delivery, there was no significant difference in the number of culture-positive patients who were born via normal spontaneous vaginal delivery and cesarean section. The distribution of subjects according to birth setting showed that there was a higher percentage of culture-positive neonates who were delivered at home and in lying-in centers. The rate of sepsis among all deliveries in the hospital during the study period was 13.8% (63/455). Of these, the percentage of culture-positive cases was significantly

lower and almost the same as those who were born but admitted in other hospitals. According to Zaidi and Huskins contributory factors to a high nosocomial infection rate in low and middle-income countries are lack of aseptic delivery techniques and hand hygiene compliance, lack of proper sterilization facilities, lack of knowledge and training regarding adequate sterilization, and overcrowding and understaffed health facilities.[23] These however were not reflected in our study.

Based on presenting clinical manifestations of patients with suspected sepsis, poor feeding, fever, respiratory distress, jaundice, and irritability were the most common findings. Respiratory distress was the most common presenting sign among culture-positive cases. A study done by Bonadio and colleagues found that change in respiratory status (distress), change in affect, and poor peripheral perfusion are indicators that best identified infants with serious bacterial infection. Poor feeding and alterations in level of alertness or activity were also included, although noted to be less sensitive indicators.[24].

Among the cases reviewed, LOS was more common than EOS, which is consistent with reports from other Asian countries. [1,8,22]

The percentage of positive blood cultures in our study was only 31.1%. Reasons for negative blood cultures include presence of other pathogens such as anaerobic organisms, viruses, and fungi. No workup however was done for these pathogens. This finding is comparable with rates reported in other developing Asian and African countries. Both gram-positive and gram-negative bacteria were isolated in our study. The incidence of gram-positive bacteria was higher compared to gram-negative bacteria. Coagulase Negative Staphylococcus (CONS) was the most common pathogen found in our study followed by *E. coli* and *Klebsiella sp.* for both EOS and LOS. Our results are similar to the results of Lazarte et. al and Baltazar, et.al which showed a high incidence of CONS and *Klebsiella* isolates from blood culture. It also parallels the results of the study done by Ballot and colleagues, which found that there is a high rate of CONS infections reported in Southeast Asia, as well as in the Middle East and Latin America. [24] In contrast, a review done by Zaidi and colleagues in 2009 revealed a higher ratio of gram-negative to gram-positive organisms (1.6:1) as etiologic agents for neonatal sepsis across Asia and the Pacific regions.[26] The same review cited *Klebsiella sp*. and *E. coli* as predominant pathogens in the majority of cases. CONS was the most common etiologic agent in our study and was considered a true pathogen upon careful assessment.

Ampicillin and gentamicin remain to be the fIrst line empiric antibiotics for neonatal sepsis in our hospital. However, in our study, most organisms showed poor sensitivity to both antibiotics. Both CONS and *S. aureus* were sensitive to vancomycin. For gram-negative organisms, susceptibility to piperacillin-tazobactam and imipenem were observed. Only *E. coli* remained susceptible to cephalosporins. The rest of the gram-negative isolates showed intermediate sensitivity to cephalosporins. The results of our study is comparable to the findings of Baltazar et. al in 2014, which showed increasing resistance of *Klebsiella* to ampicillin. Our findings also concur with the review done by Zaidi and colleagues showing high resistance (70%) of neonatal isolates from hospitals in developing countries to ampicillin and gentamicin.[23] Sensitivity to Ciprofloxacin was shown in this study particularly for *Pseudomonas, E.coli, Acinetobacter and Enterobacter.* Although fluoroquinolones (ciprofloxacin) are not recommended for use in infants and young children, they may be used in cases when bacteria show multiple resistance to standard and alternative antibiotics.[27]

We attempted to compare the 2018 Antimicrobial Resistance Surveillance Program (ARSP) data in our country with our results. In the ARSP data, *E. coli* rates of resistance against the fluoroquinolones and third generation cephalosporins (Ceftriaxone) are at 41% and 39% respectively. Resistance to carbapenems continue to rise with resistance rates to imipenem at 5%.[28] The *E.coli* isolates in our results have high resistance to ciprofloxacin (86.7%) but have low resistance (6.7%) to imipenem and 0% resistance to ceftriaxone. For *Klebsiella sp*, ARSP data reported a resistance rate of 11% for imipenem while in our results, *Klebsiella* had 0% resistance to imipenem. [28] ARSP report for *Pseudomonas aeruginosa* showed that carbapanem resistance was at 19% for imipenem.[28] *Acinetobacter baumanii* was reported to have more than 50% resistance to many antibiotics: piperacillin-tazobactam at 58%, imipenem at 56% and ciprofloxacin at 55%.[28] In our study, however, *Pseudomonas sp. and Acinetobacter* showed 0% resistance to piperacillin-tazobactam,

imipenem, amikacin, and ciprofloxacin. It should be noted that we only had 2 isolates for *Pseudomonas sp.* and 1 for *Acinetobacter baumanii*.

CONCLUSIONS AND RECOMMENDATIONS

The clinical and bacteriologic profile of neonatal sepsis is ever-changing and the most common risk factors for sepsis are low birth weight, maternal UTI, and PROM. Respiratory distress, fever, poor feeding, and irritability are the most common presenting manifestations and should prompt investigation and treatment. Predominant isolates seen were CONS, *E. coli* and *Klebsiella sp.* with resistance to first-line empiric antibiotics.

We recommend continuing studies on neonatal sepsis incidence, etiology and the changing patterns of antibiotic resistance. Periodic antibiotic susceptibility reviews in all health care facilities will greatly help in management and in decreasing rates of antibiotic resistance.

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ORIGINAL ARTICLE

Oral Azithromycin Vs Intravenous Ceftriaxone in the Treatment of Enteric Fever: A Systematic Review and Meta-Analysis

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background: Typhoid fever, also known as enteric fever, is a severe systemic illness characterized by fever and gastrointestinal manifestations that commonly affects children and young adults. It is most prevalent in South-Central Asia, Southern Africa, and Southeast Asia. Alternative drugs for the treatment of enteric fever have been studied to decrease toxicity and increase compliance. Oral azithromycin has been proposed and is widely studied as a suitable treatment alternative.

Objective: The objective of this study is to compare oral azithromycin with intravenous ceftriaxone in the treatment of uncomplicated typhoid fever in terms of cure, duration of fever, relapse, and adverse events.

Methodology: A systematic review and meta-analysis were done with eligible studies taken from PUBMED, MEDLINE, and Cochrane Clinical Trial Registry. Six studies passed the eligibility criteria and were analyzed using Review Manager 5.3.

Results: Azithromycin showed comparable results with ceftriaxone in terms of cure, duration of fever and adverse events. However, azithromycin proved superior in decreasing relapse.

Conclusion: Azithromycin is comparable to ceftriaxone in the treatment of uncomplicated typhoid fever in terms of cure, duration of fever, and occurrence of adverse events. Azithromycin likewised had a lower incidence of relapse.

Recommendations: We recommend conducting local trials in pediatric patients, to compare azithromycin with standard antibiotic regimen for typhoid fever, to help update local recommendations and expand choices for antibiotic use.

KEYWORDS: *enteric fever, azithromycin, ceftriaxone*

INTRODUCTION

Enteric fever is a severe systemic illness characterized by fever and gastrointestinal manifestations[1,2]. The organism classically responsible for typhoid fever is *Salmonella enterica* serotype Typhi (formerly *S. typhi*), while *Salmonella paratyphi, Salmonella schotmuelleri,* or *Salmonella hirschfeldii* cause paratyphoid fever. The two diseases are sufficiently similar hence are known collectively as enteric fever[3]. Enteric fever is more common in children and young adults than in older patients[4]. Worldwide, enteric fever is most prevalent in impoverished areas that are overcrowded with poor access to sanitation. Incidence estimates suggest that South-central Asia, Southeast Asia, and Southern Africa are the regions with a high incidence of *Salmonella typhi* infection[5].

Enteric fever is usually treated with a single antibacterial drug. Antibiotic selection depends on the severity of illness, local resistance patterns, feasibility of oral medications, clinical setting, and available resources. The optimal drug and duration of therapy are uncertain[6]. Main options are fluoroquinolones, third generation cephalosporins, and azithromycin. For patients with severe systemic disease, initial therapy with a parenteral agent is started. Patients with uncomplicated disease on the other hand, are started on oral antibiotics. However, some antibiotics are contraindicated for use in pediatric patients. For example, fluoroquinolones are not recommended for use in children due to toxicities such as bone marrow depression[7]. Differences in treatment regimens may vary per country due to unique resistance rates. For example, quinolone resistance have been noted in other countries, hence their antibiotic agent of choice is azithromycin[8]. Emergence of multiple drug-resistant strains seen in various countries such as India have shifted their choice of antibiotics to either azithromycin or ceftriaxone[9].

Locally, the Antimicrobial Resistance Surveillance Program (ARSP) of the Department of Health (DOH) reported that the resistance rates for typhoidal *Salmonella* to first-line drugs amoxicillin, ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole are low[10]. The DOH recommends the following first line drugs for uncomplicated typhoid fever: amoxicillin, ampicillin, chloramphenicol, or trimethoprim-sulfamethoxazole. Second line drugs are cefixime, ciprofloxacin, or azithromycin[11]. Most of these drugs are given in two to four doses per day for seven to fourteen days, except for azithromycin which is given once a day.

Azithromycin once a day for 5 days, although a second line agent, has certain advantages. These include ease of administration as it is given orally, once daily dosing, and shorter duration of treatment. This led to studies exploring azithromycin as an alternative drug in the treatment of uncomplicated typhoid fever when limitations with first line agents are encountered.

This study aimed to investigate the efficacy of oral azithromycin against parenteral ceftriaxone in the treatment of uncomplicated typhoid fever, and whether intravenous drugs may be replaced with oral azithromycin for the convenience and for better compliance among pediatric patients[12-21].

MATERIALS AND METHODS

Research Design

A systematic review and meta-analysis of studies that compared oral azithromycin with intravenous ceftriaxone for treatment of enteric fever was done. The authors followed the PRISMA statement guidelines during the preparation of this systematic review and meta-analysis and performed all steps in accordance with the Cochrane Handbook for Systematic Reviews of Interventions[12].

Data Source

A systematic search of peer-reviewed studies was conducted in three databases – MEDLINE, EMBASE and CENTRAL – from their initiation date to October 2018. Three groups of search terms were used: (1) azithromycin, (2) ceftriaxone, and (3) typhoid fever (or enteric fever). The search was conducted with no restriction by language or study design. The bibliographies of the studies were also searched for additional relevant records.

Eligibility Criteria

All studies which satisfied the following criteria were included: (1) Population: uncomplicated typhoid fever, enteric fever, paratyphoid fever; subjects aged 2 to 18 years (2) Intervention: oral azithromycin (3) Comparator: intravenous ceftriaxone (4) Outcomes: cure rate, relapse, duration of fever, and adverse effects and

(5) Study design: randomized controlled trials (RCTs) and observational studies.

The following were excluded: (1) in vitro and animal studies and (2) studies whose outcomes were not described in numerical form. Duplicates were removed prior to eligibility assessment. References were screened in two steps: the first step involved screening of titles/abstracts for matching with the inclusion criteria and the second step was screening the retrieved full-text articles for eligibility for meta-analysis.

Study Selection

Articles identified from the systematic search were exported to EndNote X9 (Thomas Reuters, 2018). Two review authors screened the title and abstract of the articles independently, and potentially relevant articles were obtained in full text and further assessed for eligibility based on the inclusion and exclusion criteria.

Data Extraction

Two independent authors extracted the relevant data from included studies. Disagreements were discussed and consensus among the reviewers was achieved. The extracted data included the following domains: (1) characters of study design 2) baseline characteristics of enrolled patients (3) risk of bias (ROB) and (4) outcomes in terms of cure, time to defervescence, relapse, and adverse events.

Risk of Bias Assessment

To assess the ROB in retrieved clinical trials, the Cochrane ROB assessment tool of the Cochrane Handbook for Systematic Reviews of Interventions was used. The bias domains were then plotted.

Statistical Analysis

The overall effect estimate was calculated as the odds ratio (with 95% CI) for dichotomous outcomes (clinical cure and relapse) and as the mean difference (95% CI) between the azithromycin and ceftriaxone groups for continuous outcomes (duration of fever). Random-effects meta-analysis was carried to pool the data, using the Mantel-Haenszel method for dichotomous outcomes, and the DerSimonian and Laird inverse-variance method for continuous outcome in Review Manager 5.3 (2011).

Ethical Considerations

The study is a research synthesis which focuses on empirical studies. It attempted to summarize and draw conclusions from statistical integration of data from separate similar published and unpublished studies that relate to the same or related research problem. No humans or animals participated in the present study. The study was presented to the Philippine Children's Medical Center Institutional Review Board and Ethics Committee and was approved.

RESULTS AND DISCUSSION

A total of 336 studies were screened from the title and abstract and 42 duplicates were removed. The remaining 294 studies were further screened. Six studies met the criteria for inclusion and subsequent data extraction. The six studies ([13,14,15,16,17,18]) included 520 patients. The risk of bias assessment of all six trials was generally low risk (Figure 1). All trials used adequate methods to randomly generate the allocation sequence and all included trials reported well-defined inclusion and exclusion criteria. However, due to the nature of administration of the drugs being studied and the subjective method of reporting symptoms as part of outcome assessment, there was no blinding, and this decreased the strength of the studies. Care must be taken when interpreting the data produced in this study.

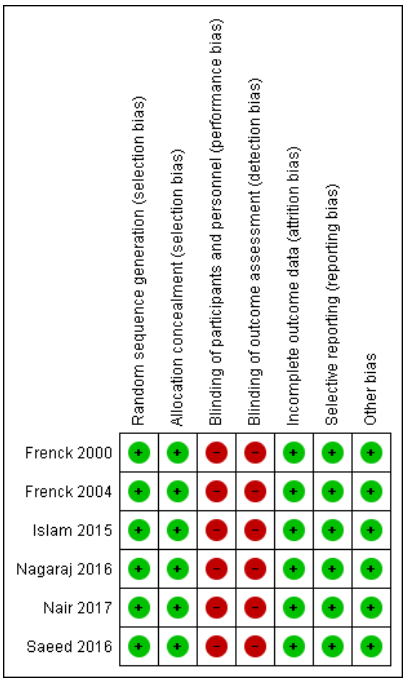

Figure 1. Risk of bias assessment

Criteria for enrolment in the included studies were patients presenting with signs and symptoms of uncomplicated typhoid fever with positive blood or stool culture for *S. typhi* or *S. paratyphi.* Dosage of azithromycin used in the studies ranged from 500 mg to 1 g per day (10-20 mg/kg/day) for five to seven days. Ceftriaxone was given at 75-100 mg/kg/day for the same duration as azithromycin.

Characteristics of Subjects

All studies included subjects who were children and adolescents with age range of 2 to 18 years. Mean age of patients was 7.01 years in the azithromycin-treated group and 6.73 years in ceftriaxone-treated group. The study population was comprised of 54% males and 46% females. A total of 259 patients were treated with azithromycin while 261 were treated with ceftriaxone (Table 1).

Table 1. Characteristics of the subjects included in the meta-analysis

Study ID	N		Mean age of patients (years)		Male/Female	
	Azithromycin	Ceftriaxone	Azithromycin	Ceftriaxone	Azithromycin	Ceftriaxone
Frenck, 2000	34	30	9.7	10.1	20/14	17/13
Frenck, 2004	32	36	3.6	3.35	19/13	20/16
Islam, 2015	50	48	6.64	6.65	Not reported	Not reported
Nagaraj, 2016	63	63	3.25	3.25	35/28	36/27
Saeed, 2016	50	50	7.47	6.68	27/23	27/23
Nair, 2017	30	34	11.4	10.4	14/16	14/20
Total	259	261	7.01	6.73	115/94	114/99

Outcome Measures

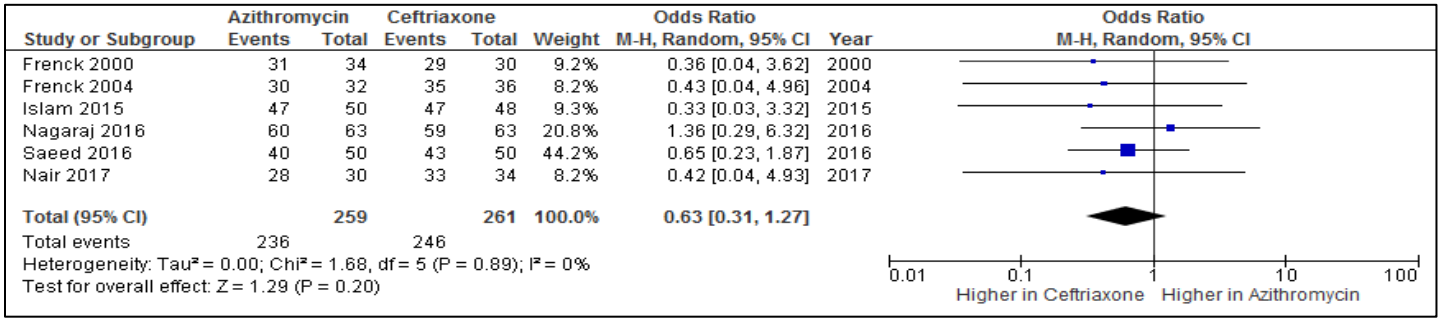

Figure 2. Forest plot for clinical cure after treatment of azithromycin versus ceftriaxone: (OR 0.63, 95% CI 0.31 to 1.27; p=0.20)

Clinical cure after treatment with azithromycin did not differ significantly from ceftriaxone (Figure 2). The pooled estimate shows that the odds of clinical cure is similar between the azithromycin and ceftriaxone group (OR 0.63, 95% CI 0.31 to 1.27; p=0.20). The level of heterogeneity is 0% (no heterogeneity).

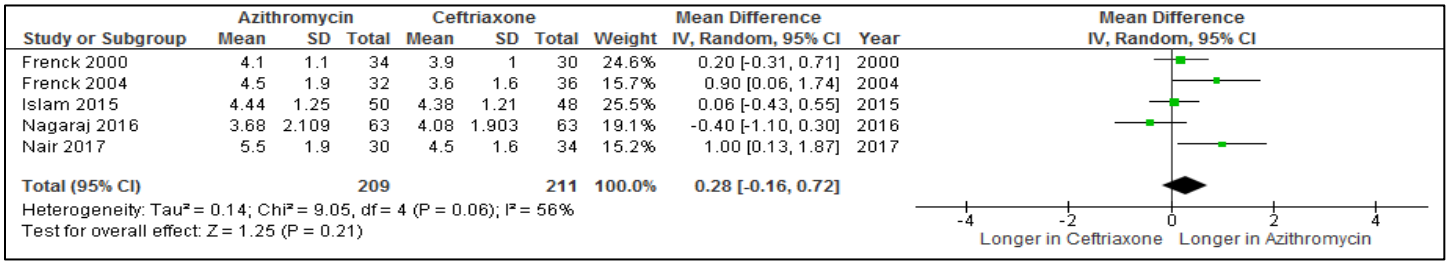

Figure 3. Forest plot for duration of fever after treatment of azithromycin versus ceftriaxone: (95% CI -0.16 to 0.72, p=0.21)

Duration of fever is the same between the azithromycin and ceftriaxone group (Figure 3). The pooled mean difference is 0.28 days (95% CI -0.16 to 0.72, p=0.21). The level of heterogeneity is 56% (moderate to substantial).

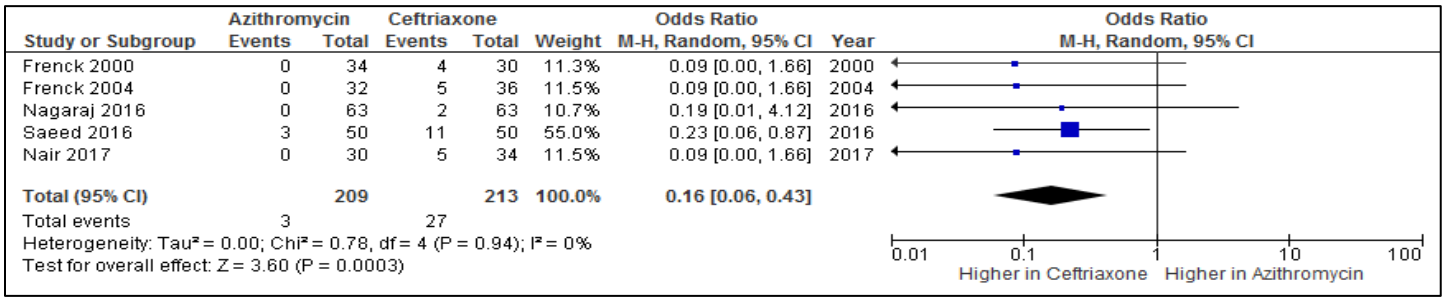

Figure 4. Forest plot for relapse after treatment of azithromycin versus ceftriaxone: (OR 0.16, 95% CI 0.06 to 0.43; p=0.0003)

There is a significantly lower incidence of relapse after treatment with azithromycin compared with ceftriaxone (Figure 4). The pooled estimate shows that the odds of relapse is 6.25 times more likely in the ceftriaxone group compared to the azithromycin group (OR 6.25, 95% CI 2.33 to 16.67, p=0.0003). The level of heterogeneity is 0% (no heterogeneity).

There were no serious adverse events reported in any of the trials. The most common adverse events reported in both treatment groups were diarrhea and vomiting (Table 2). However, these were not severe to warrant change in management. One study also concluded that it is likely that many of the gastrointestinal events were associated with typhoid fever and not with treatment[14]. Subjects with laboratory evidence of adverse events were asymptomatic and intervention was not needed[13,14]. All adverse events were self-limiting.

Table 2. List of adverse events in both treatment arms

Study ID	Clinical Adverse Event		Laboratory Adverse Event	
	Azithromycin	Ceftriaxone	Azithromycin	Ceftriaxone
Frenck, 2000	Not described	Pain on injection site (1)	Thrombocytosis (4) AST elevation (2) ALT elevation (1)	Thrombocytosis (3) AST elevation (4) ALT elevation (1)
Frenck, 2004	Vomiting (11) Diarrhea (10) Nausea (5) Abdominal pain (5) Anorexia (3) Cough (3)	Vomiting (7) Diarrhea (15) Nausea (7) Abdominal pain (5) Anorexia (6) Cough (2)	Thrombocytosis (7) AST elevation (2) ALT elevation (2)	Thrombocytosis (7) AST elevation (2) ALT elevation (5)
Islam, 2015	Not described	Not described	Not described	Not described
Nagaraj, 2016	Not described	Not described	Not described	Not described
Saeed, 2016	Not described	Not described	Not described	Not described
Nair, 2017	Vomiting (6) Diarrhea (8)	Vomiting (5) Diarrhea (12)	Not described	Not described

DISCUSSION

In other countries, alternative drugs for the treatment of enteric fever were explored due to emergence of drug-resistant strains of *Salmonella.* An orally administered drug was explored and given to patients without the risk of intravenous injections such as pain or infection. Local guidelines include oral antibiotics that are given over multiple doses with a longer duration as opposed to azithromycin. Azithromycin is a potentially useful drug in the treatment

of typhoid fever because of its high intracellular tissue penetration and long elimination half-life (72 h)[19]. This meta-analysis addresses the available evidence on the efficacy and safety of azithromycin in treating enteric fever in comparison to ceftriaxone.

Clinical cure and duration of fever was comparable for azithromycin and ceftriaxone. However, relapse was significantly lower in the subjects treated with azithromycin compared to those given ceftriaxone. Compared to ceftriaxone, azithromycin has a longer half-life and a high intracellular tissue penetration, leading to eradication of residual organisms even after completion of therapy. Azithromycin is also found to have a higher concentration in the biliary tract, which contributes further to these findings[13]. No serious adverse events were seen in both treatment arms. Most adverse events were gastrointestinal in nature, and these are not severe enough to warrant alteration of treatment. Laboratory abnormalities like elevation in liver enzymes and platelet counts (thrombocytosis) were also clinically insignificant.

In the National Antibiotic Guidelines of the Philippine DOH, in the treatment of typhoid fever, azithromycin is indicated as a second-line therapy[11]. In this meta-analysis, azithromycin has been shown to be a safe and effective drug, further expanding possible treatment choices for enteric fever when limitations with first line agents are encountered.

Aside from antibiotic treatment, efforts of the World Health Organization (WHO), UNICEF, and various national and international agencies have also focused on the prevention of typhoid and other water-borne illnesses[20]. These include boosting vaccination, water, sanitation, and hygiene programs to improve water quality and public health practices. Typhoid fever incidence rates and trends decreased proportionally with the successful implementation of public health measures.

CONCLUSION

Evidence from this meta-analysis shows that oral azithromycin is comparable with intravenous ceftriaxone for treatment of uncomplicated typhoid fever in terms of cure, duration of fever, and adverse events. However, it appears to be better than ceftriaxone in terms of preventing relapse. This latest evidence agrees with the findings of previous meta-analyses comparing azithromycin with other alternative treatments[19.21]. Adverse events are also mild and self-limiting in both treatment regimens and are not clinically significant. Azithromycin can be recommended as an alternative therapeutic option in the local setting when adverse events with first line agents are encountered.

However, because of the small number of trials eligible for this meta-analysis and the wide confidence intervals, further evidence is needed to give a strong recommendation for the preferential use of azithromycin over standard antibiotic regimens. We recommend conducting trials for pediatric patients locally, to compare azithromycin with standard antibiotic regimen for typhoid fever.

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REVIEW ARTICLE

Outbreak Response Measures for the Pediatric COVID 19 Ward at the Philippine General Hospital

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An outbreak caused by the novel SARS-CoV-2 was declared by the World Health Organization (WHO) on January 30, 2020 as a public health emergency of international concern.[1] In the Philippines, the first identified COVID 19 case was on January 16, 2020. On March 11, 2020 the WHO determined that it reached pandemic classification with widespread community transmission across the world.[1] On March 16, 2020, an enhanced community quarantine (ECQ) was initiated in the National Capital Region (NCR) in the Philippines as a measure to control the spread of COVID -19 infection.

The Philippine General Hospital (PGH) being the biggest tertiary multispecialty hospital in the Philippines was made as one of the Covid-19 Referral Hospital in the NCR.

Understanding the SARs-CoV-2 possible modes of transmission which include respiratory droplets, contact, fomites, fecal-oral route and airborne via aerosol-generating procedures,[2] the PGH-Department of Pediatrics instituted outbreak response measures. The goals were to establish an area to cohort and care for the patients with known or suspected COVID -19 infection and to prevent the risk of transmission amongst the patients, healthcare workers and allied health staff. Several infrastructure changes, setting modification of workflow and process, formulation of clinical guidelines for management of patients with COVID 19 infection and measures of infection control and environmental cleaning were made.

HOSPITAL MEASURES RELATED TO MEDICAL MANAGEMENT OF PEDIATRIC COVID PATIENTS

Infrastructure Changes

The Philippine General Hospital was built more than 100 years ago and the structure of the main wards have high ceilings and big dome shaped windows that regularly admit around 50 pediatric patients per main ward. The main pediatric ward 9, limited the admission to 20-25 patients wherein a distance of 2 meters between patients is observed as part of the infection control measure described as social distancing. Several exhaust fans were installed to direct the airflow outside.

A new pediatric COVID ward equipped as an intensive care unit comprising of 3 small wards with a capacity of 4 beds per room were designated at the 5th floor of the hospital to prevent mixing of non-COVID patients at Ward 9. Likewise, a new pediatric neonatal intensive care unit (NICU) COVID ward comprising of 3-4 bed capacity rooms at 4th floor was opened to admit all neonates born in the hospital during the pandemic.

Management of patients, staff and visitors

All new patients for admission starting April 1, 2020 were limited to life-threatening cases only. All these patients were referred to the infectious Disease Service for screening using a standard questionnaire and nasopharyngeal swab (NPS) or endotracheal (ET) aspirate were sent for SARS-CoV 2 reverse transcriptase polymerase chain reaction (RT-PCR). Patients who were suspected to have COVID infection were admitted to the COVID ward.

Teams caring for the COVID patients were not assigned to other wards until after a 14- day quarantine after their 7 days rotation in any COVID ward. They were also asked to monitor themselves for fever and other respiratory symptoms daily through web-based forms. They were advised to consult the health services for development of any symptom and assessed for the risk of transmission and appropriate management.

Only one parent and/or guardian was allowed to be in the unit as a patient's caregiver for the entire duration of hospitalization. They were asked to sign a waiver and they should be cleared of Covid-19 infection by having a negative NPS-RT PCR result, likewise, they were provided with proper personal protective equipment. Those parents who cannot stay in the unit were contacted and updated every morning through text messaging or personal as necessary. Visitors were not allowed anytime in any of the units.

FORMULATION OF CLINICAL PATHWAYS AND GUIDELINES FOR COVID MANAGEMENT

Clinical pathways on the admission of patients presenting at the Emergency Room were made. The patient will initially be assessed at pediatric emergency room triage. If the patient is assessed to have mild disease, the patient will be sent home for home quarantine x 14 days and advised. For severe/critical patients or for life-threatening/emergency cases that are non-COVID, the patient will be assessed at the pediatric emergency room for resuscitation and will be admitted to Ward 9. For cases with respiratory symptoms, especially those assessed to have pneumonia who have exposure to a COVID (+) caregiver or a household with clusters of influenza-like Illness will be referred to pediatric infectious disease service for evaluation. Specimens either nasopharyngeal swab and /or endotracheal tube aspirate for COVID-19 RT PCR should be taken aseptically. These patients will be admitted to the COVID ward.

Treatment guidelines for COVID-19 probable and confirmed cases was made with the use of experimental drugs along with parental informed consent.

MEASURES ON INFECTION CONTROL AND PREVENTION

Personal Protective Equipment

Hospital-wide comprehensive program for the use of PPE was implemented led by the Hospital Infection Control Unit (HICU). The Division of Pediatric Infectious Disease medically cleared, monitored and trained all its healthcare personnel on the use of PPE in the different pediatric areas. They were all fitted with the National Institute of Occupational Safety and Health (NIOSH)-certified N95 respirators. Deviations from the recommended PPE doffing protocol by CDC which could increase the risk of self-contamination on HCW's clothing and skin after providing care for patients is common. Errors were seen mostly during doffing practices with respect to the doffing sequence and technique, and/or use of appropriate PPE.[3,4] Safety officers were then assigned in all donning and doffing areas. They were trained to assist and identify possible break in the observance of infection control while a staff is putting on and taking off their PPEs. The presence of safety officers was perceived by the staff as a good measure to assure them that their PPE is of optimal use.

Figure 1. From left to right: Posters for PPE, Fit testing of N95 masks, Training on Donning and Doffing and as Safety officers

Installation of Dry Hydrogen Peroxide Machines

The 3 pediatric COVID ward rooms with 4 bed capacity each observing the 2 meter distancing between patients were equipped as an Intensive Care Unit. Daily cleaning and disinfection of the rooms and medical devices were observed with the use of sodium hypochlorite.[5] Since the wards cannot be closed for admission and prolonged stay of critical patients was observed , A Dry Hydrogen Peroxide (DHP) machine, a new technology by Synexis ™ was installed per room . A "No-touch" decontamination device are increasingly used as an adjunct to standard cleaning and disinfection in health care facilities. There is convincing evidence that decontamination devices reduce contamination and some evidence that their use may reduce colonization and infection with health care–associated pathogens. It has shown that at low concentrations, it disinfects more slowly but safe enough to disinfect occupied areas and does not harm electronics or other materials in the area.[6] DHP does not overwhelm the lung enzymes and has been judged by Western IRB to be a non-significant risk technology suitable for continuous use in areas occupied by patients and staff.[6,7] Further studies are needed to prove its efficacy during pandemics and other emerging infectious diseases.

Figure 2. Dry Hydrogen Peroxide (DHP) Synexis™ Machines inside Pediatric COVID wards

Infection Control when Caring for Covid-19 patients

Standard precautions are observed with the minimum PPE required for any staff caring for patients at the COVID wards include a fitted, NIOSH-certified N95 respirator, eye protection (either goggles or full-face shield), cap, gowns or hazmats, shoe covers and gloves. Double gloves was required and the outer pair should be changed between handling of patients or when contaminated. All staff were instructed to shower after doffing of PPEs and change into clean street clothes before leaving the hospital.

The patients whenever possible wears a surgical mask, and when transported should be on a designated route with minimal contact with other patients.

Selected designated equipment and supplies were brought to the COVID wards. Charting are done electronically with touch-screen devices and monitors, laptop computers, monitoring machines, 2D echo and ultrasound machines all covered with plastic wrap to decrease the risk of contamination and facilitate decontamination. Surfaces of all medical devices are cleaned regularly.

Figure 3. From left to right. Inside the COVID ward with a patient wearing appropriate PPE, medical devices covered with plastic wrap, transport of a COVID patient with decontamination of the designated route.

Pandemic outbreak involves different hierarchy of controls and modification of infrastructure and processes. Multiple stakeholders should be involved with regular meetings and constant communication to be able to institute changes in protocol and formulate hospital policies. For the past 2 months since the ECQ, the pediatric COVID ward remains to have an average of 6-9 patients per day. No nosocomial infections have been identified nor nosocomial transmission of COVID infection amongst the patients and healthcare staff at the wards. Challenges remains to be mitigated which is mostly the staff who are vulnerable to fatigue, prone to be infected with constant exposure, who needs constant assurance and support and the steady supply of the PPEs. It is therefore prudent to put containment measures as soon as the outbreak is declared to provide quality care and reduce the risk of transmission to other patients or healthcare workers.

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ORIGINAL ARTICLE

Medicinal Plants for Dermatophytosis: *Senna Alata* (Linn.) Roxb., *Allium sativum* (Linn.) and *Cymbopogon citratus* (DC.) Stapf

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ABSTRACT

Skin mycoses have been a major problem affecting millions around the globe. The threat of resistance to synthetic antifungal agents however is a major obstacle in its management. As an alternative to these, a thorough investigation of natural products is being performed to develop medicines that are effective and safe. In this review, we described three antifungal herbal plants that are available in the Philippines, namely *Senna alata* (Linn.) Roxb. (akapulko), *Allium sativum* (Linn.) (garlic) and *Cymbopogon citratus* (DC.) Stapf (lemongrass). In vitro studies showed promising results that can be used as a basis for drug formulation for community use as well as commercial products. So far, there have been no reported toxic effects from these plants. The common ground for these plants' mechanism of action was the effect of their phytochemicals in the cell membrane and cell wall organelles, inhibition of major biosynthetic pathways, and prevention of biofilm formation. Formulation and clinical studies also revealed promising results comparable to the synthetic ones.

KEYWORDS: *antifungal, dermatophytosis, Senna alata, Allium sativum, Cymbopogon citratus*

INTRODUCTION

Skin mycosis, also known as dermatophytosis, is a global health problem that infects millions around the globe. An estimated one billion people have skin, nail, and hair fungal infections[1]. While they are generally non-lethal, they greatly impact everyday life, routine and confidence of those infected.

Fungal diseases are caused by keratinophilic pathogenic fungi known as dermatophytes that can be classified into three genera based on conidial morphology: Epidermophyton, Microsporum, and Trichophyton. Some of the dermatophytes include *Trichophyton rubrum* (causative agent of tinea pedis (toes), tinea cruris (groin and inner thighs), tinea unguium (nails) and tinea corporis (skin)), *T. interdigitale* (tinea pedis and tinea cruris), *T. tonsurans* (tinea capitis (scalp, eyebrows, and eyelashes) and tinea corporis), *Microsporum audouinii* (tinea capitis) and *M. canis*[2]. Fungi invade the skin's epidermal layer, hair, and nails by producing keratinase that digest the keratinized layer of these integuments[3] and then establish themselves by thieving available nutrients for them to survive. At a molecular level, subtilisin gene (SUB) encoding serine protease catalyzes the initial contact and adherence of the fungi to the keratinized tissues of the skin[4].

In the Philippines, fungal infections were reported as the second leading cause of visits to dermatology clinics with a prevalence of 12.98%. The most frequently encountered diseases were pityriasis versicolor （25.34%), tinea corporis （22.63%）, tinea cruris （16.7%), and tinea pedis （16.38%)[5]. At present, antifungal agents such as azoles, allylamines, and tolnaftate[6] are used in the treatment of dermatophytosis. These agents inhibit ergosterol synthesis, thereby altering membrane stability and permeability[7,8]. While there are advantages to these synthetic drugs, the development of resistance to antifungal agents make it harder to cure skin mycoses. Resistance has been reported for antifungal drugs, including griseofulvin[9], azoles[10], and terbinafine[11]. Azole resistance for dermatophytes was reported to be 19% in certain countries. Factors such as host's immune status, varying geographical location, prolonged use, and misuse of antifungal drugs, fungal factors, and drug-related factors[8,12] contribute to resistance. From a molecular standpoint, resistance is conferred by the upregulation or downregulation of various cis- and trans-acting elements, chromosomal abnormalities, formation of biofilms, involvement of heat-shock protein 90 (Hsp90) and increased mRNA stability[7]. The reduced affinity of the azoles to its target molecules and increased expression of efflux pumps (ATP-binding cassette pump and major facilitator superfamily pumps)[8] contribute to azole resistance. Lastly, the appearance of superbugs such as *Candida auris,* first identified in 2009, adds to the problem as this species is generally multidrug-resistant[8].

The disadvantages of synthetic antifungal agents have made the search for other types of antifungals an ongoing race. Natural medicinal products have long been sought for its wide range of pharmacologic activity and safety. The main objective of this review is to highlight three herbal plants identified to have antifungal activity in vitro and in human studies. The authors did a thorough search for articles and reports for *Senna alata* (Linn.) Roxb*., Allium sativum* (Linn.) and *Cymbopogon citratus* (DC.) Stapf. and these are highlighted in this review.

Senna alata (Linn.) Roxb.

Senna alata (Linn.) Roxb*.,* more commonly known as candle bush, akapulko, ringworm bush, or calabra bush, has an average height of 10-15 feet with green leaves organized in alternate arrangement and a yellow flower[13]. The name ringworm bush was given because it was efficient in treating ringworm, a skin disease previously known to be caused by ringworm and other fungal diseases of the skin[14].

Akapulko is pharmacologically active as a hepatoprotective, antihelminthic, anti-inflammatory, and antimicrobial agent[15-18]. Phytochemicals present in the plant were alkaloids, flavonoids, saponin, tannin, terpenoids[19,20], anthracenosides, gallic tannins[21], anthraquinone, volatile oil[22], cardiac glycosides, gum, lipids, mucilage, phytosterol, quinone[19], coumarin[23], glycosides, phenols, steroids[20] and proanthocyanidin[24]. Some of the compounds observed in the plant and as revealed by gas chromatography – mass spectrometry (GC-MS) were aloe-emodin, cassiaindoline, and kaempferol[25,26].

In a recent review[27], the plant's ethanolic, aqueous, methanolic and hexane leaf, stem and root extracts have activities against *Aspergillus flavus, A. niger, A. parasiticius, Candida albicans, C. neoformans, Epidermophyton floccosum, M. canslaslomyces, M. canis,*

M. gypseum, M. audouinnii, T. verrucosim, T. megnini, T. mentagrophytes, T. rubrum, T. tonsurans, and *Penicillium marneffei.* In addition, its ethanolic extract showed excellent activity and is favorable in formulating a topical treatment for those infected with these fungi. A study also showed that soap formulated with akapulko ethanolic extract showed a higher reduction of viable cell count at 94.78±1.82% compared to an antiseptic soap (91.88±1.63%)[28].

The National Integrated Research Program (1996) developed a 50% akapulko lotion on medicinal plants, which underwent both non-clinical and clinical research, and which showed efficacy and safety against cutaneous fungal infections. In a systematic review and meta-analysis featuring seven random clinical trials for akapulko topical cream *S. alata* 50% lotion, the authors concluded that *S. alata* 50% lotion may be as efficacious as sodium thiosulfate 25% lotion and is as efficacious as ketoconazole 2% and terbinafine 1% creams[29].

***Allium sativum* (Linn.)**

Allium sativum of family Liliaceae, more commonly known as garlic, is a famous food seasoning and spice. The names "stinking rose," "poor man's treacle," and "nectar of God" were given to this plant due to its characteristic pungent smell. It has a history of being used as a remedy for various diseases such as leprosy, scurvy, earaches, flatulence, and epidemics such as typhus, dysentery, cholera, and influenza[30]. Garlic bulb covered with a membranous scale has an average diameter of 4-6 cm depending on the variety and the number of bulblets or cloves present[31].

Phytochemicals extracted by using different solvents from the garlic bulb include alkaloids, flavonoids, steroids, saponin, carbohydrates, glycosides[32], triterpenes, tannin[33], reducing sugars[34], terpenoids, anthraquinone, phenolics, cardiac glycosides, phlobotannin[35], carotenoids, and phytates[36]. GC-MS studies revealed that specific compounds such as ajoene, thiosulfides[37], allicin[37,38], disulfides, trisulfides, monosulfides[39], gentisic acid, chlorogenic acid, 4-hydrobenzoic acid, and p-Coumaric acid[38] can be derived from garlic as well. The major compound in garlic, allicin, is produced upon the action of alliinase to alliin and exists only after crushing or injuring the bulb[31].

In vitro analysis of the antifungal activity of garlic is summarized in Table 1. Aqueous, ethanolic, methanolic, and petroleum ether extracts and the garlic juice extract showed antifungal activity against a wide spectrum of fungal species, comparable with the controls used. Combining the extract with the commercially available antifungal agent also showed promising results. The minimum inhibitory concentration (MIC) of fluconazole against *C. albicans, C. tropicalis,* and *C. glabrata* was lower suggesting a synergistic relationship between the extract and the synthetic drug in combating these fungi[40]. Synergism between ketoconazole and the extract was also observed against *T. metagrophytes, T. rubrum, T. verrucosum, M. canis,* and *E. flocossum* [41]. The Ilocos garlic has also shown good antifungal activity against *Saccharomyces cerevisiae, C. albicans. M. canis, T. rubrum,* and *T. mentagrophytes* [42]

Table 1. In vitro analysis of the antifungal activity of Allium sativum against different fungal pathogens.

Organism	**Extract/Media/Technique**	**Result**	
		MIC (mg/mL)	**Inhibition**
Alternaria alternata	Aqueous, PDA, AWD	6.25	11.7±0.3mm[43]
	Methanolic, PDA, AWD	156.2	11.2±0.2mm[43]
A. flavus	Petroleum ether, SDA, CPAD	2.5	38±1.26 mm[44]
	Aqueous, SDA, CPAD	2.5	23±0.82 mm[44]
A. niger	Garlic Juice, SDA, ADD		41±4.0 mm > clotrimazole (22.5±1.5 mm)[45]
	Ethanolic, PDA, PFT		93.03% mycelial growth inhibition at 200 mg/mL[46]
	Aqueous, PDA, AWD	3.12	22.5±0.3 mm[43]
	Methanolic, PDA, AWD	312.5	8.4±0.1 mm[43]
	Petroleum ether, SDA, CPAD	2.5	16±1.09 mm [44]

	Aqueous, SDA, CPAD	2.5	14±1.63 mm [44]
	Methanolic, SDA, PPT		42 mm inhibition at 100 mg/mL[47]
A. parasiticus	Aqueous, PDA, AWD	12.5	11.7±0.1mm[43]
	Methanolic, PDA, AWD	156.2	11.7±0.3mm[43]
A. ustus	Ethanolic, PDA, PFT		100% inhibition at 200 mg/mL[46]
C. albicans	Garlic juice CPAD		41 mm (Ilocos garlic)[42]
	Ethanolic CPAD		53 mm at 10% (Ilocos garlic)[42]
	Garlic Juice, SDA, ADD		28±1.0 mm > Clotrimazole (27.5±0.5mm)[45]
	Methanolic, SDA, ADD	12.5	29 mm[32]
	Methanolic, SDA, PPT		37 mm inhibition at 100 mg/mL[47]
	Petroleum ether, SDA, CPAD	2.5	23±0.05 mm[44]
	Aqueous, SDA, CPAD	2.5	16±1.41 mm[44]
	Aqueous, SBM	3.125 [40]	
C. glabrata	Aqueous, SBM	1.56 [40]	
C. krusei	Aqueous, SBM	6.25 [40]	
C. paralopsis	Methanolic, SDA, ADD	3.125	21.8 mm[32]
C. tropicalis	Methanolic, SDA, ADD	6.25	30 mm[32]
	Aqueous, SBM	0.78 [40]	
Curvularia lunata	Petroleum ether, SDA, CPAD	2.5	45±1.34 mm[44]
	Aqueous, SDA, CPAD	2.5	45±1.15 mm[44]
E. floccosum	Aqueous, SDA, AWD		6 mm < Nystatin (25mm)[48]
	Ethanolic, SDA, AWD		12 mm < Nystatin (25mm)[48]
	Methanolic, SDA, AWD		13.33 mm < Nystatin (25mm)[48]
Fusarium	Aqueous, SDA, AWD	> 20	2.4 mm at 5 mg/mL 4.2 mm at 10 mg/mL 9.5 mm at 20 mg/mL [49]
	Ethanolic, SDA, AWD	2.5	4.1 mm at 2.5 mg/mL 6.2 mm at 5 mg/mL 10.1 mm at 10 mg/mL 14.3 mm at 20 mg/mL [49]
F. oxysporum	Aqueous, PDA, AWD	3.12	22.6±0.1mm [43]
	Methanolic, PDA, AWD	156.2	10.4±0.1mm [43]
M. canis	Garlic juice CPAD		37 mm (Ilocos garlic)[42]
	Ethanolic CPAD		33 mm at 10% (Ilocos garlic) [42]
Penicillium	Ethanolic, PDA, PFT		92.97% mycelial growth inhibition at 200 mg/mL[46]
Rhizopus	Aqueous, SDA, AWD	> 20	4.3 mm at 5 mg/mL 5.2 mm at 10 mg/mL 10.4 mm at 20 mg/mL [49]
	Ethanolic, SDA, AWD	5.00	5.2 mm at 2.5 mg/mL 6.4 mm at 5 mg/mL 9.1 mm at 10 mg/mL 12.2 mm at 20 mg/mL [49]
S. cerevisiae	Garlic juice, CPAD		41 mm (Ilocos garlic)[42]
	Ethanolic, CPAD		53 mm at 10% (Ilocos garlic)[42]

T. rubrum	Ethanolic, CPAD		42 mm at 10% (Ilocos garlic) [42]
	Aqueous, SDA, AWD		18.33 mm < Nystatin (31mm)[48]
	Ethanolic, SDA, AWD		23.33 mm < Nystatin (31mm)[48]
	Methanolic, SDA, AWD		30.67 mm < Nystatin (31mm)[48]
T. metagrophytes	Ethanolic CPAD		40 mm at 10% (Ilocos garlic) [42]
	Aqueous, SDA, AWD		18 mm < Nystatin (30mm)[48]
	Ethanolic, SDA, AWD		24 mm < Nystatin (30mm)[48]
	Methanolic, SDA, AWD		28.33 mm < Nystatin (30mm)[48]
T. verrucosum	Aqueous, SDA, AWD		15.67 mm < Nystatin (28mm)[48]
	Ethanolic, SDA, AWD		23.67 mm < Nystatin (28mm)[48]
	Methanolic, SDA, AWD		24.67 mm < Nystatin (28mm)[48]

Legend: SDA – Sabouraud Dextrose Agar; PDA – Potato Dextrose Agar; AWD – Agar Well Diffusion; ADD – Agar Disk Diffusion; CPAD – Cup Plate Agar Diffusion; PPT – Pour Plate Technique; PFT – Poisoned Food Technique; SBM – Standard Broth Microdilution

The molecular mechanism of action of the garlic extract was determined using transmission electron microscopy, scanning electron microscopy, and proteomics. The extract induced several changes in the fungi such as damaged cell wall and cell membrane, formation of vacuoles, cytoplasmic granulation, cytoplasmic loss, destroyed hyphae, destroyed cellular organelles, and pseudohyphae development[38,39,50,51]. *In vitro,* the lag phase of the fungi was longer compared to the control, and the exponential phase was inhibited[51,52]. Allicin can induce cell membrane damage and impede lipid biosynthesis[50]. In addition, allicin, when combined with Amphotericin B and flucytosine, destroyed the fungal membrane and lowered its adhesive force[53]. Allyl alcohol, on the other hand, lengthened the lag phase, affected the cell wall and cell membrane, decreased cytoplasmic volume, reduced glutathione and rate of oxygen consumption, and increased cytoplasmic granulation, the concentration of reactive oxygen species and mitochondrial membrane potential[51]. Proteomic analyses revealed differentially expressed proteins involved in major fungal metabolic pathways, including drug metabolism, redox processes, pathogenesis, cellular response to stress, cell cycle, DNA replication, gene expression processes, protein modification, synthesis of nucleotides and certain signaling pathways[52].

Clinical studies on garlic's antifungal ability have been conducted in the Philippines. In a study conducted at MCU-FDTMF Hospital, 36 patients were randomly assigned to either garlic cream or ketoconazole treatment for superficial mycoses. They were instructed to apply the medications thinly on the affected area twice a day for one month. Weekly evaluations of microscopic clearance of fungi by KOH smear, relief of subjective complaint of itchiness, relief of objective sign of scaling, shortening of treatment duration, and adverse reactions were observed. Garlic cream was successful and equally effective as ketoconazole in terms of conversion of positive into negative KOH smears and in alleviating subjective complaints such as itchiness. Ketoconazole had no side effects while the garlic cream presented with erythema and burning sensation in the area applied, albeit tolerable[54].

Another clinical study comparing garlic cream and ketoconazole, enrolled 72 patients with Tinea Versicolor, confirmed through positive KOH (potassium hydroxide) smear. After a two-week period, the smear conversion rate was similar between the two groups, and no adverse reactions were observed nor reported[55].

The safety and effectiveness of 0.6% ajoene gel compared with 1% terbinafine cream for the treatment of 60 soldiers with clinical and mycological diagnosis of either tinea corporis or tinea cruris was also studied. After thirty days of treatment, the ajoene and terbinafine groups resulted in 77 and 75 percent healing rates, respectively. Sixty days after treatment, the healing rates were 73% and 71% for the groups treated with ajoene and terbinafine, respectively.

Several trials have shown good clinical evidence on the potential of garlic as an effective antifungal

agent[56]. As an oral rinse, garlic mouthwash was compared to nystatin mouthwash for denture stomatitis and given to patients for four weeks. The changes in the length and width of erythema were found to be significant for both treatments. Moreover, greater satisfaction with the use of garlic was seen compared with nystatin[57].

***Cymbopogon citratus* (DC.) Stapf**

Known as lemongrass, *Cymbopogon citratus* belongs to the family of Poaceae and is characterized by a lemon-like odor. It is used as a food flavoring and is common in teas, soups, and curries[58]. It is a perennial herb that grows approximately two meters in height with short rhizomes that can be a means of propagation. The plant has reported antimicrobial, anti-inflammatory, and antioxidant properties and has been added to pesticides, insecticides, cosmetics, perfumes, and pharmaceuticals[59]. In the Philippines, it is traditionally used in the control of diabetes and for cleansing[60]. The phytochemical constituents observed in lemongrass include alkaloids, tannins, flavonoids [60,61], saponins, phenols, carbohydrates, reducing sugars[62], anthraquinones, steroids[63], terpenoids[64], volatile oil[66], unsaturated fats[60], phlorotannins, cardiac glycosides[66], triterpenoids, and phytosterols[67]. GC-MS analysis of essential oils extracted from lemongrass showed three major components arranged from the most to the least abundant: geraniol (37.70 - 52.80%), neral (31.52 – 36.65%) and β-myrcene (3.73% - 11.41%)[68-70].

Majority of the in vitro antifungal analysis of lemongrass was done on the plant's essential oil. Essential oil is approximately 1-2% of the lemongrass's dry weight[71]. In several studies summarized in Table 2, liquid and vapor phases of the essential oil were compared for its antifungal activity. Better antifungal activities were observed in vapor phases, which might suggest that the volatile compounds present were more effective in inhibiting fungal growth. In vitro analysis of an antifungal cream containing 2.5% and 3.0% lemongrass oil had higher efficiency compared to the commercially available creams that contain clotrimazole, isoconazole, and nitrate as its active ingredient[72]. Silicon rubber surfaces coated with essential oil and prepared using hypromellose ointment showed a 45-76% decrease in biofilm formation. The oil inhibited biofilm formation of the two strains of *C. tropicalis* (U71 and V89)[73].

Table 2. In vitro analysis of the antifungal activity of Cymbopogon citratus (DC.) Stapf against different fungal pathogens.

Organism	**Extract, Media, Technique**	**Result**	
		MIC	**Inhibition**
A. flavus	LGEO (liq), PDA, Plate Assay	0.9 μL/mL	Total inhibition of fungal growth at 1.0 μL/mL observed for 8 days[74]
	LGEO (liq), SDA, DDA		90 mm[69]
	LGEO (vap), SDA, VDT		90 mm[69]
	LGEO (liq), PDA, PFT	5 μL/mL	- Spore production reduced by 23.2% at 1 μL/mL - Spore germination reduced by 79.7% at 4 μL/mL - 100% inhibition of Aflatoxin B1 production[70]
A. fumigatus	LGEO (liq), SDA, DDA		90 mm[69]
	LGEO (vap), SDA, VDT		90 mm[69]
A. niger	LGEO (liq), SDA, DDA		An1 - 90 mm; An2 – 59 mm[69]
	LGEO (vap), SDA, VDT		An1 – 59 mm; An2 – 75 mm[69]
	LGEO (liq), PDA, PFT	5 μL/mL	- Spore production reduced by 38.9% at 1 μL/mL - Spore germination reduced by 91.3% at 4 μL/mL[70]

A. ochraceus	LGEO (liq), PDA, PFT	5 µL/mL	- Spore production reduced by 45.6% at 1 µL/mL - Spore germination reduced by 84% at 4 µL/mL[70]
A. parasiticus	LGEO (liq), PDA, PFT	5 µL/mL	Spore production reduced by 39.2% at 1 µL/mL Spore germination reduced by 88.2% at 4 µL/mL[70]
A.s terreus	LGEO (liq), SDA, DDA		90 mm[69]
	LGEO (vap), SDA, VDT		90 mm[69]
C. albicans	LGEO (liq), SDA, DDA		Ca1 - 80 mm; Ca2 – 90 mm; Ca3 – 90 mm; Ca4 – 45 mm[69]
	LGEO (vap), SDA, VDT		Ca1-Ca4 – 90 mm[69]
	Chloroform, SDA, DDA	Leaf – 32 µg/mL Root – 38 µf/mL[65]	
	LGEO (liq), PDA, DVA	288 mg/L	80 mm inhibition at 20 µL LGEO liquid phase[75]
	LGEO (vap), PDA, DVA	32.7 mg/L	Complete inhibition at 40 µL LGEO vapor phase[75]
C. dubliniensis	LGEO (liq), SDA, BDM	0.43 mg/mL	80% biofilm formation inhibition at MIC; Reduced adhesion to acrylic at 1.7 mg/mL concentration[76]
C. parapsilosis	LGEO (liq), SDA, DDA		Cp1 - 90 mm; Cp2 – 18 mm[69]
	LGEO (vap), SDA, VDT		Cp1 - 90mm; Cp2 – 25 mm[69]
C. tropicalis	LGEO (liq), SDA, DDA		Ct1 and Ct2 - 90 mm[69]
	LGEO (vap), SDA, VDT		Ct1 and Ct2 - 90mm[69]
E. floccosum	LGEO (liq), SDA, DDA	115 µg/mL	90 mm[72]
M. furfur	LGEO (liq), Mycosel medium, ADT	0.62 µL/mL[68]	
M. globose	LGEO (liq), Mycosel medium, ADT	0.31 µL/mL[68]	
M. obtusa	LGEO (liq), Mycosel medium, ADT	0.62 µL/mL[68]	
M. sloofiae	LGEO (liq), Mycosel medium, ADT	0.31 µL/mL[68]	
M. sympodialis	LGEO (liq), Mycosel medium, ADT	1.52 µL/mL[68]	
Mi. gypseum	LGEO (liq), SDA, DDA	235 µg/mL	90 mm[72]
Mucor	LGEO (liq), SDA, DDA		42 mm[69]
	LGEO (vap), SDA, VDT		90mm[69]
	Ethanolic	Stem – 1.10 mg/mL[66]	
Penicillium	LGEO (liq), SDA, DDA		90 mm[69]
	LGEO (vap), SDA, VDT		90 mm[69]
	Ethanolic	Leaf – 0.70 mg/mL	

		Stem – 1.10 mg/mL[66]	
T. mentagrophytes	LGEO (liq), SDA, DDA	122.5 μg/mL	90 mm[72]
T. rubrum	LGEO (liq), SDA, DDA	135 μg/mL	90 mm[72]

Legend: LGEO (liq) – Lemongrass essential oil (liquid); LGEO (vap) – Lemongrass essential oil (vapor); SDA – Saboraud Dextrose Agar; DDA – Disk Diffusion Assay; VDT – Vapour Diffusion Technique; PFT – Poisoned Food Technique; ADT – Agar Dilution Technique; BDM – Broth Dilution Method; DVA – Disc Volatilization Assay

The mechanism of action of lemongrass essential oil (LGEO) lies in its ability to disrupt the cell membrane. In a report, both vapor and liquid phases caused disturbed fungal membranes. The most significant effect at a minute MIC, however, was observed in the vapor phase[75]. In another report, the citral component of LGEOs prevented the formation of pseudohyphae and chlamydoconidia in *C. albicans*[77]. In a review of the mechanism of action of the lemongrass essential oils, it was found that LGEO target structures such as the cell membrane, cell wall and mitochondria. Moreover, these compounds affect cell growth and morphology, block efflux pumps, increase reactive oxygen species production and prevent biofilm formation, mycotoxin synthesis, and quorum sensing[78].

A study was conducted in South Africa to investigate the safety and efficacy of lemon juice and lemongrass in the treatment of oral thrush in HIV/AIDS patients versus gentian violet aqueous solution 0.5%, which is the standard of care[79]. Ninety patients were enrolled in the study, but only 82 had complete and acceptable data. Thirty patients were enrolled in the lemongrass arm, but only 17 completed the study. Based on the intention to treat analysis, the difference between lemongrass and gentian violet was not statistically significant. In the per-protocol analysis, lemongrass was significantly better than gentian violet aqueous solution 0.5% in treating oral thrush in an HIV-positive population.

In the Philippines, clinical trials for antifungal creams formulated with lemongrass extracts have been performed[80]. A double-blind, randomized controlled trial on the effectiveness of 10% lemongrass oil vs. 1% clotrimazole solution against tinea corporis and tinea cruris was conducted. Ninety-six patients with clinically and mycologically diagnosed tinea corporis and/or tinea cruris were assigned randomly to apply either 10% lemongrass oil or 1% clotrimazole solution twice daily for four weeks. There was no statistically significant difference in terms of complete cure at four weeks between the two groups (p=1.0, Fisher's exact test). There was no recurrence two weeks post-treatment in both groups. Erythema and burning sensation from the application of lemongrass were observed in two patients.

In another randomized clinical study comparing lemongrass 10% cream versus Clotrimazole 1% cream in the treatment of superficial fungal skin infections at Quezon City General Hospital[81], it was found that after two weeks, both treatments showed statistically significant improvement from baseline. The clotrimazole group showed faster resolution of lesions. No adverse reaction was observed or reported in the lemongrass group.

CONCLUSION

Akapulko (Senna alata), garlic (Allium sativum), and lemongrass (Cymbopogon citratus) have demonstrated their antifungal effects both in vitro and in clinical studies. An Akapulco, 50% formulation, has undergone Phase 1 to 3 clinical trials and awaits further commercialization. Lemongrass and garlic preparations are good alternatives as topical antifungal agents and may be used in the community and further developed into commercial preparations.

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GUIDELINES

Interim Guidelines on the Screening, Assessment and Clinical Management of Pediatric Patients with Suspected or Confirmed Coronavirus Disease 2019 (COVID-19)

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INTRODUCTION

The emergence of the novel coronavirus SARS-CoV-2 and the subsequent declaration by the World Health Organization of the coronavirus disease 2019 (COVID-19) pandemic has greatly impacted the lives of many all over the world. As the total number of reported cases increase globally, the number of pediatric cases have also steadily increased over the past several months. This has led to an expansion of the wealth of scientific and clinical knowledge on COVID-19 in children.

This rapid advice has been updated from the previous version (version 2, released 12 April 2020) as new knowledge on pediatric COVID-19 has become available in recent literature. It aims to provide guidance to pediatricians, general and family practitioners, and other healthcare professionals caring for children on how to assess and treat pediatric patients with suspected or confirmed COVID-19. These guidelines were formulated based on information available at the time of its release, and shall be updated as new data becomes available.

This rapid advice is divided into three parts: part 1 discusses basic concepts on COVID-19 in children, including local epidemiology, disease transmission, risk factors, clinical manifestations, and classification of severity; part 2 mainly focuses on proper screening and triaging of children; and part 3 largely focuses on basic concepts of management.

PART 1. COVID-19 IN CHILDREN

I. LOCAL EPIDEMIOLOGY AND BURDEN OF ILLNESS IN CHILDREN

As of 16 August 2020, the Department of Health has recorded 10,873 confirmed COVID-19 cases aged 19 years and below, of whom 51.6% were males. Majority of cases were between 15-19 years old, comprising 40.7% of total cases, followed by the 10-14 years age group at 23.2%, 0-4 years age group at 20%, and 5-9 years age group at 16.1%.

A total of 64 deaths were recorded among confirmed cases 19 years and below, giving a case fatality rate of 0.6%. Among the deaths, 48.4% were seen in the 0-4 years old age group, followed by the 15-19 age group with 29.7% of total deaths.

II. INCUBATION PERIOD

The incubation period of the SARS-CoV-2 virus is on average 5-6 days, but can last up to 14 days. Transmission of disease may occur during the pre-symptomatic and symptomatic phase of illness – infectiousness begins from 2.3 days before symptom onset and peaks at 0.7 days before symptom onset. The virus may be detected for a median of 20 days up to 37 days after symptom onset, but infectiousness has been observed to decline significantly 8 days after the onset of symptoms, and live virus could no longer be cultured beyond this period. Asymptomatic infection has been described in literature, with a wide range of reported incidence ranging from 1% to 78% depending on the population studied. How asymptomatic infection drives transmission is a subject that has yet to be elucidated.

III. TRANSMISSION

COVID-19 is primarily transmitted through inhalation of infected respiratory droplets, or by contact of the mucosal surfaces of the eyes, nose and mouth after touching contaminated objects and surfaces. Airborne transmission may also occur in certain situations where viral particles are aerosolized through aerosol-generating procedures such as non-invasive positive pressure ventilation (NIPPV, BiPAP and CPAP), endotracheal intubation and extubation, cardiopulmonary resuscitation (CPR), open suctioning of airway secretions, high frequency oscillatory ventilation, tracheostomy, chest physiotherapy, nebulizer treatment, sputum induction, nasogastric tube placement and bronchoscopy.

Other possible modes of transmission have been reported in literature. Prolonged viral shedding in stool of infected children has been documented, but there is limited evidence at present on whether viral RNA shed in stools is infectious and whether fecal viral shedding plays a role in the dissemination of infection.

Recent published data have shown evidence of transplacental transmission of SARS-CoV-2 from mother to infant, despite prior claims that vertical transmission does not occur. Evidence of placental infection with SARS-CoV-2 was seen in a mother infected with COVID-19, with the neonate also testing positive for COVID-19 on nasopharyngeal and rectal swab RT-PCR. A systematic review on vertical transmission of COVID-19 also concluded that vertical transmission of infection cannot be excluded in several of the reported cases. Transmission via breastmilk has also been investigated, and although viral RNA particles have been isolated in breastmilk, the viability of these viral particles have not been proven and transmission via breastmilk has yet to be confirmed.

Children have been shown to be infected via close contact with people infected with SARS-CoV-2. In a study on the spread of COVID-19 in family clusters with confirmed COVID-19 infection in children, 79% of households had an adult family member diagnosed with COVID-19 before the onset of symptoms in the COVID-19-infected child. In only 8% of households did the child develop symptoms first before any other household contact. This supports earlier findings that children are mainly infected within familial clusters. Evidence has also shown that children with COVID-19 are capable of transmitting the disease to adults and to other children. Yet despite these findings, the exact role of children in the extent of disease transmission has yet to be clearly determined and would need to be further investigated.

IV. RISK FACTORS

Several risk factors have been identified that predispose children to COVID-19 infection. In the systematic review by Hoang et al., a cohort of 655 patients were identified to have the following underlying conditions that predisposed the patients to COVID-19 infection:

- Immunosuppression (30.5%)
- Respiratory conditions (21%)
- Cardiovascular conditions (13.7%)
- Complex congenital malformations (10.7%)
- Hematologic conditions (3.8%)
- Neurologic conditions (3.4%)
- Obesity (3.4%)
- Prematurity (3.4%)
- Endocrine/metabolic conditions (2.1%)
- Renal conditions (1.7%)
- Gastrointestinal conditions (0.5%)

V. CLINICAL MANIFESTATIONS OF COVID-19 IN CHILDREN

A systematic review of children with COVID-19 by Hoang et al. has described the most common symptoms seen (Table 1). The two most common manifestations are fever (59.1%) and cough (55.9%). No symptoms were seen in 19.3% of infected children.

Table 1. Clinical symptoms of COVID-19 in children (n=2,445)

Clinical symptoms	%
Fever	59.1
Cough	55.9
Rhinorrhea, nasal congestion	20.0
Myalgia, fatigue	18.7
Sore throat	18.2
Shortness of breath, dyspnea	11.7
Abdominal pain, diarrhea	6.5
Vomiting, nausea	5.4
Headache, dizziness	4.3
Pharyngeal erythema	3.3
Decreased oral intake	1.7
Rash	0.25
Asymptomatic	19.3

Several reports have been made about children testing positive for current or recent infection with SARS-CoV-2, and presenting with a severe inflammatory syndrome with Kawasaki disease-like features. This syndrome has since been named **Multisystem Inflammatory Syndrome in Children (MIS-C).** The case definition for this syndrome is as follows:

US Centers for Disease Control and Prevention (CDC) Case Definition for Multisystem Inflammatory Syndrome in Children (MIS-C)

- An individual aged < 21 years presenting with fever[a], laboratory evidence of inflammation[b], and evidence of clinically severe illness requiring hospitalization, with multisystem (>2) organ involvement (cardiac, renal, respiratory, hematologic, gastrointestinal, dermatologic or neurological); AND
- No alternative plausible diagnoses; AND
- Positive for current or recent SARS-CoV-2 infection by RT-PCR, serology, or antigen test; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms

Additional comments:

- Some individuals may fulfill full or partial criteria for Kawasaki disease but should be reported if they meet the case definition for MIS-C
- Consider MIS-C in any pediatric death with evidence of SARS-CoV-2 infection

[a] Fever > 38.0°C for ≥ 24 hours, or report of subjective fever lasting ≥ 24 hours

[b] Including, but not limited to, one or more of the following: an elevated C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen, procalcitonin, d-dimer, ferritin, lactic acid dehydrogenase (LDH), or interleukin 6 (IL-6), elevated neutrophils, reduced lymphocytes and low albumin

VI. CLASSIFICATION OF SEVERITY OF COVID-19 IN CHILDREN

The following classification of severity of COVID-19 in children is adopted from Dong et al.:

Table 2. Classification of severity of COVID-19 in children

Classification	Description
Asymptomatic infection	A child with a positive 2019-nCoV nucleic acid test, without any clinical symptoms and signs, and normal chest imaging.
Mild infection	A child with symptoms of acute upper respiratory tract infection, including fever, fatigue, myalgia, cough, sore throat, runny nose, and sneezing. Physical examination shows congestion of the pharynx and no auscultatory abnormalities. Some cases may have no fever, or have only digestive symptoms such as nausea, vomiting, abdominal pain and diarrhea.
Moderate infection	A child with frequent fever and cough, mostly dry cough followed by productive cough, with or without wheezing, but no shortness of breath. Physical examination shows

	abnormal auscultatory findings and no hypoxemia. Chest x-ray findings reveal pneumonia. Some cases may have no clinical signs and symptoms, but chest CT scan shows lung lesions, which are subclinical.
Severe infection	A child with early respiratory symptoms such as fever and cough, may be accompanied by gastrointestinal symptoms such as diarrhea. The disease progresses after around 1 week, and dyspnea occurs, with central cyanosis. Oxygen saturation is less than 92%, accompanied by other manifestations of hypoxia.
Critical condition	Children who quickly progress to acute respiratory distress syndrome (ARDS) or respiratory failure, and may also have shock, encephalopathy, myocardial injury or heart failure, coagulation dysfunction, and acute kidney injury. Organ dysfunction can be life-threatening.

In the same study by Dong et al. of 2,143 confirmed and suspected cases of pediatric COVID-19 in China, 4.4% were found to be asymptomatic, 50.9% had mild disease, and 38.8% had moderate disease, accounting for 94.1% of total cases. The rate of severe and critical cases was 5.2% and 0.6% respectively.

In contrast to adult patients with COVID-19, most children and adolescents present with mild to moderate symptoms; only a small percentage of patients develop severe and critical manifestations. The mortality rate in children was reported to be at 0.09% in one systematic review. Several theories have been formulated to attempt to explain the difference in severity and susceptibility of children compared to adults (table 3). Further studies are needed to find more evidence supporting these theories.

Table 3. Theories regarding the severity and susceptibility of children to COVID-19

Factor	Theory
ACE2 receptor	The ACE2 receptor is necessary for viral entry into cells. The development, function, or activity of this protein might be less in children. ACE2 receptors are upregulated in those with COPD or hypertension, which may partially explain more serious disease in those with comorbid conditions.
Role of other viruses	Children are susceptible to a wide variety of viral illnesses. Presence of these viruses on epithelial surfaces can limit infection of SARS-CoV-2 through competition. Also, cross-reactive antibodies resulting from other viral infections, including non-SARS coronaviruses, may be partially protective against SARS-CoV-2.
Reduced exposures	Children may have fewer opportunities than adults to be exposed to the virus or to those with COVID-19. Additionally, compared to adults, children have had less lifetime exposure to toxins such as cigarette smoke and air pollution, factors which may affect the health of an individual's epithelium.
Aging of the immune system	Natural involution of the thymus over time leads to a decline in circulating naïve T cells. Due to this normal process, immune systems in adults are less able to be adaptive than those of children.
Innate versus adaptive immune system	The innate immune system, which acts earlier than the adaptive immune response, is more active in children, and may prevent more serious illness.

Inflammation	There is evidence that the levels of various proinflammatory cytokines are higher in adults. This may mean that adults experience a more pronounced inflammatory response than children with a similar exposure to SARS-CoV-2.

PART 2. SCREENING AND ASSESSMENT

I. SCREENING A CHILD FOR COVID-19

A. Investigate whether the child has had any symptoms of **influenza-like illness (ILI)** - sudden (within 3 days) onset of fever ≥ 38°C and cough or sore throat - for which no other plausible alternative etiology can be considered.

Likewise, determine if the child presents with features compatible with **Severe Acute Respiratory Infection (SARI)**, defined as an acute respiratory infection with onset during the previous 7 days requiring overnight hospitalization. A SARI case must meet the ILI definition AND any of the following:

a) shortness of breath or difficulty of breathing;
b) severe pneumonia of unknown etiology, acute respiratory distress, or severe respiratory disease possibly due to novel respiratory pathogens (such as COVID-19)

Symptoms frequently seen in children with COVID-19 are listed in Table 1 (see above). The two most common manifestations are fever (59.1%) and cough (55.9%).

B. Exposure evaluation

Assess the child's travel history or history of close contact:

1. Evaluate if the child has been in close contact with sick individuals or suspect, probable or positive COVID-19 patients, whether from home or during travel. *Contact* is defined by the WHO as a person who has experienced any one of the following exposures **during the 2 days before and the 14 days after the onset** of symptoms of a probable or confirmed case:
   a. Face-to-face contact with a probable or confirmed case within 1 meter and for at least 15 minutes;
   b. Direct physical contact with a probable or confirmed case;
   c. Direct care for a patient with probable or confirmed COVID-19 disease without using recommended personal protective equipment; OR
   d. Other situations as indicated by local risk assessments

   Note: For confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample that led to confirmation was taken.
2. Take note if the child resides in or has travelled within the last 14 days to areas with localized transmission or local communities under quarantine. Check DOH updates to confirm if the child's community is classified as such. Note also if there is clustering of influenza-like illnesses in the home, neighborhood or area.

C. Clinical evaluation

Assess the child's clinical status, taking note of either rapid progression or worsening symptoms despite compliance with standard treatment and absence of defined etiology.

D. Laboratory evaluation

Ancillary laboratory tests may aid in the screening and triaging of children presenting with symptoms, and may aid in assessing the severity of symptoms and need for further management. (Common laboratory tests and characteristic findings are discussed in part 3 clinical management.)

E. If any of the following: exposure evaluation, clinical evaluation or ancillary laboratory tests (particularly imaging procedures) is positive, **the diagnosis of COVID-19 should be considered** (Figure 1).

F. If none of the features described above is present, the child is considered to have an **Acute Respiratory Infection**. Screen for pre-existing comorbidities contributory to and/or causative

of the current complaint (e.g. asthma, risk factors for aspiration). Take note also of pre-existing immunocompromising conditions that may predispose to a more severe condition (malignancy, congenital immunodeficiencies, HIV/AIDS, severe acute malnutrition, congenital heart/lung/kidney disease, intake of immunosuppressant drugs, etc.). If these exist, assess the need for inpatient care and manage accordingly. If none of these conditions are present, treat the child as having an acute respiratory infection and follow "Home Interventions" guidelines as described in Part 3.

II. CASE DEFINITIONS FOR COVID-19

After screening the child for COVID-19, classify the child according to the case definitions for COVID-19 (see also Appendix A for case definitions).

Table 4. Updated WHO Case Definitions for COVID-19 (07 August 2020)

Category	Criteria
SUSPECT CASE **(two suspected case definitions A or B)**	**A.** A person who meets the <u>clinical</u> **AND** <u>epidemiological</u> criteria: <u>Clinical criteria</u>: 1. Acute onset of fever AND cough; **OR** 2. Acute onset of ANY THREE OR MORE of the following signs or symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status. **AND** <u>Epidemiological criteria</u>: 1. Residing or working in an area with high risk of transmission of the virus: for example, closed residential settings and humanitarian settings, such as camp and camp-like settings for displaced persons, anytime within the 14 days prior to symptom onset; **OR** 2. Residing in or travel to an area with community transmission anytime within the 14 days prior to symptom onset; **OR** 3. Working in health setting, including within health facilities and within households, anytime within the 14 days prior to symptom onset. **B.** A patient with severe acute respiratory illness (SARI: acute respiratory infection with history of fever or measured fever of ≥ 38°C; and cough; with onset within the last 10 days; and who requires hospitalization).
PROBABLE CASE	**A.** A patient who meets clinical criteria above AND is a contact of a probable or confirmed case, or epidemiologically linked to a cluster of cases which has had at least one confirmed case identified within that cluster. **B.** A suspected case (described above) with chest imaging showing findings suggestive of COVID-19 disease* * Typical chest imaging findings suggestive of COVID-19 include the following: • Chest radiography: hazy opacities, often rounded in morphology, with

	peripheral and lower lung distribution • Chest CT: multiple bilateral ground glass opacities, often rounded in morphology, with peripheral and lower lung distribution • Lung ultrasound: thickened pleural lines, B lines (multifocal, discrete, or confluent), consolidative patterns with or without air bronchograms **C.** A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause. **D.** Death, not otherwise explained, in an adult with respiratory distress preceding death AND who was a contact of a probable or confirmed case or epidemiologically linked to a cluster which has had at least one confirmed case identified within that cluster.
CONFIRMED CASE	A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

Source: World Health Organization. Public health surveillance for COVID-19. Interim guidance. 07 August 2020. Accessed at https://www.who.int/publications/i/item/who-2019-nCoV-surveillanceguidance-2020.7.

III. DISEASE SEVERITY CLASSIFICATION CRITERIA

A child for whom the diagnosis of COVID-19 is considered should further be classified according to disease severity. Table 5 lists categories specified in the WHO Clinical Management of COVID-19 (27 May 2020), which have recently been adopted by the Department of Health.

Table 5. COVID-19 Disease Severity

Mild disease		Symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia.
Moderate disease	Pneumonia	Child with clinical signs of non-severe pneumonia (cough or difficulty breathing + fast breathing and/or chest indrawing) and no signs of severe pneumonia. Fast breathing (in breaths/min): • < 2 months: ≥ 60 • 2–11 months: ≥ 50 • 1–5 years: ≥ 40 Adolescent or adult with clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) but no signs of severe pneumonia, including SpO_2 ≥ 90% on room air While the diagnosis can be made on clinical grounds, chest imaging (radiograph, CT scan, ultrasound) may assist in diagnosis and identify or exclude pulmonary complications.
Severe disease	Severe pneumonia	Child with clinical signs of pneumonia (cough or difficulty in breathing) + at least one of the following: • Central cyanosis or SpO_2 < 90%; severe respiratory

		distress (e.g. fast breathing, grunting, very severe chest indrawing); general danger sign: inability to breastfeed or drink, lethargy or unconsciousness, or convulsions • Fast breathing (in breaths/min): ○ < 2 months: ≥ 60 ○ 2–11 months: ≥ 50 ○ 1–5 years: ≥ 40 Adolescent or adult with clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO_2 < 90% on room air While the diagnosis can be made on clinical grounds, chest imaging (radiograph, CT scan, ultrasound) may assist in diagnosis and identify or exclude pulmonary complications.
Critical disease	Acute respiratory distress syndrome (ARDS)	**Onset**: within 1 week of a known clinical insult (i.e. pneumonia) or new or worsening respiratory symptoms.

		Chest imaging: (radiograph, CT scan, or lung ultrasound): bilateral opacities, not fully explained by volume overload, lobar or lung collapse, or nodules. **Origin of pulmonary infiltrates**: respiratory failure not fully explained by cardiac failure or fluid overload. Need objective assessment (e.g. ECG) to exclude hydrostatic cause of infiltrates / edema if no risk factor present. **Oxygenation impairment in adolescents/adults**: a) Mild ARDS: 200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg (with PEEP or CPAP ≥ 5 cmH_2O) b) Moderate ARDS: 100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg (with PEEP ≥ 5 cmH_2O) c) Severe ARDS: PaO_2/FiO_2 ≤ 100 mmHg (with PEEP ≥ 5 cmH_2O) **Oxygenation impairment in children**: note OI and OSI, use OI when available. If PaO_2 not available, wean FiO_2 to maintain SpO_2 ≤ 97%

		to calculate OSI or SpO_2/FiO_2 ratio: • Bilevel (NIV or CPAP) ≥ 5 cmH_2O via full face mask: PaO_2/FiO_2 ≤ 300 mmHg or SpO_2/FiO_2 ≤ 264 • Mild ARDS (invasively ventilated): 4 ≤ OI < 8 or 5 ≤ OSI < 7.5 • Moderate ARDS (invasively ventilated): 8 ≤ OI < 16 or 7.5 ≤ OSI < 12.3 • Severe ARDS (invasively ventilated): OI ≥ 16 or OSI ≥ 12.3
Critical disease	Sepsis	**Adolescents/adults**: acute life-threatening organ dysfunction caused by a dysregulated host response to suspected or proven infection. Signs of organ dysfunction include: altered mental status, difficult or fast breathing, low oxygen saturation, reduced urine output, fast heart rate, weak pulse, cold extremities or low blood pressure, skin mottling, laboratory evidence of coagulopathy, thrombocytopenia, acidosis, high lactate, or hyperbilirubinemia.
		Children: suspected or proven infection and ≥ 2 age-based systemic inflammatory response syndrome (SIRS) criteria, of which one must be abnormal temperature or white blood cell count.
	Septic shock	**Adolescents/adults**: persistent hypotension despite volume resuscitation, requiring vasopressors to maintain MAP ≥ 65 mmHg and serum lactate level > 2 mmol/L. **Children**: any hypotension (SBP < 5th centile or > 2 SD below normal for age) or two or three of the following: altered mental status; bradycardia or tachycardia (HR < 90 bpm or > 160 bpm in infants and heart rate < 70 bpm or > 150 bpm in children); prolonged capillary refill (> 2 sec) or weak pulse; fast breathing; mottled or cool skin or petechial or purpuric rash; high lactate; reduced urine output; hyperthermia or hypothermia
Other complications that have been described in COVID-19 patients include acute, life-threatening conditions such as: acute pulmonary embolism, acute coronary syndrome, acute stroke and delirium. Clinical suspicion for these complications should be heightened when caring for COVID-19 patients, and appropriate diagnostic and treatment protocols available.		

Table Notes:
When PaO_2 is not available, $SpO_2/FiO_2 \leq 315$ suggests ARDS (including in non-ventilated patients).

Oxygenation Index (OI) is an invasive measurement of the severity of hypoxemic respiratory failure and may be used to predict outcomes in pediatric patients. It is calculated as follows: percentage of fraction of inhaled oxygen multiplied by the mean airway pressure (in mmHg), divided by the partial pressure of arterial oxygen (in mmHg). Oxygen saturation index (OSI) is a non-invasive measurement and has been shown to be a reliable surrogate marker of OI in children and adults with respiratory failure. OSI replaces PaO_2 with oxygen saturation as measured by pulse oximetry (SpO_2) in the OI equation.

The SOFA score ranges from 0 to 24 and includes points related to six organ systems: respiratory (hypoxemia defined by low PaO_2/FiO_2); coagulation (low platelets); liver (high bilirubin); cardiovascular (hypotension); central nervous system (low level of consciousness defined by Glasgow Coma Scale); and renal (low urine output or high creatinine). Sepsis is defined by an increase in the sepsis-related SOFA score of ≥ 2 points. Assume the baseline score is 0 if data are not available.

SIRS criteria: abnormal temperature (> 38.5°C or < 36°C); tachycardia for age or bradycardia for age if < 1 year; tachypnea for age or need for mechanical ventilation; abnormal white blood cell count for age or > 10% bands

Source: World Health Organization. Clinical Management of COVID-19. Interim Guidance. 27 May 2020. Accessed at https://www.who.int/publications/i/item/clinical-management-of-covid-19.

PART 3. CLINICAL MANAGEMENT

Since there is no specific antiviral proven to be effective for COVID-19 at this time, management remains focused on providing best supportive care, management of co-existing conditions and treatment of possible bacterial co-infections.

I. PATIENTS WITH MILD SYMPTOMS

COVID-19 testing MAY be done for these children if testing kits are available in the facility, but in settings where kits are limited, priority must be given to those with severe symptoms. The child can then be sent home after the specimen has been collected. In any circumstance that the child's condition deteriorates, or upon the discretion of the physician, advise inpatient management.

Home interventions for children with mild COVID-19

Children with non-severe disease – and in some cases with stable underlying comorbidities – do not require hospital interventions unless there is concern for rapid deterioration or an inability to promptly return to hospital. Laboratory confirmation of COVID-19 is not necessary for patients with mild symptoms because it will not change the management. Home management is recommended and should focus on appropriate supportive treatment, prevention of transmission of the virus to others, as well as monitoring for clinical deterioration, which will eventually prompt inpatient management (See Appendix B Sample Symptom Monitoring Form). Isolation to contain or prevent virus transmission within the household and community should be prioritized. Where feasible, a communication link with health care providers should be made for the duration of the home care until the child's symptoms have completely resolved.

Isolation

- Children should stay at home and try to separate themselves from other people in the household.
- Place the child in a well-ventilated single room (e.g. open windows, use electric fans for ventilation, may use air conditioner if available) ideally with its own bathroom, where feasible.
- Confine activities of the child in his/her room. If not possible, limit shared space and movement of the child in the house.
- Assign one person who is in good health as primary caretaker of the child (see section on *Caregiver*).
- Other household members not caring for the child should stay in a different room, or if not feasible, must always maintain a distance of at least 1 meter from the child.
- Do not allow visitors until the child has completely recovered and has no signs or symptoms of respiratory tract infection.
- The child should use dedicated dishes, drinking glasses, cups, eating utensils, towels, and beddings.
- Children 2 years of age and older should be properly instructed on how to wear a mask. The child's mask should securely cover the nose and mouth. Masks should not be worn when eating or drinking, and should not be touched when worn. All household members should also wear a surgical face mask when in the same room as the child or when interacting inside the home as much as possible.
- Children younger than 2 years old should NOT wear masks due to risk of suffocation. A mask is also not recommended in the following situations: if the child has difficulty breathing when wearing it, if the child has a cognitive or respiratory impairment giving them a difficult time tolerating the mask, if the mask is a possible choking or strangulation hazard, and if wearing a mask causes the child to touch their face more frequently.
- Try to find the right size of mask for your child's face and be sure to adjust it for a secure fit. The regular adult-sized face mask may be too large for a small child. N95 masks are not recommended for children and should be reserved for healthcare workers at increased risk of exposure to COVID-19.
- Children older than 2 years old may use a face shield together with a face mask. Ensure that the use of a face shield does not pose a risk of suffocation for the child. Neonates and children less than 2 years old should NOT use a face shield. Face shields must be thoroughly disinfected using alcohol or detergent solution then air-dried after every use.

- The child and all household members should practice hand hygiene (handwashing or use of hand disinfection) following contact with the child suspected or confirmed to have COVID-19.
- Teach the child to cover his/her mouth and nose during coughing or sneezing using tissue, inner part of the elbow or sleeves, followed by hand hygiene.

Caregiver

- Ideally, assign one person of good health, non-elderly, and with no underlying comorbidities and immunocompromising conditions, to avoid undue risk to the caregiver.
- Caregivers should wear a surgical mask that covers their nose and mouth when in the same room as the patient. DO NOT touch or handle masks during use. Once wet or dirty with secretions, remove the mask WITHOUT touching the front and replace immediately with a dry mask. DO NOT reuse masks. Cloth masks do not provide adequate protection in this setting and should NOT be used.
- Caregiver should use disposable gloves when handling oral or respiratory secretions, stool or urine. Wash and disinfect hands after removing gloves.

Hygiene and Sanitation

- Proper hand washing with soap and water for at least 20 seconds should be performed in these situations:
    - Before and after contact with the child, especially after handling the child's secretions
    - Before and after preparing the child's food / feeding the child
    - After assisting the child in using the toilet or diaper-changing, and after bathing the child
    - If hands are visibly dirty
- Use disposable paper towels or clean cloth towels (with frequent replacements) to dry hands.
- Avoid direct contact with the child's secretions and stool.
- The toilet should be flushed with the lid down to prevent droplet splatter and aerosol clouds.
- Clean and disinfect surfaces frequently touched in the room as well as toilet surfaces using regular household soap or detergent. Ensure cleaning agents are properly labeled and stored beyond the child's reach, to prevent accidental ingestion/poisoning.

Laundry and Disposal of Soiled Linen and Diapers

- Waste generated during home care (including diapers, tissue/wipes, etc.) should be placed into a waste bin with a lid in the child's room. The trash bag must be tightly sealed before disposal.
- Do not shake dirty laundry; this minimizes the possibility of dispersing the virus through the air.
- Clothes/beddings/pillows/stuffed toys used by the child must be washed separately.
- Machine washing with warm water and laundry detergent is recommended. If machine washing is not possible, soiled linen can be soaked in hot water and soap in a large drum using a stick to stir and being careful to avoid splashing. The drum should then be emptied, and the linens soaked in 0.05% chlorine for approximately 30 minutes. The laundry should then be rinsed with clean water. If still dirty, soiled linen may be washed thoroughly using regular laundry soap/household detergent and warm water, then allowed to dry under the sun.
- If stools are on surfaces of linen or towels, the stool should be carefully removed with paper towels and immediately safely disposed of in a toilet or latrine. Then the soiled linen or towels should be treated as soiled linens.
- Wear disposable gloves and face masks when handling soiled items. Place all used disposable gloves, face masks, and other contaminated items in a lined container before disposing of them with other household waste.
- Wash hands (with soap and water or an alcohol-based hand sanitizer) immediately after handling these items. Soap and water should be used preferentially if hands are visibly dirty.

Home Therapies

- Specific medications against COVID-19 are still under investigation. Studies are still currently being evaluated, consolidated, and reviewed to ensure that recommendations are evidence-based.
- Antipyretics such as paracetamol may be given to make the febrile child more comfortable. The use of ibuprofen has not been shown to be associated with worse clinical outcomes compared to paracetamol in one study of adult patients with COVID-19. However, more studies are needed to ascertain the safety of ibuprofen in children with COVID-19.
- The child may be prescribed empiric antibiotic treatment according to his or her physician's clinical judgment. Antibiotics should be used rationally based on existing national guidelines for PCAP and respiratory tract infections.
- Home nebulization should be avoided unless the child's physician decides that it is indicated, because the risk of infection transmission via droplet nuclei or aerosols may increase during nebulizer treatments. Use a metered-dose inhaler if necessary.
- While getting essential vitamins and minerals such as Vitamin C, Vitamin D3 and Zinc from supplements may help bolster the immune system, emphasis must be made on providing a balanced diet and proper nutrition, as well as adequate hydration.
- Steam inhalation, or the practice of inhalation of water vapor by leaning over a bowl of boiling water, has been shown to be ineffective in treating and preventing COVID-19. In addition, it has been found to be associated with scald burns.

Emotional and Mental Support

- If the child can comprehend, parents are encouraged to talk to the child about their condition in a way they can understand, giving reassurance that they are being observed closely at home with the supervision of their doctor.
- Limit the family's exposure to news coverage, including social media. Children may misinterpret what they see and hear, and thus can be frightened about something they do not understand.
- Continue with the child's regular routine while under quarantine at home and allow time for learning activities and simple play if the child feels well enough for it. Observe limits in screen time as recommended for the child's age.

Monitoring

- The caregiver should be instructed to record the child's symptoms using the symptom monitoring form (Appendix B), and should notify the healthcare provider if the child's symptoms worsen or if one of the child's contacts develops symptoms. It may be necessary to bring the child to the nearest health care facility for proper assessment if symptoms worsen or if no improvement is seen in 2-3 days at home.

II. PATIENTS WITH MODERATE, SEVERE OR CRITICAL SYMPTOMS

All patients with moderate, severe or critical symptoms should be admitted, would be assumed as having COVID-19 and should be tested for such (see "Diagnostics" below). Alternatively, if the facility is not equipped to handle COVID-19 patients, referral to a COVID-19 referral center must be done.

A. In-patient Management

1. The child should be admitted in the hospital and placed in an isolation room or in a dedicated COVID-19 ward/floor, as soon as possible.
2. A dedicated healthcare worker should be in full Personal Protective Equipment (cap, N95 mask, goggles, face shield, full impermeable gown, gloves, and shoe covers) when handling the patient. Proper donning and doffing of PPEs and infection control measures should be observed at all times.
3. Specimen collection must be performed by a knowledgeable medical worker. Ensure that assistance is available as the child may be uncooperative during the procedure. Collect a nasopharyngeal swab (NPS) and / or an oropharyngeal swab (OPS), and if possible, a lower respiratory tract specimen. Samples

must be sent to the Research Institute for Tropical Medicine (RITM) or to a DOH-accredited laboratory through the proper channels. Case investigation forms (CIF) must be accurately filled out for proper documentation.

4. The WHO recommends standard, contact, and droplet precautions with eye and face protection, with addition of airborne precautions as needed during aerosol-generating procedures.

B. Diagnostics

1. **Molecular-based assays**

Nucleic acid amplification testing using the **reverse transcriptase polymerase chain reaction (RT-PCR)** is the preferred method for diagnosing SARS-CoV-2 infection. Appropriate specimens include samples collected from the upper (pharyngeal swabs, nasal swabs, nasopharyngeal secretions) and/or lower airways (sputum, airway secretions, bronchoalveolar lavage fluid). The Department of Health advices the collection of both nasopharyngeal and oropharyngeal specimens. For patients for whom it is clinically indicated (e.g. those receiving invasive mechanical ventilation), a lower respiratory tract aspirate or bronchoalveolar lavage sample should be collected and tested as a lower respiratory tract specimen.

Among the upper respiratory tract specimens, nasopharyngeal and nasal swabs have the highest sensitivity. In a study by Wang et al. of 1,078 specimens collected from 205 adult patients with confirmed COVID-19 infection, RT-PCR positivity was highest in bronchoalveolar lavage specimens (93%), followed by sputum (72%), nasal swab (63%), pharyngeal swab (32%), feces (29%) and blood (1%). None of the urine specimens tested positive.

A similar study by Yuan et al. of 212 children comparing the viral load in throat and anal swab has shown that 78 of 212 patients were confirmed with SARS-CoV-2 infection according to the positive results obtained from either throat or anal swabs. Of the 78 patients, 17 were positive on anal swabs, 37 were positive on throat swabs, and 24 were positive on both. The RT-PCR positivity rate was 78.2% for throat swabs vs 52.6% for anal swabs.

SARS-CoV-2 preferentially proliferates in type II alveolar cells (AT2) and peak of viral shedding appears 3 to 5 days after the onset of disease. Median duration of viral RNA detection was 20 days and the longest observed duration of viral shedding was 37 days in survivors. Appropriate respiratory specimens should be collected as soon as possible once a suspect COVID-19 case is identified, regardless of the time of symptom onset. A positive test for SARS-CoV-2 confirms the diagnosis of COVID-19. If initial testing is negative but the suspicion for COVID-19 remains, resampling and testing from multiple respiratory tract sites is recommended.

Results of RT-PCR assays may be affected by the adequacy of sample, collection, handling and transport of specimen, and timing of sample collection in relation to symptom onset. Kucirka et al. reported that on day 1 from exposure, the sensitivity of RT-PCR is 0%. Before symptom onset (on the average, day 4 from exposure), the sensitivity is at 33%. On the day of symptom onset (typically day 5 from exposure), the sensitivity is at 62%. This further increases to 80% on the 3rd day of symptoms (or average of day 8 from exposure). Sensitivity decreases to 34% on day 21 of exposure. The sensitivity is highest 3 days after symptom onset on average, or 8 days after exposure.

The timing of RT-PCR testing in infants born to COVID-19 positive mothers is discussed in the Philippine Obstetrical and Gynecological Society (POGS)-Philippine Pediatric Society (PPS) guidelines on the clinical approach to the management of

COVID-19 in pregnancy and the newborn (revised May 7, 2020).

2. **Serologic Tests**

Specific antibodies (IgM and IgG) are produced after SARS-CoV-2 infection and can be detected by a variety of methods from the blood, e.g. lateral flow immunochromatographic assay (LFIA), enzyme linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), etc. Determining unique viral protein targets to reduce cross-reactivity to other coronaviruses is a challenge and can affect test sensitivity and specificity.

According to a Cochrane systematic review by Deeks et al., pooled results for IgG, IgM, IgA, total antibodies and IgG/IgM all showed low sensitivity during the first week from onset of symptoms (less than 30.1%), rising in the second week, and reaching their highest values in the third week. The combination of IgG/IgM had a sensitivity of 30.1% at day 1 to day 7 from the time of exposure, 72.2% at 8 to 14 days, 91.4% at 15 to 21 days, and 96% at 21 to 35 days. There are insufficient studies to estimate sensitivity of tests beyond 35 days post-symptom onset.

A systematic review by Bastos et al. compared the diagnostic accuracy of different methods of serological tests (ELISA, LFIA, and CLIA). The pooled sensitivity of ELISA was 84.3%, of LFIA was 66%, and of CLIA was 97.8% However, the study also reported a high or unclear risk of bias in 98% of the studies, and results were not stratified by the timing of sample collection in relation to symptom onset in 67% of the studies.

At present, it is still unknown whether antibodies persist following infection and whether the presence of antibodies confers protective immunity against future infection.

To date, serologic testing is not recommended as a standalone test for diagnosing COVID-19, and must be done always in conjunction with RT-PCR testing. Rapid point-of-care LFIAs are not recommended due to its low sensitivity and high false negative rates. The laboratory-based immunoassays CLIA and ELISA are the preferred tests for antibody determination, and this is best done on the third week onwards from the onset of symptoms.

Currently, there are several Philippine FDA-registered IgM/IgG antibody tests. The DOH has released guidelines on the use of these serologic tests (see DOH Department Memorandum 2020-0151, Interim Guidelines on Expanded Testing for COVID-19, released 31 March 2020). However, it must be emphasized that these tests are not recommended for use in diagnosing acutely ill patients nor for screening of patients. Their use should be limited mainly for seroprevalence studies in identified populations or areas, and not as standalone tests for the diagnosis of COVID-19.

3. **Ancillary Laboratory Tests**

Preliminary laboratory tests are listed below. The possible results seen in patients with COVID-19 are based on recently published studies. Other tests may be ordered depending on the child's presentation and upon the physician's discretion.

a. **Complete blood count (CBC)**

In the systematic review by Hoang et al., the complete blood count picture seen in children with COVID-19 is as follows:

Table 6. Complete blood count picture in children with COVID-19

Parameter	Mean
Leukocytes (normal range: 4.0-12.0 x 10^3/uL)	7.1 x 10^3/uL
Neutrophils (normal range: 54-62%)	44.4%
Lymphocytes (normal range: 25-33%)	39.9%

Hemoglobin (normal range: 11.5-14.5 g/dL)	12.9 g/dL
Platelets (normal range: 150-450 x 10^3/uL)	272.5 x 10^3/uL

The WBC count is generally normal, however, lymphopenia has been frequently reported, with a median absolute lymphocyte count (ALC) of 1,201 cells/uL (normal ALC 1,500-3,000 cells/uL).

Platelet count may be normal. However, thrombocytopenia has been reported in several case reports of COVID-19 patients presenting with fever, initially assessed to have dengue fever based on positive serology. The presentation of fever and thrombocytopenia is important to recognize in the local setting where dengue fever is common.

b. **Inflammatory markers**

Among the inflammatory markers investigated, procalcitonin, D-dimer and interleukin-6 were found to be elevated. Increased procalcitonin levels may be seen in patients with severe COVID-19 without bacterial co-infection; however, a rapid rise or significantly elevated procalcitonin may also indicate secondary bacterial infection.

Table 7. Inflammatory markers in children with COVID-19

Parameter	Mean
C-reactive protein (CRP) (male normal range: 0.6-7.9 mg/L) (female normal range: 0.5-10 mg/L)	9.4 mg/L
Procalcitonin (normal value: ≤ 0.15 ng/mL)	0.25 ng/mL
Erythrocyte sedimentation rate (ESR) (normal range: 0-20 mm/h)	14.1 mm/h
D-dimer (normal value: < 0.4 mg/L)	0.7 mg/L
Lactate dehydrogenase (normal range: 150-500 U/L)	276.6 U/L
Fibrinogen (normal range: 220-440 mg/dL)	224.2 mg/dL
Interleukin-6 (normal value: ≤ 1.8 pg/mL)	26.1 pg/mL
Ferritin (normal range: 10-60 ng/mL)	51.6 ng/mL
Creatine kinase Normal range for age: 6 months to 2 years (male) 50–292 U/L 6 months to 2 years (female) 38–260 U/L 3–5 years (male) 59–296 U/L 3–5 years (female) 42–227 U/L 6–8 years (male) 54–275 U/L 6–8 years (female) 50–231 U/L 9–11 years (male) 55–324 U/L 9–11 years (female) 52–256 U/L 12–14 years (male) 63–407 U/L 12–14 years (female) 45–257 U/L 15–17 years (male) 68–914 U/L 15–17 years (female) 45–458 U/L adult normal range: 5-130 U/L	197.9 U/L

c. **Arterial Blood Gas (ABG) or pulse oximetry**

Obtaining an arterial blood gas analysis or perfoming pulse oximetry can be done to assess the severity of hypoxemia in patients with pneumonia. An oxygen saturation at room air of < 95% may indicate pneumonia; a value < 90% may indicate severe pneumonia.

d. Other tests to determine alternative etiology or secondary infection

Whenever possible, it is advised to determine an alternative etiology for the patient's symptoms. However, co-infections with COVID-19 have been

documented, and tests that are positive for other bacterial or viral pathogens do not rule out COVID-19.

Consider the following diagnostic tests, depending on the patient's presenting signs and symptoms:

- **Bacterial and fungal cultures** (blood, stool, urine and other appropriate specimens) to test for bacterial or fungal infection, ideally collected before start of antimicrobial or antifungal therapy
- **Dengue NS1 and dengue serologic tests (IgM, IgG)** must be requested for patients who present with symptoms of dengue. Take note, however, that symptoms of dengue and COVID-19 overlap, and that there have been reported cases of confirmed COVID-19 patients with false positive dengue NS1 and serology
- Rapid antigen detection tests for specific bacterial or viral pathogens
- Multiplex respiratory or gastrointestinal panel tests

4. Imaging studies

a. **Chest x-ray**

Chest x-ray is the recommended first line imaging modality in children suspected to have COVID-19 presenting with respiratory symptoms. However, this modality has limited sensitivity and specificity, hence, a negative chest x-ray does not exclude pulmonary involvement in patients with laboratory-confirmed COVID-19, nor does it indicate absence of infection in cases of suspected COVID-19 not yet confirmed by RT-PCR.

Table 8. Chest x-ray findings in children with COVID-19

Classification	Chest x-ray findings	Suggested reporting language
Typical findings of pediatric COVID-19	Bilateral distribution peripheral and/or subpleural ground glass opacities and/or consolidation	Imaging findings are commonly seen with COVID-19 pneumonia in children. Differential diagnosis also includes other viral or atypical pneumonia.
Indeterminate findings of pediatric COVID-19	Unilateral peripheral or peripheral and central ground glass opacities and/or consolidation, bilateral peribronchial thickening and/or peribronchial opacities, or multifocal or diffuse ground glass opacities and/or consolidation without specific distribution	Imaging findings can be seen with COVID-19 pneumonia in children. However, they are nonspecific and differential diagnosis includes both infectious and non-infectious etiologies.
Atypical findings of pediatric COVID-19	Unilateral segmental or lobar consolidation, central unilateral or bilateral ground glass opacities and/or consolidation, single round consolidation i.e., round pneumonia with or without air bronchogram, pleural effusion, or	Imaging findings are atypical or uncommonly reported in cases of COVID-19 pneumonia in children. Recommend consideration of alternative diagnosis.

	lymphadenopathy	
Negative for pediatric COVID-19	No CXR findings suggestive of pneumonia	No CXR findings present to suggest pneumonia (Note: CXR has limited sensitivity for COVID-19, especially in early stages)

b. **Chest CT scan**

Chest CT scan is not recommended as the initial diagnostic test in pediatric patients suspected to have COVID-19. Chest CT scan findings of COVID-19 in the pediatric population are not pathognomonic but may be suggestive of the diagnosis in the appropriate clinical setting. It may be considered in patients with a worsening clinical course who are not responding appropriately to therapy, or to further investigate a specific pulmonary condition.

Table 9. Chest CT scan findings in children with COVID-19

Classification	Chest CT scan findings	Suggested reporting language
Typical findings of pediatric COVID-19	Bilateral, peripheral and/or subpleural ground glass opacities and/or consolidation in lower lobe predominant pattern	Imaging findings are commonly seen with COVID-19 pneumonia in children. Differential diagnosis also includes other viral or atypical pneumonia, hypersensitive pneumonitis, and eosinophilic lung disease. In addition, fungal infection in immunocompromised children when "halo" sign is present.
Indeterminate findings of pediatric COVID-19	Unilateral peripheral or peripheral and central ground glass opacities and/or consolidation, bilateral peribronchial thickening and/or peribronchial opacities, multifocal or diffuse ground glass opacities and/or consolidation without specific distribution, or the "crazy paving" sign	Imaging findings can be seen with COVID-19 pneumonia in children. However, non-specific and differential diagnosis includes infectious and non-infectious etiologies.
Atypical findings of pediatric COVID-19	Unilateral segmental or lobar consolidation, central unilateral or bilateral ground glass opacities and/or consolidation, discrete small nodules, lung cavitation, pleural effusion, or lymphadenopathy	Imaging findings are atypical or uncommonly reported in cases of COVID-19 pneumonia in children. Recommend consideration of alternative diagnosis.
Negative for pediatric COVID-19	No chest CT findings suggestive of pneumonia in children	No CT findings present to suggest pneumonia (Note: CT may be negative in the early stages of COVID-19).

c. **Chest ultrasound**

Chest ultrasound has been used as an alternative to chest x-ray and chest CT scan in the diagnosis of pneumonia in COVID-19 patients due to its ease of use at point-of-care, absence of radiation exposure, and lower cost than CT scan. Chest CT scans performed in COVID-19 patients have been shown to have a strong correlation with chest ultrasound.

The following are ultrasonographic features seen in COVID-19 pneumonia:

- Thickening of the pleural line with pleural line irregularity
- B lines in a variety of patterns including focal, multi-focal, and confluent
- Consolidations in a variety of patterns including mutifocal small, non-translobar, and translobar with occasional mobile air bronchograms
- Appearance of A lines during recovery phase
- Pleural effusions are uncommon

C. Experimental Therapeutic Interventions for Severe Suspected, Probable or Confirmed COVID-19 in Children

Since the SARS-COV-2 is a newly detected virus and COVID-19 cases were only diagnosed in January 2020, there is limited data on the treatment and prevention of this illness in adults and children, and many of the clinical trials are still ongoing. Ethically, new drugs are tested first in adults before testing them in children unless there is an important reason to do so, such as if the disease is only seen in children. Based on observational data in 2,143 children from China, COVID-19 disease is less severe in children compared to adults and has lower mortality rates. Asymptomatic cases were 4.4%, mild cases were seen in 50.9%, moderate cases in 38.8% while severe and critical cases totaled 5.2%. Thus, research in adults should be prioritized before those in children. Antiviral agents are recommended ONLY in severe cases because the majority of children are either asymptomatic or experience mild disease only. Prophylaxis in children is also not recommended at the moment because of this.

The use of investigational drugs should be discussed with the parents or legal guardian of the child, carefully explaining the potential clinical benefits and potential adverse reactions of these investigational drugs. A signed informed consent form should be obtained by the clinician (see Appendix D for sample informed consent form).

Table 10. Experimental Therapeutic Interventions for Severe Suspected, Probable or Confirmed COVID-19 in Children

DRUG	INDICATION	DOSING REGIMEN / DURATION	ADVERSE EFFECTS
ANTIVIRAL			
Remdesivir (for clinical trial or compassionate use only, informed consent from the parent or legal guardian must be sought	Treatment of COVID-19 in hospitalized patients • With SpO_2 < 94% on ambient air or those who require supplemental oxygen • On mechanical ventilator or ECMO (extracorporeal membrane oxygenation)	**3.5 kg to < 40kg:** LD of 5mg/kg on day 1 followed by 2.5mg/kg once daily from day 2 onwards via IV infusion over 30-120 minutes **40 kg and higher:** Adult dose: LD of 200mg on day 1, 100mg from day 2	• Transient elevations in AST or ALT after multiple days of therapy • Mild reversible PT prolongation without INR change or hepatic effects • Drug vehicle is SBECD (sulfobutylether beta-cyclodextrin sodium),

before initiation of treatment)		onwards via IV infusion over 30-120 minutes **Duration:** • 5 days for those not on mechanical ventilation or ECMO and clinically improving • 10 days for those on mechanical ventilation, on ECMO and not clinically improving Formulation: 100mg/vial	which has been associated with renal toxicity • GI symptoms (e.g., nausea and vomiting) **Drug Interaction**: • Coadministration of Remdesivir and Chloroquine or Hydroxychloroquine sulfate is not recommended based on in vitro data showed an antagonistic effect
ADJUNCTIVE TREATMENT			
Dexamethasone	Severe COVID-19 • On mechanical ventilation • ARDS • Shock/cardiac dysfunction • With substantially elevated LDH, D-dimer, IL-6, IL-2R, CRP, and/or ferritin	0.15 mg/kg PO or IV once daily (max. dose: 6mg) Up to 10 days or until discharge Formulation: 4mg/mL, 2mL ampoule	• Adrenal suppression • Immunosuppression (activation of latent infections, secondary infections) • Hyperglycemia • Psychiatric disturbances • Increased blood pressure • Peripheral edema • Myopathy (particularly if used with neuromuscular blocking agents) • Delayed viral clearance (as shown in past outbreaks of SARS and MERS)
Tocilizumab (for clinical trial or compassionate use only, informed consent from the parent or legal guardian must be sought before initiation of treatment)	May be considered in the following: • Severe/critical COVID-19 pneumonia with hyperinflammation or cytokine storm • Rapid worsening of respiratory gas exchange • Age 2 years old and above • No other viral/fungal infection, TB, bacterial sepsis, hepatitis B	8 mg/kg/dose IV, given as 1-hour infusion Additional dose may be given 12 hours after the first if clinical status worsens or with no improvement Maximum dose: 800 mg/dose	• Increased serum cholesterol • Increased ALT and AST • Hypertension • Skin rash • Diarrhea • Leukopenia, neutropenia, thrombocytopenia • Headache • Upper respiratory tract infection, nasopharyngitis

Intravenous immunoglobulin (IVIG)	Multisystem Inflammatory Syndrome in Children (MIS-C)	1-2 g/kg over 8-12 hours	• Hypersensitivity reaction, including anaphylaxis • Infusion reactions: Headache, chills, myalgia, wheezing, tachycardia, lower back pain, nausea, hypotension • Renal failure • Thromboembolism • Aseptic meningitis syndrome • Hemolysis • Transfusion-related acute lung injury • Transmission of infectious pathogens

See Appendix C for the rationale for recommendations.

***Disclaimer*:** Recommendations were made based on the best available evidence. As the knowledge on this disease is still evolving, these recommendations may change as more evidence becomes available.

III. DISCHARGE FROM ISOLATION AND DISCONTINUATION OF TRANSMISSION-BASED PRECAUTIONS

The World Health Organization has presented an updated recommendation reflecting recent findings that persistently positive RT-PCR tests do not necessarily indicate infectiousness (WHO criteria for releasing COVID-19 patients from isolation, 17 Jun 2020). These updates are reflected in the current DOH recommendations which employ a combination of time- and symptom-based strategies, stating that repeat RT-PCR testing is no longer a prerequisite for discontinuation of quarantine, isolation or transmission-based precautions.

A. **Symptomatic patients with confirmed or probable COVID-19** can be discharged from isolation and discontinue transmission-based precautions once the following criteria are fulfilled:
   1. The Clinically recovered based on evaluation by a physician
   2. Absence of COVID-19 symptoms for at least 3 days
   3. Has completed 14 days of isolation, counting from onset of illness

B. **Asymptomatic patients with confirmed or probable COVID-19** can be discharged from isolation and discontinue transmission-based precautions once the following criteria are fulfilled:
   1. Clinically recovered based on evaluation by a physician
   2. Remained symptom-free for 14 days
   3. Has completed 14 days of isolation, counting from date of positive test

C. For patients **suspected of having COVID-19**, CDC recommends that discontinuation of isolation and empiric transmission-based precautions in COVID-19 suspects can be made upon receipt of at least one negative SARS-CoV-2 RT-PCR test performed by a certified laboratory testing facility. If a higher level of clinical suspicion for COVID-19 exists, consider maintaining isolation and transmission-based precautions and

performing a second SARS-CoV-2 RT-PCR. If a COVID-19 suspect case is never tested, the decision to discontinue isolation and transmission-based precautions can be made based upon using the strategy described above for symptomatic confirmed or probable cases.

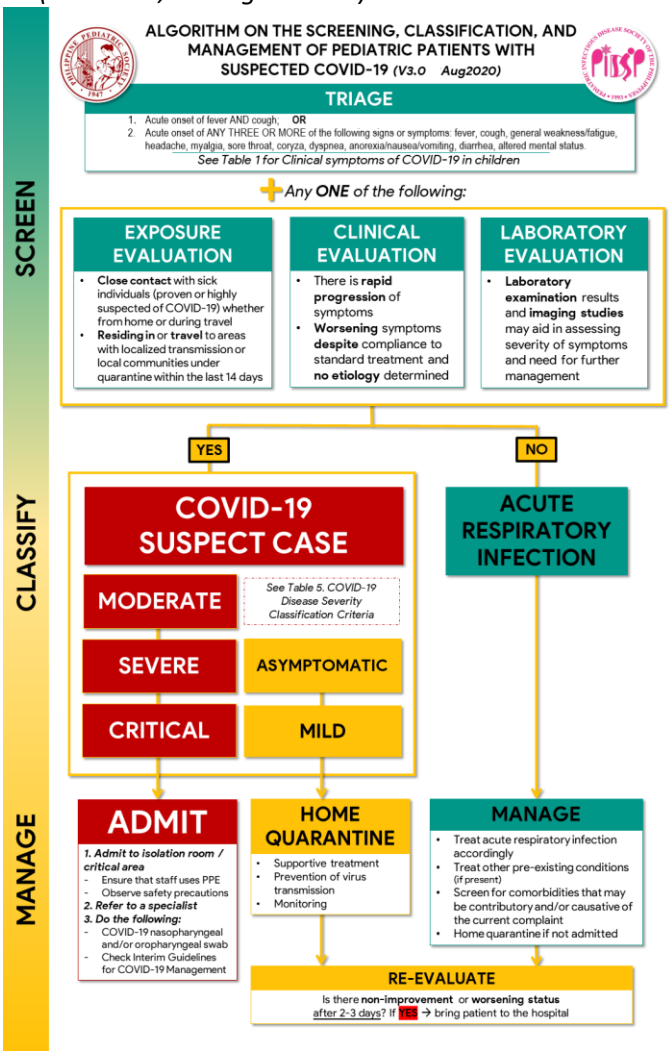
Ultimately, clinical judgement and suspicion of SARS-CoV-2 infection determine whether to continue or discontinue empiric transmission-based precautions.

For symptomatic patients discharged to home prior to completion of the 14 day period, the decision to send the patient home should be made in consultation with the patient's healthcare team and local health authorities. It should include considerations of the home's suitability for and patient's ability to adhere to the isolation recommendations.

After discharge, ensure that the following considerations are kept in mind:

- See section on *Home Interventions* (above) for advise on infection control, hygiene and monitoring in the home setting.
- Follow-up in 2 to 4 weeks after discharge.
- Once fully recovered, ensure that the child's immunizations are up to date. Consult the child's healthcare provider for proper scheduling.

Figure 1. Algorithm on the screening, classification and management of pediatric patients with suspected COVID-19 (Version 3, 20 August 2020)



Appendix A. Case Definitions for Surveillance

Source: World Health Organization. Public health surveillance for COVID-19. Interim guidance. 07 August 2020. Accessed at https://www.who.int/publications/i/item/who-2019-nCoV-surveillanceguidance-2020.7

SUSPECT CASE
(two suspected case definitions A or B)

A. A person who meets the clinical **AND** epidemiological criteria:

Clinical criteria:

1. Acute onset of fever AND cough;

OR

2. Acute onset of ANY THREE OR MORE of the following signs or symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status.

AND

Epidemiological criteria:

1. Residing or working in an area with high risk of transmission of the virus: for example, closed residential settings and humanitarian settings, such as camp and camp-like settings for displaced persons, anytime within the 14 days prior to symptom onset;

OR

2. Residing in or travel to an area with community transmission anytime within the 14 days prior to symptom onset;

OR

3. Working in health setting, including within health facilities and within households, anytime within the 14 days prior to symptom onset.

B. A patient with severe acute respiratory illness (SARI: acute respiratory infection with history of fever or measured fever of ≥ 38°C; and cough; with onset within the last 10 days; and who requires hospitalization).

PROBABLE CASE

A. A patient who meets clinical criteria above AND is a contact of a probable or confirmed case, or epidemiologically linked to a cluster of cases which has had at least one confirmed case identified within that cluster.

B. A suspected case (described above) with chest imaging showing findings suggestive of COVID-19 disease*

* Typical chest imaging findings suggestive of COVID-19 include the following:
  - Chest radiography: hazy opacities, often rounded in morphology, with peripheral and lower lung distribution
  - Chest CT: multiple bilateral ground glass opacities, often rounded in morphology, with peripheral and lower lung distribution
  - Lung ultrasound: thickened pleural lines, B lines (multifocal, discrete, or confluent), consolidative patterns with or without air bronchograms.

C. A person with recent onset of anosmia (loss of smell) or ageusia (loss of taste) in the absence of any other identified cause.

D. Death, not otherwise explained, in an adult with respiratory distress preceding death AND who was a contact of a probable or confirmed case or epidemiologically linked to a cluster which has had at least one confirmed case identified within that cluster.

CONFIRMED CASE

A person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms.

Definitions:

1. Close Contact

Contact is defined by the WHO as a person who has experienced any one of the following exposures **during the 2 days before and the 14 days after the onset** of symptoms of a probable or confirmed case:

a. Face-to-face contact with a probable or confirmed case within 1 meter and for at least 15 minutes;
b. Direct physical contact with a probable or confirmed case;
c. Direct care for a patient with probable or confirmed COVID-19 disease without using recommended personal protective equipment; OR
d. Other situations as indicated by local risk assessments

Note: For confirmed asymptomatic cases, the period of contact is measured as the 2 days before through the 14 days after the date on which the sample that led to confirmation was taken.

2. Influenza-like Illness (ILI)

A condition with sudden onset (within 3 days of presentation and fever should be measured at the time of presentation) of fever ≥ 38°C and cough or sore throat in the absence of other diagnoses

3. Severe Acute Respiratory Infection (SARI)

An acute respiratory infection with onset during the previous 7 days requiring overnight hospitalization. A SARI case must meet the ILI definition AND any one of the following: (a) shortness of breath or difficulty of breathing; (b) severe pneumonia of unknown etiology, acute respiratory distress, or severe respiratory disease possibly due to novel respiratory pathogens (such as COVID-19).

Appendix B. Sample Symptom Monitoring Form

(Adapted from WHO and CDC recommendations by the "PH COVID-19 Health Care Workers' Chat Group" Team in collaboration with PSPHP, and Foundation of Family Medicine Educators)

Name: _____

Quarantine period: _____ to _____

Instructions: Monitor the child twice a day (AM and PM). Put a check (✓) if symptoms are present. For fever, write down the exact temperature of the child.

Week ___	Date _____		Date _____		Date _____		Date _____		Date _____		Date _____		Date _____	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
No symptoms														
Fever (write temp)														
Cough														
Sore throat														
Difficulty of breathing														
Runny nose														
Diarrhea														
Vomiting														
Abdominal pain														
Fatigue														
Headache														
Muscle pains														
Other symptoms 1. 2. 3.														
Medicines given 1. 2. 3.														

Important contact numbers to remember:

DOH COVID-19 Hotline: (02) 894-COVID or (02) 894-26843 or 1555

Provincial/City/Municipality COVID-19 Hotline: (contact details)

Hospital Emergency Room: (name of hospital and contact details)

Pediatrician: (contact details / email address)

Appendix C. Rationale for Recommendations of the Experimental Therapeutic Interventions for Severe Suspected, Probable or Confirmed COVID-19 in Children

Drugs with Anti-SARS-COV-2 Activity

1. Remdesivir

Remdesivir is an intravenous (IV) investigational nucleotide prodrug of an adenosine analog. It has demonstrated in vitro activity against severe acute respiratory syndrome coronavirus 2 (SARS-COV-2, and in vitro and in vivo activity (based on animal studies) against SARS-CoV and the Middle East Respiratory Syndrome (MERS-CoV). Remdesivir binds to the viral RNA dependent RNA polymerase, inhibiting viral replication through premature termination of RNA transcription.

The first published report concerning Remdesivir compassionate use described clinical improvement in 36 of 53 hospitalized patients (68%) with severe COVID-19. On May 1, 2020, the US FDA issued EUA (Emergency use Authorization) of Remdesivir to allow emergency use of the agent for severe COVID-19 (confirmed or suspected) in hospitalized adults and children.

EUA of Remdesivir was based on the preliminary data analysis of the Adaptive COVID-19 Treatment trial (ACTT) last April 29, 2020. The analysis included 1,063 hospitalized patients with advanced COVID-19 and lung involvement, showing that patients who received Remdesivir recovered faster than similar patients who received placebo (31% faster recovery time vs. placebo ($P<0.001$). The median time to recovery was 11 days in patients treated with Remdesivir compared with 15 days in the placebo group. This effect was not observed in patients with mild to moderate disease: time to recovery was 5 days for both the Remdesivir group and the placebo group. For patients with severe disease who constitute approximately 90% of the study population, time to recovery was 12 days in the Remdesivir group and 18 days in the placebo group. However, there was no difference seen in time to recovery in patients who started Remdesivir when they were already on mechanical ventilation or ECMO.

The safety and effectiveness of Remdesivir for COVID-19 treatment have not been fully evaluated in pediatric patients. It is available through a PhilFDA Drug Emergency Use (DEU) Authorization for adults and children and through a compassionate use program for patients aged <18 years with COVID-19.

Recommendation: Remdesivir may be used to treat pediatric patients with severe COVID-19 disease in a clinical trial setting or for compassionate use. Informed consent must be obtained prior to prescribing Remdesivir for pediatric COVID-19 patients.

2. Hydroxychloroquine/Chloroquine

Hydroxychloroquine and chloroquine are antimalarial drugs which were used widely in endemic areas before the era of resistance. These drugs are also used for their immunomodulatory effects of treating autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. In vitro studies have revealed their direct antiviral activity against SARS-COV-2 by inhibiting receptor binding and membrane fusion. Hydroxychloroquine was found to be more potent than chloroquine in antiviral action with an EC50 of 0.72 µM versus 5.47µM for chloroquine. In addition, their strong immunomodulatory effects are hoped to prevent the cytokine storm seen in COVID-19 patients. An article by Gao announced preliminary findings from clinical trials in China involving 100 patients showing that chloroquine prevented exacerbations of pneumonia, promoted virus free conversion, and shortened the disease course. No details were provided on the patients, but this prompted the inclusion of chloroquine in the Chinese National Health Commission Guidelines on Diagnosis, Treatment, and Prevention of Pneumonia caused by COVID-19. Researchers in France published preliminary

results of a non-randomized study using hydroxychloroquine in 20 patients showed a higher reduction of viral carriage on the 6th day compared to controls and more efficient viral reduction when azithromycin was added. A small trial in patients with mild COVID-19 disease was recently published, which showed patients on hydroxychloroquine had a shorter time to recovery for fever and cough as well as a higher proportion of improved pneumonia compared to those in the control group. Azithromycin was added as it showed higher viral clearance in the French study.

Unfortunately, subsequent trials did not show any benefit (time to temperature normalization, duration of hospitalization, and mortality) in the use of hydroxychloroquine in hospitalized patients with COVID 19. The RECOVERY Trial is an open-label, adaptive design RCT conducted in the UK to test a range of drugs, including hydroxychloroquine, for treating patients hospitalized with COVID-19. On June 5, 2020, the chief investigators of the RECOVERY TRIAL in the UK announced the discontinuation of the hydroxychloroquine arm due to a lack of benefit. There was no significant difference between the 1,542 patients who received hydroxychloroquine compared with3,132 patients who received standard of care alone, for the 28-day mortality (25.7% vs. 23.5%, hazard ratio 1.11, 95% CI 0.98–1.26) along with no difference between hospitalization duration. On June 17, 2020, the World Health Organization decided to stop the hydroxychloroquine arm based on new data from the SOLIDARITY and RECOVERY trials and other evidence that showed no benefit for patients with COVID-19. In some trials, increased incidence of gastrointestinal adverse effects was noted, while observational trials reported a higher incidence in ventricular arrhythmias and prolongation of QTc intervals in adults given hydroxychloroquine.

Several ongoing trials in adults, as well as six trials using hydroxychloroquine or chloroquine in children, are continuing. Results from these trials shall determine the role of these aminoquinolines in the management of COVID-19.

Recommendation: Hydroxychloroquine and chloroquine are not recommended to be routinely given to children with COVID-19.

3. Lopinavir/Ritonavir

Lopinavir/ritonavir is a protease inhibitor licensed for use in combination with other antiretroviral drugs for the treatment of HIV-1 in adults, adolescents, and children above the age of 2 weeks. It was previously used in the treatment of SARS-CoV and MERS-CoV infections; this was the initial basis for its use against SARS-CoV-2 as well. However, recent studies have shown no benefit in patients for which this drug was used to treat COVID-19.

A randomized, controlled, open-label trial that evaluated LPV/r in addition to standard care in hospitalized adults with confirmed SARS-CoV-2 infection showed no benefit with LPV/r treatment beyond standard care. Results showed treatment with LPV/r was not associated with a difference from standard care in the time to clinical improvement (hazard ratio for clinical improvement, 1.24; 95%CI 0.90 to 1.72). The RECOVERY Trial, which included LPV/r among the drugs evaluated against COVID-19, also showed no benefits for patients given this treatment. The study compared 1596 patients randomized to LPV/r with 3376 patients randomized to usual care alone. There was no significant difference in the primary endpoint of 28-day mortality (22.1% LPV/r vs. 21.3% usual care (relative risk 1.04 [95% CI 0.91-1.18]) and the results were consistent in the different subgroups of patients. There was also no evidence of beneficial effects on the risk of progression to mechanical ventilation or length of hospital stay. The SOLIDARITY Trial spearheaded by the WHO has also released a statement stating that it has discontinued the trial's hydroxychloroquine and LPV/r arms. The recommendation was based on SOLIDARITY trial interim results and from a review of the evidence from all trials presented at the 1-2 July WHO Summit on COVID-19 research and innovation, which showed LPV/r produce little or no reduction in the mortality of hospitalized COVID-19 patients when compared to standard of care.

Recommendation: Lopinavir/Ritonavir is not recommended to treat children with COVID-19.

Adjunctive Therapy for Suspected and Confirmed COVID-19

1. Dexamethasone

The safety and efficacy of dexamethasone or other corticosteroids as treatment modalities for COVID-19 have not been sufficiently evaluated in the pediatric population. However, data extrapolated from adult studies have shown benefits in survival for severely ill patients.

Preliminary results from the Randomised Evaluation of COVID-19 therapy (RECOVERY) trial in the UK, showed initial findings that dexamethasone reduced the risk of 28-day mortality by 35% in patients on ventilator support (RR 0.65, 95%CI 0.48-0.88, p=0.0003) and by 20% in patients receiving oxygen support (RR 0.80, 95%CI 0.67-0.96, p=0.0021) compared with those receiving usual care. There was no added benefit in patients not requiring respiratory support (RR=1.22, 95%CI 0.86-1.75, p=0.14). The pediatric arm of the RECOVERY trial and other studies in children are ongoing.

Patients with severe COVID-19 can develop a systemic inflammatory response that can lead to lung injury and multisystem organ dysfunction. It has been hypothesized that the anti-inflammatory effects of corticosteroids might prevent or mitigate these complications. However, the benefits from corticosteroids should be balanced with its possible adverse effects such as delayed viral clearance, as seen in the SARS and MERS outbreaks, worsening of clinical outcomes, including secondary bacterial infection and mortality.

In a meta-analysis of 1 small cohort study and 7 RCTs for non-COVID-19, ARDS showed a reduction in mortality (RR 0.72, 95%CI=0.55 to 0.93). In patients with severe COVID-19 without ARDS, there was very low-quality evidence of an increase in mortality with corticosteroid use (HR 2.30, 95%CI=1.00 to 5.29). Observational data from SARS and MERS studies showed very low-quality evidence of a small or no reduction in mortality, as well as a delay in viral clearance.

Recommendation: Dexamethasone may be given for severe COVID-19 in children, specifically those on mechanical ventilation, acute respiratory distress syndrome (ARDS), shock/cardiac dysfunction, substantially elevated LDH, D-dimer, IL-6, IL-2R, CRP, and or/ferritin).

2. Tocilizumab

Tocilizumab is a recombinant humanized anti-IL6 antibody licensed for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, and giant cell arteritis. It is also used for the induction of the rapid reversal of cytokine release syndrome (CRS), a form of cytokine storm caused by chimeric antigen T– cell (CART) immunotherapy. Since it can bind to the IL-6 receptor with high affinity, it can prevent IL-6 from binding to its receptor, rendering it incapable of immune damage to target cells, and alleviating the inflammatory responses.

In a prospective, open-label study that enrolled 63 adult patients, all of the patients received antiretroviral protease inhibitors (lopinavir/ritonavir 45/63 patients, darunavir/cobicistat 18/63 patient) and either tocilizumab IV (8 mg/kg) or tocilizumab SQ (324 mg); within 24 hours after this initial dose, a second dose was administered to 52 of the 63 patients. Following administration of tocilizumab, fever resolved except for one patient, and CRP, ferritin, and D-dimer levels declined. The ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) improved. Tocilizumab administration within 6 days from admission in the hospital was associated with an increased likelihood of survival (HR 2.2 95%CI 1.3–6.7, p<0.05). No moderate or severe adverse events were reported. The overall mortality rate was 11% (7 of 63 patients). No details were provided regarding the rate of secondary infections after tocilizumab use.

Similar results were noted in a retrospective cohort study of 21 hospitalized patients with severe or critical COVID -19 pneumonia who received tocilizumab plus standard of care, body temperatures of all patients returned to normal after

one day. Peripheral oxygen saturation, inflammatory markers, and chest computed tomography (CT) scan showed improvement. Serious adverse events were not noted, and there were no deaths reported.

In another retrospective cohort study, 544 patients (tocilizumab n=179, standard of care n=365), tocilizumab treatment was also associated with a reduced risk of invasive mechanical ventilation or death compared with standard care group however higher cases of new infections (24 (13%) of 179 patients treated with tocilizumab, versus 14 (4%) of 365 patients treated with standard of care alone) such as bacterial, viral, invasive fungal infections, and tuberculosis, hepatitis B and herpes simplex 1 reactivation.

Cases of anaphylaxis, severe allergic reactions, severe liver damage and hepatic failure, and intestinal perforation have been reported after long term tocilizumab administration in patients without COVID-19.

While the results of some studies were promising, there were no studies done in children, and the results of several ongoing clinical trials should be awaited prior to its routine clinical application.

Recommendation: Tocilizumab should not be routinely used for patients with severe pneumonia due to COVID-19 in patients with cytokine storm or hyperinflammation except in a clinical trial setting or for compassionate use. Informed consent must be obtained prior to prescribing tocilizumab for pediatric COVID-19 patients.

3. Intravenous Immunoglobulin

IVIGs are sterile, purified IgG products manufactured from pooled human plasma and usually contain more than 95% unmodified IgG, which has intact Fc-dependent effector functions and only trace amounts of immunoglobulin A or immunoglobulin M. The evidence of the efficacy of IVIG in both the adult and pediatric population is still limited.

The use of IVIG has been reported in a few cohort studies of adult COVID-19 patients, and even less in pediatric patients. There are no randomized controlled trials or efficacy data available. However, IVIG has been widely used in children for the treatment of several conditions, including Kawasaki disease, for which it has generally been shown to be safe.

Reports from the US, Italy, and the UK have demonstrated the use of IVIG in pediatric patients with COVID-19 and multisystem inflammatory syndrome in children (MIS-C), especially those with a Kawasaki disease-like presentation. Most of these patients received high-dose IVIG, and the majority of these patients improved and had recovery of cardiac function.

It has been hypothesized that earlier administration of IVIG, given between 7-10 days after infection, may help interrupt the cytokine storm and enhance immune function. However, more data are needed to support this theory. In a recent pre-print release, a retrospective study of 58 adult cases of severe or critical COVID-19 in Wuhan, China compared the outcomes of patients given IVIG $\leq$48 h of admission, and those started >48 h of admission. There was a statistically significant difference in 28-day mortality between the $\leq$48 h group (23.3%) and in the >48 h group (57.1%) (p=0.009). There was also a significantly shorter length of hospital stay in the $\leq$48 h group (11.50 $\pm$ 1.030) than in the >48 h group (16.96 $\pm$ 1.620 days) (p=0.0055), a shorter length of ICU stay (9.533 $\pm$ 1.089 vs 13.50 $\pm$ 1.632, p=0.0453), and a lower proportion of patients needing mechanical ventilation (6.67% vs 32.14%, p=0.016) in the $\leq$48 h group.

In another multicenter cohort study that included 325 critical adult patients with COVID-19, it showed no difference in the 28-day and 60-day mortality with IVIG in the overall cohort. However, in the subgroup analysis, IVIG was associated with a significant reduction in the 28-day mortality in patients with critical COVID-19. Earlier administration (admission $\leq$7 days) with a high dose (>15 g/d) exhibited a significant reduction of 60-day mortality in these critical patients. However, these

patients received numerous other treatments, which limit the interpretation of findings. These studies support earlier administration of IVIG.

Recommendation: IVIG should not be routinely given for pediatric COVID-19. However, it can be given to patients presenting with multisystem inflammatory syndrome.

Supportive Treatment

1. Zinc

Zinc is an important micronutrient supporting growth and normal function of the immune system. Zinc deficiency results in dysfunction of both humoral and cell-mediated immunity and increases susceptibility to infectious diseases. Children who are living in low-income settings are often undernourished and zinc-deficient. In the Philippines, the prevalence of zinc deficiency in the young population is as follows: pre-school children six months to < 5 years, 21.6%; school children 6 to 12 years, 30.8%; and adolescents 13 to 19 years, 28.9%. Zinc deficient children are at increased risk of restricted growth, and developing diarrheal diseases, as well as respiratory tract infections such as acute lower respiratory tract infections. Zinc supplement given to zinc-deficient children could reduce measles-related morbidity and mortality caused by lower respiratory tract infections. Zinc supplementation has a role in the early cure of pneumonia, and it also decreased the total hospital stay of children with severe pneumonia. It reduced the number of days of acute lower respiratory Tract Infection (ALRI) in Thai children, as well as their stay in the hospital. Zinc supplementation has been shown to reduce the duration and limit the complications of diarrhea in children by increasing intestinal fluid absorption, supporting mucosal integrity, and enhancing immune response. Increasing the concentration of intracellular zinc with zinc-ionophores like pyrithione can efficiently impair the replication of a variety of RNA viruses. In addition, the combination of zinc and pyrithione at low concentrations inhibits the replication of SARS-coronavirus. Previous *in vitro* study has shown that chloroquine, an antimalarial agent, acts as a zinc ionophore in human ovarian cancer cells. Zinc supplement may affect not only COVID-19-related symptoms like diarrhea and lower respiratory tract infection but also on the SARS-CoV-2 virus itself.

Recommendation: Zinc may be given as supportive treatment in pediatric patients with severe COVID-19.

2. Vitamin D

Vitamin D is not only a nutrient but also a hormone, which can be synthesized in our body with the help of sunlight. In addition to its role in maintaining bone integrity, it also stimulates the maturation of many cells, including immune cells. Vitamin D boosts immune defenses and reduces excessive inflammation. Low levels of vitamin D are associated with respiratory tract infections. Children with acute pneumonia may be vitamin D deficient. The mean intake of vitamin D among Filipino school children aged 6-12 years and adolescents aged 13-18 years was far below the Adequate Intake. The overall prevalence of combined vitamin D deficiency (<50 umol/L) and insufficiency (51-75 umol/L) was 48.7% among Filipino adults.

Vitamin D reduces the risk of RTIs through several mechanisms. Vitamin D helps maintain tight junctions, gap junctions, and adherens junctions. Several studies discussed how viruses disturb junction integrity, increasing infection by the virus, and other microorganisms. This action by viruses is an important reason why viral infections progress to pneumonia. Vitamin D enhances natural cellular immunity partly through induction of antimicrobial peptides, including human cathelicidin and defensins, and by reducing the cytokine storm induced by the innate immune system. Cathelicidins exhibit direct antimicrobial activities against gram-positive and gram-negative bacteria, fungi, and enveloped viruses like CoVs. The innate immune system generates both proinflammatory and anti-inflammatory cytokines in response to viral and bacterial infections, as observed in COVID-19 patients. Vitamin D supplementation may be used as an adjunct to antibiotics for the treatment of acute childhood pneumonia. Although there is no direct evidence that Vitamin D will help in COVID-

19 disease, it is recommended because many children are Vitamin D deficient, and enhancing their immunity in respiratory tract infections is deemed beneficial.

Recommendation: Vitamin D_3 may be given as supportive treatment to all pediatric patients with severe COVID-19.

Appendix D. Informed Consent Template

INFORMED CONSENT FOR OFF-LABEL USE OF MEDICATION/S AND/OR USE OF INVESTIGATIONAL DRUG/S FOR COVID-19

Dr. _____ *[Name of physician]* is offering to treat you, your child (in which case the word "you" will refer to "your child" throughout this document), or the person you represent (in which case the word "you" will refer to the person you are representing) with _____ ***[Name of unapproved drug, device, or biologic]*** because you have been clinically diagnosed with probable or confirmed SARS-CoV2 infection, called COVID-19, and there are no standard acceptable drugs at present.

What you should know about this treatment using COVID-19 investigational drug
This treatment has not been approved by the Food and Drug Administration.
For drugs approved for medical use by the Philippine Food and Drug Administration (FDA), the manufacturers' packaging labels, or inserts, state the condition or conditions for which they may be used. Physicians may opt for off-label drug use when convinced that it is for the patient's best interests, and the patient is well-informed and expresses his/her consent for its use, its composition, contraindications, and side effects.
This treatment is considered experimental.
This treatment is not research and you will not be considered a research subject.
Someone will explain this treatment to you.
You give consent to get this treatment.
Whether or not you get this treatment is up to you.
You can choose not to get this treatment.
You can agree to get this treatment now and later change your mind.
If you do change your mind, contact your doctor right away.
Whatever you decide it will not be held against you.
Feel free to ask all the questions you want before you decide.

How long will this treatment last?
We expect that the experimental treatment will last _____ *[days/until a certain event]*.

What happens if I get this treatment?
[Tell the patient what to expect using lay language and simple terms.]

Is there any way this treatment could be bad for me?
[Describe the risks of the treatment]
This treatment may hurt you in ways that are unknown. These may be a minor inconvenience or may be so severe as to cause death.
If you are or become pregnant, this treatment may hurt your baby or your pregnancy in ways that are unknown. These may be a minor inconvenience or may be so severe as to cause death.

Can this treatment help me?
We cannot promise that this treatment will cure you. The goal of this treatment is to _____. ***[Describe the potential benefits of the treatment]***
What else do I need to know?
Efforts will be made to limit your personal information, including medical records, to people who have a need to review this information. Organizations that may inspect and copy your information include appropriate representatives of the _____***[Name of hospital]***, and the FDA or appropriate government agency.
If you are injured or made sick from taking part in this treatment, medical care will be provided. Generally, this care will be billed to you or your insurance. However, it is possible that your insurance will not pay for the care, because the treatment is experimental or with use of investigational drug. Contact your doctor for more information.

Who can I talk to?
If you have questions, concerns, or complaints, or think the treatment has hurt you, you can talk to your doctor at _____ ***[Insert contact information]***

This treatment is subject to oversight by this hospital's Institutional Ethics/ Review Board/ Committee. If you have questions about your rights or any unresolved question, concerns, or complaints, talk to them at _____ ***[Insert contact information]***.

Your signature documents your permission to take part in this experimental treatment.

Signature of person providing consent (patient, legally authorized representative, parent, or guardian)	Date
Printed name of patient	
Printed name of person providing consent, if patient is unable to consent	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

*Informed Consent Form replicated from Philippine Society for Microbiology and Infectious Diseases (PSMID) Interim Guidance on the Clinical Management of Adult Patients with Suspected or Confirmed COVID-19 Infection, *Version 3.1, as of July 20, 2020*

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GUIDELINES

Infection Control and Prevention of COVID-19 Transmission: Interim Recommendations for Schools in the Philippine Setting (as of August 6, 2020)

THIS GUIDANCE AIMS TO SUPPORT PHYSICIANS WHO COLLABORATE WITH SCHOOLS AND GOVERNMENT IN CREATING INFECTION CONTROL POLICIES FOR SCHOOL RE-ENTRY IN THE TIME OF COVID-19 PANDEMIC, WHILE TAKING INTO CONSIDERATION THE OVER-ALL HEALTH OF EVERYONE, BASED ON AVAILABLE EVIDENCE. THE GUIDANCE IS DYNAMIC AND MAY CHANGE DEPENDING ON THE RAPIDLY EVOLVING KNOWLEDGE, DATA, AND UNDERSTANDING OF SARS-COV-2 IN THE COUNTRY.

Important Considerations:

1. Decisions for school resumption must always be in full cognizance of the risks vs. benefits of doing so, with constant re-evaluation of such decision based on ongoing risks and the success of implementing mitigation strategies in each school setting.
2. School policies should be adaptable to changes in response to new information, with administrators willing to refine approaches when specific policies are not working.
3. Strategies to be implemented are those that can be revised and adapted depending on the transmission of the virus in local community and school settings, and on the available resources that can be sustained through the schoolyear, in collaboration with the local public health authorities.
4. Policies should be feasible, practical and appropriate for the developmental stage of the students.
5. Special considerations and accommodations to account for the diversity of the student population should be made, particularly for the vulnerable and disadvantaged populations (i.e., medically fragile, living in poverty, developmentally challenged, and having special health care needs or disabilities) with the goal of safe reopening of schools.
6. Whenever possible and feasible, on-line learning, depending on the class and or subject matter, is the preferred teaching method, over classroom teaching.

BACKGROUND

School closures have been one of the public health measures implemented to control the outbreak of COVID-19 in the Philippines and in the rest of the world. As of this writing, the Philippine government has set, as a condition for the opening of face-to-face classes, the availability of a vaccine against COVID-19. Some countries, however, have resumed, or have plans of resuming face-to-face classes as their cases of COVID-19 decrease. Some advocates have called for the opening of schools in areas in the Philippines where there are no cases, such as in isolated areas and islands. Successful implementation of health safety protocols to reduce the risk of transmission is of paramount importance once face-to-face classes resume.

Studies conducted on COVID-19 in children show that majority of cases are mild and that children are less likely than adolescents and adults to get infected and to have severe disease. Current observations demonstrate that children are less infectious than adults, and the role of children in transmission is still unclear. There is also equivocal evidence on the impact of school closures on the control of the epidemic.

UNESCO identifies the following six key dimensions to assess the readiness of schools to reopen and to provide basis for planning: policy, financing, safe operations, learning, reaching the most marginalized, and well-being/protection. The risk of potential spread of COVID-19 in the school setting must be weighed against the significant academic, social, emotional, and other benefits that schools provide to children.

This document addresses relevant issues on infection control and prevention of transmission of SARS-CoV-2 in schools, and serves as a complement to recommendations from other authorities and policy-making bodies to guide decision-making for school resumption.

Addressing all issues pertinent to infection prevention during school opening is necessary. With regard to risk-prevention, the disease transmission rates at the local community level should be analyzed - where widespread local transmission of COVID-19 is occurring, schools are recommended to remain closed (i.e. risk outweighs benefit). As for areas with no or low disease transmission, as determined by the relevant health authorities such as the Inter-Agency Task Force on Emerging Infectious Diseases (IATF-EID) or the local Epidemiology Bureau, gradual resumption of face-to-face classes may be considered if: the school has appropriate policies and protocols in place for preventing transmission among its students and staff, school staff is well-trained on implementing these health protocols, and children, parents, and the community are well-informed of these plans. Engineering and administrative controls, as well as use of appropriate personal protective equipment, are recommended.

PREVENTING/REDUCING THE RISK OF TRANSMISSION IN THE SCHOOL SETTING

General Considerations:

SARS-CoV-2, the virus causing COVID-19, is primarily spread from person to person via droplet transmission. Thus, strategies to reduce infections are designed to protect against this mode of spread, including hand washing, physical (1-2 meter) distancing, face (nose, mouth and eyes) coverings, and disinfection. Furthermore, the interventions recommended below are most effective when observed altogether to optimize their cumulative benefits for infection prevention and control in the school setting. School authorities are expected to be models and examples of these health practices at all times.

Good Personal Hygiene

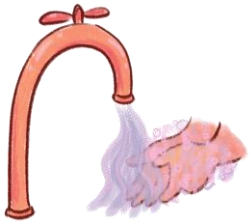
School administrators should promote, demonstrate, and monitor regular hand-washing and positive hygiene behavior. Information dissemination via regular announcements, posting easy-to-understand visual reminders in conspicuous places, encouraging good hand and respiratory hygiene practices, as well as providing information on COVID-19 and prevention of its transmission, should be done in a sustained manner. Spot checkers, i.e. designated personnel at critical locations, may call the attention of students and staff to remind them about hand and respiratory hygiene. Specific class sessions to orient and remind students and staff on infection control measures and good personal hygiene should be done frequently, including demonstrations and return demonstrations of proper procedures.

Note: Infection prevention and good hygiene should extend to the home. Parents should be informed of the practices used in the school and these should also be practiced at home.

Handwashing

- Ensure soap and clean water are available at easily accessible hand washing stations.
- Encourage frequent and thorough hand washing (at least 20 seconds).
- Provide alcohol-based hand sanitizers in toilets, classrooms, halls, and near entrances and exits, where possible.
- Ensure adequate, clean and separate toilets for girls and boys.

Suggested messaging to reinforce handwashing are the following: Wash your hands often, especially before and after eating; after blowing your nose, coughing, or sneezing; after going to the bathroom/ toilets/latrines, and whenever your hands are visibly dirty. If soap and water are not readily available, use an alcohol-based hand sanitizer with at least 60% alcohol. Always wash hands with soap and water, if hands are visibly dirty.



Cough etiquette

- Ensure that everyone covers their mouth and nose when they cough and sneeze, using a tissue, handkerchief, or one's inner elbow.
- Direct everyone to place used tissues straight into the garbage can. Bins should be provided in every classroom for used tissues, and should be emptied regularly (ideally, use hands-free covered bins).
- Avoid touching one's eyes, nose and mouth
- Always wash hands with soap and water, or use a hand sanitizer after coughing and/or sneezing.

Use of Masks

The use of masks is part of a comprehensive package of prevention and control measures that can limit the spread of certain respiratory viral diseases, including COVID-19. Masks can be used either for protection of healthy persons (worn to protect oneself when in contact with an infected individual) or for source control (worn by an infected individual to prevent onward transmission). Notwithstanding, the use of a mask alone is insufficient to provide an adequate level of protection or source control, and other personal and community level measures should also be adopted to suppress transmission of respiratory viruses. Whether or not masks are used, compliance with hand hygiene, physical distancing and other infection prevention and control (IPC) measures are critical to prevent human-to-human transmission of COVID-19.

Indirect evidence for the use of masks (medical or other) by healthy individuals in the wider community from evidence in studies studying household transmission suggest that such individuals would need to be in close proximity to an infected person in a household or at a mass gathering (where physical distancing cannot be achieved), to become infected with the virus.

Cloth face coverings are meant to protect other people in case the wearer is unknowingly infected but does not have symptoms. To some extent, they also protect the wearer's nose and mouth from inhaling virus from an infected person who might not be wearing a mask, or is too close physically. An (acrylic) eye shield protects the wearer from virus from infected people coughing or sneezing towards the wearer. For younger children who cannot tolerate wearing face masks, they may be exempt from doing so provided that physical distancing is observed.

- Masks should be worn at school by students and staff. Masks should also be worn when going outside the school and when riding public transportation. In addition, whenever possible, wearing of face shields should be encouraged to increase protection. Should teachers find it difficult to teach with a face covering, he/she may opt to use a face shield while the students in class maintain physical distancing and wear their own masks.
- Cloth face coverings may serve as substitutes for medical grade masks. Face coverings may be challenging for students (especially younger students) to wear in all-day settings such as schools. Face coverings should be worn by staff and students (particularly older students) as

feasible, and are most essential in times when physical distancing is difficult.

- Individuals should be frequently reminded not to touch the face covering and to wash their hands frequently especially before and after wearing a mask.
- Information should be provided to staff, students, and students' families on proper use, removal, and washing of cloth face coverings. Frequent demonstrations by teachers on proper donning and doffing of face masks would be useful.
- *Note:* Cloth face coverings should NOT be placed on:
    - Children younger than 2 years old
    - Anyone who has trouble breathing or is unconscious
    - Anyone who is incapacitated or otherwise unable to remove the cloth face covering without assistance

Screening Prior to Entry

Health screening (including temperature, symptoms, and exposure history) should be done daily for students and staff in schools that have re-opened. On the other hand, pre-testing (with either serology or PCR) of all children and staff prior to school reopening is not feasible nor recommended at this time, as the results of such tests only demonstrate if the person is infected at that specific moment of testing.

- Any child who has tested positive for SARS-CoV-2, or is experiencing symptoms (see Table 1), or has had close contact with a person who has tested positive or has COVID-19 symptoms, should stay home and not go to school at all. The school authorities, or teacher, are informed by the parent promptly, and medical care and advise is sought for the child though his/her physician. The same applies to teachers and other school staff.
- Parents/caregivers should be provided with a list of symptoms to check before sending their children to school. Prior to school entry, health and safety guidelines include temperature checks and reporting symptoms upon arrival at the school and before entering the classroom. Having students take their own temperatures may be considered in schools with enough equipment to do so, to reduce congestion at entryways.
- Staff should wear appropriate protective gear when taking students' temperature, such as face shields, masks and gloves, and clean thermometers after each use; these materials should be provided by the school or local health authorities.
- Avoid having large groups of students gather at the entrances or exits of the school premises; this may happen when screening is done in these areas. Staggering the time of the start and dismissal of classes of each group should be done.
- School policies regarding temperature screening must balance the practicality of performing these procedures for large numbers of students and staff, the risk of transmission in schools, and the possible lost instructional time when such screenings are conducted.

Physical Distancing

Physical distancing, sometimes referred to as social distancing, reduces the risk of droplet transmission.

- Class sizes should be reduced to accommodate seating students 1-2 meters apart. The group or class to which the student belongs to, serves as his/her cohort, and serves as a "bubble" to which they may have interactions and to which they have a traceable contact history, should the need arise. Classes may also be held at larger venues to accommodate spacing. Open-air venues have been found to entail a lower risk of infection transmission.
- Innovative scheduling, including staggering classes, so that students can come and leave the

school in batches, may decrease the number of exposures of one student to others, and properly enables the suggested physical distancing. Holding shorter face-to-face class durations (i.e. only in the morning, or only in the afternoon, or classes only on alternate days), complemented by online instructions, may be done to accommodate a smaller class size.

- If possible, a separate entrance and exit gate should be designated, with unidirectional paths clearly marked to minimize congestion/crossing of students, staff, and others.
- Parents and non-essential visitors should not be allowed within the school premises unless with special permission from a designated school authority.
- Whenever possible, avoid situations where long lines form, or encourage the appropriate 1-2 meter distancing between students, by placing markings on the floor. Minimize close student contact when outside of classrooms by putting signs and barriers for uni-directional hallways (e.g. put physical guides/tape, on floors or signages on sidewalks to create one-way routes).
- It is suggested that teachers be the ones to move between classrooms, instead of students, to reduce crowding and opportunities for conversation between students during classroom transfers.
- Students who may find it difficult to follow physical distancing instructions (i.e. younger children, children with cognitive disabilities) should not attend physical classes and should be accommodated via other means of instruction.
- There should be a minimum number of teachers and adults in staff rooms, where they should maintain the required 1-2 meter distance, and refrain from mingling with others for more than 15 minutes. Activities that involve mixing of classes and other large activities/gatherings should be avoided. Visitors should be discouraged; one parent/caregiver, per student, may accompany a child to school, as necessary.
- Physical distancing during travel to and from the school should also be maintained, as well as other health protocols relevant to transportation as formulated by authorities.

Mealtimes

It is of utmost importance to make students and teachers realize that the highest risk of infection will occur when people remove their masks and eye shields when it is time to eat. As such, meals are best taken alone, with no one beside and in front of anyone who is eating.

- Meals should preferably be done in the classroom to minimize movement and mixing.
- Each student should have his/her own utensils; utensils should not be shared.
- Meals and drinks should also not be shared. It is preferred that students bring their own food to school.
- The use of water fountains should be avoided to prevent cross-contamination.
- Food preparation and safety standards, as prescribed, should be adhered to.
- Conversation between students, while eating, should be totally avoided, while their masks are off.
- Mealtimes should be staggered to minimize interaction between groups. When meals are distributed, this may be done in the classroom; if a designated area i.e. a canteen is used, the use of the area should be limited to one group to avoid mixing of students.
- Acrylic barriers in the canteen tables may be installed, or an alternate seating arrangement can be designed, with markings on seats and tables, to minimize exposure of students when masks are removed while eating.

Ventilation

There is little evidence that ventilation directly reduces the risk of disease transmission, but many studies suggest that insufficient ventilation increases disease transmission. There is insufficient data to estimate the minimum ventilation requirements in schools, offices and other non-hospital buildings, to prevent the spread of airborne infection. Likewise, no technical specifications and standards for air-conditioning systems to reduce the risk of COVID-19 transmission in indoor spaces are available.

- Classrooms and other school rooms should be properly ventilated, and indoor air should have appropriate egress outdoors by opening windows and doors. Consider utilizing outdoor spaces whenever possible. Do not open windows and doors if doing so poses a safety or health risk (e.g., risk of falling debris, triggering asthma symptoms) for certain children using the facility.
- Guidance from the European Centers for Disease Control (ECDC) include: 1) maintenance of air-conditioning systems according to the manufacturer's current instructions, particularly in relation to the cleaning and changing of filters; 2) energy- saving settings, such as demand-controlled ventilation controlled by a timer or CO2 detectors, should be avoided; 3) direct air flow should be diverted away from groups of individuals to avoid pathogen dispersion and transmission from infected subjects; 4) avoid the use of air recirculation as much as possible.

Cleaning and Disinfection

- Daily cleaning of classrooms, as well as of libraries, cafeterias, toilets, pantries, gymnasiums, auditoriums, lockers, and other facilities in the school should be done.
- If possible, eliminate the need to use frequently-touched surfaces. For example, classroom doors can be left open rather than opening the door when entering and leaving.
- Clean and disinfect frequently touched surfaces, including desks, chairs, other furniture, commonly shared items, and the floor, at least, once daily or as often as possible; this may be done after classes are dismissed so that no students are exposed to disinfectant chemicals; students should NOT be handling disinfectants.
- For disinfection, diluted household bleach (mixing 1 part of household bleach containing 5.25% sodium hypochlorite with 99 parts of water) may be used; leave for 15-30 minutes,

rinse with water and wipe dry afterwards. For metallic surfaces, disinfect with 70% alcohol.

- Proper protective equipment should be worn by disinfecting staff.
- Standard precautions should be adopted when providing first aid to students who may become unwell while in class such as use of gloves and an apron when dealing with blood or body fluids/substances. If a child spreads droplets by sneezing or coughing, clean surfaces with disinfectant wipes immediately.
- Outdoor playgrounds/natural play areas only need routine maintenance, and hand hygiene should be emphasized before and after use of these areas. Play equipment with high-touch surfaces, such as railings, handles, etc., should be cleaned and disinfected regularly.
- Resources and equipment for regular cleaning and disinfection must be accessible to the appropriate staff.

Staff Training

- Develop detailed protocols on hygiene measures, including handwashing, respiratory etiquette, use of protective equipment, cleaning procedures for facilities and safe food preparation practices.
- Train administrative staff and teachers, on implementing physical distancing, screening, school hygiene practices, and increase staff at schools as needed. Teachers and school staff must serve as examples of best practices and must be consistent in observing and implementing infection control measures. A phased opening, wherein implementation problems are assessed and addressed, should be considered.
- Cleaning staff should also be trained on disinfection and equipped with personal protective equipment.

- Whenever feasible, it is encouraged that school personnel are also trained to do contact tracing in coordination with local health authorities, should the need arise.

Special Considerations for the Sick and Vulnerable

- Children with underlying medical conditions that render them vulnerable to infection and disease should not be allowed to attend face-to-face classes, at the present time. Consultation with a medical professional should be done, if the parents or teachers are unsure whether a child with a medical condition is considered vulnerable to COVID-19 or not, should he/she attend school.
- Teachers and staff who are 65 years of age or older, or those 60 to 64 years with underlying medical conditions, or are pregnant, are considered vulnerable and should not attend school.
- The presence of any signs or symptoms or significant exposure to a COVID-19 positive case should dictate that the student/staff not attend school to prevent spread to others.
- When a child is sick, the requirement for a doctor's note to allow return to class is deemed necessary, to reduce the possibility of the child leaving the home when he/she is symptomatic.
- Children or staff who develop symptoms (fever, cough or sore throat) while at school should be isolated in an appropriate area or school clinic with appropriate adult supervision, and collected by a parent as soon as possible.
- Proper education and information is necessary to remove stigma on those who were sick. They should be handled in a sensitive manner so that they do not feel "dirty".
- Creating clear and flexible policies on missing classes may remove the reluctance for taking a leave of absence for both students and staff, as well as enable continuing learning if the need for quarantine happens. Generous opportunities for making up for missed classes may also encourage students and staff to be more forthcoming with regards to screening for symptoms.
- All children and school staff are encouraged to remain updated with regard to their immunization requirements, especially with vaccines against outbreak-prone and respiratory diseases.

Testing, Contact Tracing, and Isolation

- Symptomatic students and staff should be tested for COVID-19 to facilitate contact tracing and quarantine of contacts. Schools must coordinate with the local government unit's Department of Health or city/town health personnel to have access to PCR testing and to initiate contact tracing by the concerned local authorities. Whenever possible, schools are encouraged to do their own contact tracing as regards potential exposures in the school setting.
- Schools should be prepared to follow public health guidance regarding exclusion and isolation protocols for sick children and staff identified at the time of arrival, or throughout the school day. In the event of a confirmed or suspected case of COVID-19 among students or staff, the school should have, in place, guidance on communication protocols, designated physical space for temporary isolation of the involved person prior to going home, and appropriate cleaning and contingency plans for closing classrooms or schools, based on identified cases and in compliance with public health guidelines.
- Return-to-school guidelines for students and staff should follow official policies of the Department of Health. Children and school staff who were previously sick and suspected or confirmed to have COVID-19 may be released from isolation and may return to school provided the following criteria are met:
  - Completed 14 days of isolation or quarantine, **AND**
  - 14 days have passed since the symptoms first appeared, with clinical recovery (afebrile for at least 3 days and without respiratory symptoms), **OR**
  - 14 days after a positive COVID-19 RT-PCR if with no symptoms, **OR**

- 14 days after the last exposure to a known case of COVID-19 case.

Note: Repeat COVID-19 testing is NOT routinely recommended to document recovery and should not be a requirement for school readmission.

Importance of Communication and Transparency

- School authorities must provide accurate, timely, scientific evidence-based information regularly to their staff, students, and parents. Education about the pandemic and infection control measures is essential to successful implementation of these strategies.
- School administrators should inform parents, staff, and local health facilities what measures are being taken by the school. Regular updates of these efforts should be done in a transparent and prompt manner.
- Provide age-appropriate information on COVID-19 and its prevention by hygiene, physical distancing, use of masks and other measures, via television or posters, as feasible based on the resources of the school.
- Communicate and monitor developments with local health authorities, employees, and families regarding cases, exposures, and updates to policies and procedures.

When to Consider Closing

While the decision to open classes is vested upon the Department of Education, school authorities should be empowered to decide if they should temporarily close their facilities, should the need arise, especially in the context of preventing further spread. Taiwan, for example, follows procedures that it used during the H1N1 influenza outbreak. If one or more students or staff in a class is confirmed to have COVID-19, that class is suspended for 14 days; in high school this applies to all classes the person attended. If two or more cases are confirmed in a school, the school is closed for 14 days. If one third of schools in a city or district are closed, then all schools must close. This example may be considered in coordination with national policies on school closures.

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