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JOINT EDITORIAL

CALL FOR EMERGENCY ACTION TO LIMIT GLOBAL TEMPERATURE INCREASES, RESTORE BIODIVERSITY, AND PROTECT HEALTH

Wealthy nations must do much more, much faster

Lukoye Atwoli, Editor in Chief, East African Medical Journal
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Damián Vázquez, Editor in Chief, Pan American Journal of Public Health

Correspondence:
Laurie Laybourn-Langton
Email:
laurie.laybourn@ukhealthalliance.org

The UN General Assembly in September 2021 will bring countries together at a critical time for marshalling collective action to tackle the global environmental crisis. They will meet again at the biodiversity summit in Kunming, China, and the climate conference (COP26) in Glasgow, UK. Ahead of these pivotal meetings, we—the editors of health journals worldwide—call for urgent action to keep average global temperature increases below 1.5°C, halt the destruction of nature, and protect health.

Health is already being harmed by global temperature increases and the destruction of the natural world, a state of affairs health professionals have been bringing attention to for decades.[1] The science is unequivocal; a global increase of 1.5°C above the pre-industrial average and the continued loss of biodiversity risk catastrophic harm to health that will be impossible to reverse.[2,3] Despite the world's necessary preoccupation with COVID-19, we cannot wait for the pandemic to pass to rapidly reduce emissions.

Reflecting the severity of the moment, this editorial appears in health journals across the world. We are united in recognising that only fundamental and equitable changes to societies will reverse our current trajectory.

The risks to health of increases above 1.5°C are now well established.[2] Indeed, no temperature rise is "safe." In the past 20 years, heat related mortality among people aged over 65 has increased by more than 50%.[4] Higher temperatures have brought increased dehydration and renal function loss, dermatological malignancies, tropical infections, adverse mental health outcomes, pregnancy complications, allergies, and cardiovascular and pulmonary morbidity and mortality.[5,6] Harms disproportionately affect the most vulnerable, including among children, older populations, ethnic minorities, poorer communities, and those with underlying health problems.[2,4]

Global heating is also contributing to the decline in global yield potential for major crops, falling by 1.8-5.6% since 1981; this, together with the effects of extreme weather and soil depletion, is hampering efforts to reduce undernutrition.[4] Thriving ecosystems are essential to human health, and the widespread destruction of nature, including habitats and species, is eroding water and food security and increasing the chance of pandemics.[3,7,8]

The consequences of the environmental crisis fall disproportionately on those countries and communities that have contributed least to the problem and are least able to mitigate the harms. Yet no country, no matter how wealthy, can shield itself from these impacts. Allowing the consequences to fall disproportionately on the most vulnerable will breed more conflict, food insecurity, forced displacement, and zoonotic disease—with severe implications for all countries and communities. As with the COVID-19 pandemic, we are globally as strong as our weakest member.

Rises above 1.5°C increase the chance of reaching tipping points in natural systems that could lock the world into an acutely unstable state. This would critically impair our ability to mitigate harms and to prevent catastrophic, runaway environmental change.[9,10]

GLOBAL TARGETS ARE NOT ENOUGH

Encouragingly, many governments, financial institutions, and businesses are setting targets to reach net-zero emissions, including targets for 2030. The cost of renewable energy is dropping rapidly. Many countries are aiming to protect at least 30% of the world's land and oceans by 2030.[11]

These promises are not enough. Targets are easy to set and hard to achieve. They are yet to be matched with credible short and longer term plans to accelerate cleaner technologies and transform societies. Emissions reduction plans do not adequately incorporate health considerations.[12] Concern is growing that temperature rises above 1.5°C are beginning to be seen as inevitable, or even acceptable, to powerful members of the global community.[13] Relatedly, current strategies for reducing emissions to net zero by the middle of the century implausibly assume that the world will acquire great capabilities to remove greenhouse gases from the atmosphere.[14,15]

This insufficient action means that temperature increases are likely to be well in excess of 2°C,[16] a catastrophic outcome for health and environmental stability. Critically, the destruction of nature does not have parity of esteem with the climate element of the crisis, and every single global target to restore biodiversity loss by 2020 was missed.[17] This is an overall environmental crisis.[18]

Health professionals are united with environmental scientists, businesses, and many others in rejecting that this outcome is inevitable. More can and must be done now—in Glasgow and Kunming—and in the immediate years that follow. We join health professionals worldwide who have already supported calls for rapid action.[1,19]

Equity must be at the centre of the global response. Contributing a fair share to the global effort means that reduction commitments must account for the cumulative, historical contribution each country has made to emissions, as well as its current emissions and capacity to respond.

Wealthier countries will have to cut emissions more quickly, making reductions by 2030 beyond those currently proposed[20,21] and reaching net-zero emissions before 2050. Similar targets and emergency action are needed for biodiversity loss and the wider destruction of the natural world.

To achieve these targets, governments must make fundamental changes to how our societies and economies are organised and how we live. The current strategy of encouraging markets to swap dirty for cleaner technologies is not enough. Governments must intervene to support the redesign of transport systems, cities, production and distribution of food, markets for financial investments, health systems, and much more. Global coordination is needed to ensure that the rush for cleaner technologies does not come at the cost of more environmental destruction and human exploitation.

Many governments met the threat of the COVID-19 pandemic with unprecedented funding. The environmental crisis demands a similar emergency response. Huge investment will be needed, beyond what is being considered or delivered anywhere in the world. But such investments will produce huge positive health and economic outcomes. These include high quality jobs, reduced air pollution, increased physical activity, and improved housing and diet. Better air quality alone would realise health benefits that easily offset the global costs of emissions reductions.[22]

These measures will also improve the social and economic determinants of health, the poor state of which may have made populations more vulnerable to the covid-19 pandemic.[23] But the changes cannot be achieved through a return to damaging austerity policies or the continuation of the large inequalities of wealth and power within and between countries.

COOPERATION HINGES ON WEALTHY NATIONS DOING MORE

In particular, countries that have disproportionately created the environmental crisis must do more to support low and middle income countries to build cleaner, healthier, and more resilient societies. High income countries must meet and go beyond their outstanding commitment to provide $100bn a year, making up for any shortfall in 2020 and increasing contributions to and beyond 2025. Funding must be equally split between mitigation and adaptation, including improving the resilience of health systems.

Financing should be through grants rather than loans, building local capabilities and truly empowering communities, and should come alongside forgiving large debts, which constrain the agency of so many low income countries. Additional funding must be marshalled to compensate for inevitable loss and damage caused by the consequences of the environmental crisis.

As health professionals, we must do all we can to aid the transition to a sustainable, fairer, resilient, and healthier world. Alongside acting to reduce the harm from the environmental crisis, we should proactively contribute to global prevention of further damage and action on the root causes of the crisis. We must hold global leaders to account and continue to educate others about the health risks of the crisis. We must join in the work to achieve environmentally sustainable health systems before 2040, recognising that this will mean changing clinical practice. Health institutions have already divested more than $42bn of assets from fossil fuels; others should join them.[4]

The greatest threat to global public health is the continued failure of world leaders to keep the global temperature rise below 1.5°C and to restore nature. Urgent, society-wide changes must be made and will lead to a fairer and healthier world. We, as editors of health journals, call for governments and other leaders to act, marking 2021 as the year that the world finally changes course.

COMPETING INTERESTS:

We have read and understood BMJ policy on declaration of interests and FG serves on the executive committee for the UK Health Alliance on Climate Change and is a Trustee of the Eden Project. RS is the chair of Patients Know Best, has stock in UnitedHealth Group, has done consultancy work for Oxford Pharmagenesis, and is chair of the Lancet Commission of the Value of Death. None further declared.

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This editorial is being published simultaneously in many international journals. Please see the full list here: https://www.bmj.com/content/full-list-authors-and-signatories-climate-emergency-editorial-september-2021

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REVIEW ARTICLE

A REVIEW OF PNEUMONIA IN THE PHILIPPINES

Jaime A. Santos, MD

Former Head, Infectious Disease Section
Philippine Children's Medical Center

Correspondence:
Dr. Jaime A. Santos
Email: jimson2@mac.com

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

This review article gives an overview of pneumonia in the Philippines, with focus on childhood pneumonia. Its primary objective is to provide information on epidemiology, etiology, economic burden, risk factors and prevention of pneumonia. A review of literature was done to gather information about the disease, with emphasis on local data. In the Philippines, pneumonia is the third leading cause of death across all ages and is the most common cause of death among children<5 years of age. A prospective study on Invasive Pneumococcal Disease conducted in the Philippines looked at the incidence of chest x-ray–confirmed pneumonia (N=5,940) in three hospitals over a 2-year period. The highest incidence was seen in those 28 days to <6 months of age at two sites and those 6–12 months of age in another site. Risk factors include not exclusively breastfeeding infants <6 months, undernutrition, zinc deficiency, crowding and exposure to indoor air pollution, low birth weight, poverty and socio-economic factors, presence of underlying comorbidities and immunodeficiency states. CAP ranks number one in processed Philippine Health Insurance (PhilHealth) claims, showing the huge economic burden. Therefore, rationalizing its management with simple standardized guidelines, exclusive breastfeeding for 6 months and continued breastfeeding with appropriate complementary feeding, improving indoor air pollution, and promoting vaccination are effective interventions.

KEYWORDS: *Pneumonia, Pneumococcal disease, Pneumococcal Conjugate Vaccine*

INTRODUCTION

Pneumonia, here defined as inflammation of the lungs, usually secondary to infection, is a major cause of morbidity and mortality worldwide. It is associated with tachypnea, fever, and lung parenchymal involvement evident on either physical examination or chest x-ray as infiltrates. It affects all ages with the greatest burden in children below 5 years of age.[1]

Based on evidence from the 1970s and 1980s, the World Health Organization (WHO) recently revised the classification of childhood pneumonia, in the context of managing patients, into two simple categories: (a) pneumonia with fast breathing and/or chest indrawing and (b) severe pneumonia with any general danger sign. The first requires only home therapy with amoxicillin but the second requires referral and healthcare facility admission for injectable therapy.[2] In the Philippines, among pediatricians, the risk classification system first introduced in the 2004 Joint Pediatric Infectious Disease Society of the Philippines (PIDSP) and Philippine Academy of Pediatric Pulmonology (PAPP) Clinical Practice Guidelines for pediatric community-acquired pneumonia (PCAP) and carried over up to the second 2016 PAPP update is widely used. This risk classification has four categories: (a) PCAP A and (b) PCAP B are non-severe, with PCAP B patients having comorbidities, inability to drink, or malnutrition; (c) PCAP C is severe or moderate risk requiring hospitalization; and (d) PCAP D is very severe or high risk requiring critical care.[3]

MATERIALS AND METHODS

A review of literature was done to gather information about the disease in terms of its epidemiology, etiology, economic burden, risk factors and prevention.

RESULTS AND DISCUSSION

Epidemiology

According to WHO, pneumonia is the single most common cause of death in children, estimated at 1.2 million every year.[4] This represents 18% of all deaths below 5 years of age worldwide.[5] The large majority of deaths occur in low to middle income countries.

Pneumonia and diarrhea together lead to 2 million child deaths annually and these two diseases have been the major focus of attention to reduce childhood morbidity and deaths.[4]

Among countries in Southeast Asia, the Philippines has a relatively high age-standardized death rate of 126.05 per 100,000 population as of 2017.[6] Pneumonia is the third leading cause of death in the country across all ages, next only to cardiac diseases and cancer.[7] In Filipino children below 5 years of age, it is the most common cause of death accounting for about 14% of all causes of mortality.[8]

There is a dearth of studies on the incidence of childhood pneumonia in the Southeast Asian region and in the Philippines. The only prospective study on Invasive Pneumococcal Disease conducted in the Philippines also looked at the incidence of chest x-ray–confirmed pneumonia (using WHO criteria) from 5,940 subjects in three hospitals (Philippine Children's Medical Center [PCMC], Philippine General Hospital [PGH] and Research Institute for Tropical Medicine [RITM]) over a 2-year period.[9] The incidence of chest x-ray–confirmed pneumonia ranged from 633.74 (PCMC) to 1,683.59 (PGH) per 100,000. The highest incidence was seen in those 28 days to <6 months of age at two sites (2,166.16 and 3,891.94 per 100,000) and in those 6-12months of age at the RITM (3,847.52 per 100,000). As the study noted, the results cannot be generalized to the whole country as this was a hospital-based study.[10]

Etiology

Determining the etiology of childhood pneumonia has remarkably been difficult because of certain challenges. These include the use of varying case definitions for pneumonia, lack of pulmonary specimens because very young children do not expectorate, and lung taps are invasive; suboptimal accuracy of assays using non-pulmonary specimens; and difficulty in interpreting results including multiple pathogens identified and equivocal chest x-rays when done.[11] There are several ongoing pneumonia etiology studies. The most extensive is the initially 7-country Pneumonia Etiology Research for Child Health (PERCH) study initiated in 2008 and funded by the Bill & Melinda Gates Foundation.

It aims to update our knowledge of pneumonia etiology using standardized case definitions and methodologies, employing an extensive array of specimens from varying socio-economic settings, and utilizing modern laboratory tests including multiplex real-time polymerase chain reaction for over 30 pathogens.[12,13]

Historical and recent data on etiology of pneumonia, however, indicate that the percentage of bacterial compared with viral etiology tends to be higher in low to middle income countries and that among the bacterial causes, *Streptococcus pneumoniae* tends to predominate together with *Haemophilus influenzae* type b and *Moraxella catarrhalis*. Atypical bacteria causing pneumonia include *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and *Legionella pneumophila* in older patients. Depending on the geographic area, the role of *Staphylococcus aureus*, including community-acquired methicillin-resistant strains, can also be considerable.[14]

In the Philippines, detection of etiology is site-limited and data on viruses are even more limited. The advent of molecular methods has increased the recognition of a variety of viral agents causing pneumonia, but the most important are respiratory syncytial virus (RSV) and influenza virus.[15] In a study on viral etiology of pediatric pneumonia patients in the Cordillera Administrative region from 2009 to 2010, RSV was the most prevalent (of 377 nasopharyngeal/oropharyngeal swabs tested, 106 or 28.1% were positive for a viral isolate, of which 93 were RSV).[16] Viral detection in upper respiratory tract (URT) specimens, however, has low specificity because finding a virus in the URT does not necessarily mean it is the cause of the lower respiratory tract infection.[17] With the outbreak of measles cases in the country and other areas of the world, measles virus has once again become important as a cause. Currently, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has become a significant pathogen causing pneumonia in view of the Coronavirus disease 2019 (COVID-19) pandemic that has also affected the Philippines.[18] It is expected that this will account for a large number of cases when data are assessed in the near future. This is because the burden of COVID-19 pneumonia, based on worldwide experience, continues to increase, and because tools enabling SARS-CoV-2 diagnosis specifically, reverse-transcription polymerase chain reaction (RT-PCR), are now more widely available.[19]

Pathogens similar to those seen in children are encountered in adults in varying proportions and this recognition serves as the basis for guidelines for empiric therapy of adult community-acquired pneumonia (CAP).[20] A recent hospital-based study looked at the etiology of adult CAP (535 subjects) in a rural setting in Leyte, Philippines over a 2-year period. Bacterial detection rate was higher (40%) compared with viral detection (13%). *H. influenzae* (12%) was the most commonly detected bacterium followed by *Klebsiella pneumoniae* (11.6%) and *S. pneumoniae* (10.5%). Influenza virus (5%) was the most commonly detected virus. An underlying disease was found in more than half of patients with pulmonary tuberculosis accounting for 22%.[21] Even in children presenting with severe pneumonia, tuberculosis can play a role as it might be a direct cause of severe pneumonia or might be an underlying comorbidity increasing the risk of secondary bacterial infection.[22] This is true even for pediatric patients. SARS-CoV-2 causes pneumonia in children, though confirmed pediatric COVID-19 cases are smaller in numbers compared to adult COVID-19.[23,24]

Economic Burden

The economic burden of pneumonia in the Philippines is huge. Looking at the Philippine Health Insurance Corporation (PhilHealth) claims that were processed, CAP ranks number one and for severe CAP (CAP III) alone, a total of PHP 10,938,863,732 was paid in 2018.[25]

A local study on the economic burden of PCAP in children 3 months to <19 years of age using the societal perspective looked at both healthcare and non-healthcare costs at two tertiary private hospitals in the Philippines with 2012 as the reference year. Although the exact economic burden was not determined due to lack of specific number of PhilHealth claims in the age group, the figures obtained were considerable.

The estimated healthcare-related cost for PCAP-C was PHP 24,332 to PHP 75,409, and for PCAP-D, PHP 77,460 to PHP 121,301 in those without mechanical ventilation and PHP 97,993 to PHP 141,834 in those with mechanical ventilation. The authors concluded that there is a huge disparity between the PhilHealth case rates and the results of the study, so that the economic burden is much higher than PhilHealth claims data would suggest.[26]

A similar study by the same authors on CAP in adults, concluded that based on the number of PhilHealth claims for 2012 and the estimated healthcare cost, the economic burden of pneumonia in 2012 was PHP 8.48 billion for CAP, moderate risk (MR) and PHP 643.76 million for CAP, high risk (HR). This is again, much higher than figures based on PhilHealth estimates alone would suggest.[27]

Risk Factors

There are known risk factors for developing pneumonia. These include not exclusively breastfeeding infants younger than 6 months, undernutrition, zinc deficiency, crowding and exposure to indoor air pollution, low birth weight, poverty and socio-economic factors, presence of underlying comorbidities, e.g., cardiac or lung disease, immunodeficiency states including human immunodeficiency virus (HIV) infection, neuromuscular and gastrointestinal disorders like reflux.[4,28]

In a questionnaire-based study in Biliran Island, Philippines, a history of asthma, low socio-economic status and long travel time to the healthcare facility estimated by cost-distance analysis were significantly associated with higher occurrence of pneumonia-like episodes.[29] In a secondary analysis of a pneumococcal conjugate vaccine (PCV) trial among children <2 years of age in Bohol, a distance of 12 km from Bohol Regional Hospital, was associated with a decreased hazard ratio for radiographic pneumonia in PCV-vaccinated, compared with the placebo group. For children living 1 km from the hospital, there was little difference.[30]

Underlying viral infections like influenza, RSV and measles can predispose to secondary bacterial pneumonia as previously noted. A recent prospective cohort study done among 3,851 Filipino children below 5 years of age likewise showed that risk for subsequent acute respiratory infections was significantly enhanced after infections with adenovirus, influenza A virus, parainfluenza virus type 4 and rhinovirus species C.[31]

RECOMMENDATIONS

The WHO/UNICEF integrated Global Action Plan for Pneumonia and Diarrhea has specific targets for 2025 for children less than 5 years of age with respect to pneumonia, which are to: (a) reduce mortality from pneumonia to less than three per 1,000 live births, and (b) reduce the incidence of severe pneumonia by 75% compared to 2010 levels. The framework adapted is the Protect, Prevent and Treat framework: (a) protecting children through good health practices, (b) preventing children from becoming ill through universal immunization, HIV prevention and a healthy environment, and (c) treating ill children appropriately.[32]

To these ends, rationalizing the management of pneumonia with simple standardized guidelines, exclusive breastfeeding for 6 months and continued breastfeeding with appropriate complementary feeding, improving indoor air pollution, and promoting vaccination are interventions that have been shown to work. The pandemicity and novelty of COVID-19, however, has required more stringent measures, for example, quarantine, physical distancing and careful use of personal protective equipment. The current vaccine-preventable causes of pneumonia are measles, influenza, *Bordetella pertussis*, *H. influenzae* type b (Hib) and *S. pneumoniae*. The major causes of death from influenza and measles is pneumonia, and the case fatality rate of severe pneumonia in the presence of measles is more than double than that of severe pneumonia without measles.[33]

While measles, pertussis, influenza and Hib vaccines have been used for a longer period of time, their coverage needs to be enhanced. As for the PCV, the recent WHO position continues to recommend its inclusion in national immunization programs because of extensive impact data on pneumonia reduction. Its use should be complementary to control measures, such as appropriate case management, exclusive breastfeeding for the first 6 months of life and reducing known risk factors, such as indoor air pollution and tobacco smoke.[34] All of these recommendations, together with tuberculosis control, HIV prevention strategies and more accurate surveillance and monitoring, are of urgent importance in the Philippine setting.

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CASE REPORT

PURULENT PERICARDITIS SECONDARY TO METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN A PREVIOUSLY HEALTHY INFANT: A CASE REPORT

Aaron G. Tulay, MD [1]
Elizabeth E. Gallardo, MD [1, 2]

[1] Department of Pediatrics, Saint Louis University-Hospital of the Sacred Heart
[2] Department of Pediatrics, Section of Pediatric Infectious Diseases, Saint Louis University-Hospital of the Sacred Heart

Correspondence:
Dr. Aaron G. Tulay
Email: aaront296@gmail.com

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ABSTRACT

Purulent pericarditis with cardiac tamponade caused by community-acquired methicillin-resistant *Staphylococcus aureus* is rare and fatal. There are limited data in children in the current antibiotic era, and available reports usually involve patients with immune dysfunction and prior thoracic instrumentation or has a thoracic focus of infection. Rapid recognition and treatment are paramount in the survival of patients. We report a case of purulent pericarditis with cardiac tamponade secondary to community-acquired MRSA in a previously healthy 10-month-old male infant who presented with fever, pallor, shock, and cardio-respiratory distress. CBC showed leukocytosis with neutrophilia, markedly elevated inflammatory markers, and cardiomegaly on chest radiography. The ECG showed diffuse concave ST-segment elevation, low QRS voltages on precordial leads, and electrical alternans consistent with pericarditis with probable significant pericardial effusion confirmed by 2D echocardiography with note of cardiac tamponade. He was managed effectively with pericardiostomy in combination with a 4-week course of vancomycin. Blood and pericardial fluid culture grew MRSA. This case underscores the organism's lethality and its potential to infect immunocompetent children without predisposing factors. The value of early recognition, prompt initiation of treatment and management is of utmost importance.

KEYWORDS: *Purulent pericarditis, Pericardiostomy, CA-methicillin resistant Staphylococcus aureus, Case report*

INTRODUCTION

Purulent pericarditis is defined as an infection of the pericardium characterized by frank pus in the pericardial space.[1] It is a fatal infection requiring immediate detection and treatment. In the advent of antibiotics, this has become rare with limited epidemiologic data and accounts for only <1% of all reported cases of infectious pericarditis. This classically affects children with a compromised immune system or those with a history of thoracic instrumentation, surgery, and trauma rather than in healthy children. While a number of pediatric literature reports of purulent pericarditis, there is limited data on community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) as the etiology in children.[1-8] There have been studies demonstrating an increase in the incidence of various community-acquired MRSA infections affecting the pediatric population such as soft tissue infections, sepsis, and pneumonia. We report a case of a previously well immunocompetent 10-month-old male infant with purulent pericarditis secondary to a community-acquired methicillin-resistant *Staphylococcus aureus* with characteristics of cardiac tamponade. Specifically, this paper aims to examine its clinical manifestations, emphasize the importance of early recognition, and the benefit of a team approach in its successful management.

CASE REPORT

A previously healthy 10-month-old male infant was admitted in our tertiary hospital presenting with five days history of intermittent febrile episodes with a maximum temperature of 38.9^{o}C with no other associated manifestations such as poor intake, irritability, cough, colds, difficulty of breathing, vomiting, diarrhea, nor skin lesions. Outpatient consultation was done, and he was prescribed with amoxicillin syrup at 30 mg/kg/day for an undisclosed diagnosis. A day before his admission, he manifested with pallor, tachypnea, and poor oral intake.

The patient was born term via normal spontaneous vaginal delivery with good APGAR score and was discharged after 24 hours from birth. Newborn screening result was normal, and he was exclusively breastfed until 6 months of age with introduction of complementary feeding at 7 months of age. He has no previous hospitalizations nor history of recurrent infections. Prior consultations with a medical practitioner were for well-baby check-ups. Patient had his primary series of vaccination at a health center with the last vaccine dose administered 5 months prior to this admission. Interruptions of vaccinations were due to the lockdowns imposed for the control of COVID-19 pandemic. The patient is developmentally at par with age. His household contacts were apparently healthy with no noteworthy recurrent skin lesions. Family history is likewise unremarkable.

On arrival at the ER, the patient was diaphoretic, pale, and in cardiopulmonary distress. He was febrile at 39^{o}C, tachycardic at 193 beats per minute, tachypneic with a respiratory rate of 80 breaths per minute, systolic blood pressure was 80 mmHg, and his oxygen saturation at room air was 95%. Patient is adequately nourished with weight of 8 kg, length of 70 cm (z-score 0), and the mid-upper arm circumference (MUAC) was 13 cm. Head was normocephalic with no scalp lesions, no conjunctival, nasal, and aural discharges, no oral mucosal ulcerations or inflammation, no cervical lymphadenopathy, with no jugular vein distention. Chest findings included subcostal and intercostal retractions with fine bibasal rales. Heart sounds were muffled, with no murmurs and no friction rub. He had no abdominal distention, no hepatosplenomegaly, with normoactive bowel sounds. On further physical examination, extremities were cold and clammy with weak peripheral pulses and a capillary refill time of 3 seconds. Considerations at this point were myopericarditis, community-acquired pneumonia, and septic shock. Initial laboratory workup showed significant leukocytosis (WBC: 25,000/mm^3) with neutrophilic predominance, markedly elevated C-reactive protein (CRP) -249.69 mg/L and procalcitonin -10.87 ng/ml with normal cardiac enzymes. Chest x-ray (Fig.1A) revealed cardiomegaly (CT ratio: 0.63) and minimal streaky densities on the right lower lobe signed out as right lower lobe pneumonia, which was

incongruent with the patient's degree of distress. Electrocardiography (Fig. 2) demonstrated sinus tachycardia, diffuse concave ST-segment elevation, low QRS voltages on precordial leads, and electrical alternans consistent with pericarditis with a probable significant pericardial effusion; cardiac tamponade was also considered at this point. The patient was started on ceftriaxone (61 mg/kg/day q12 hours) and penicillin (150,000 units/kg/day q6 hours); fluid resuscitation at 20 cc/kg was performed, and dopamine drip at 5 mcg/kg/min was initiated. A stat 2D-echocardiography by an on-call pediatric cardiologist showed a low ejection fraction (38%) and confirmed the presence of a moderate circumferential pericardial effusion with right atrial and ventricular collapse (Fig.3) consistent with tamponade physiology. Emergency pericardiostomy (Fig.4) with pericardial drain placement was done for both therapeutic and diagnostic reasons. Intraoperatively, the pericardial sac was noted to be tensed and upon opening, about 70 cc of purulent fluid was initially drained from the pericardial space (Fig.4). The cardiac surface was noted to be pale with fibrin deposits, pericardial tissue samples were taken for histopathologic studies. Resolution of diaphoresis, return of full pulses, normal heart sounds and improvement of tachycardia and tachypnea were observed during the immediate post-operative period. Because of the purulent nature of the effusion, penicillin was discontinued, and clindamycin (37.5 mg/kg/day q8 hours) was added to the antibiotic regimen.

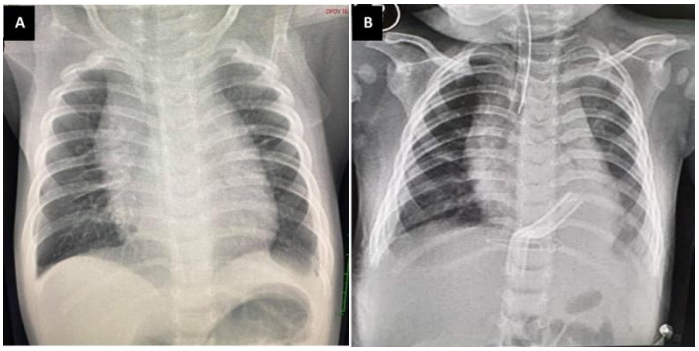

Figure 1: **A**. Initial Chest X-ray showing cardiomegaly with a computed cardiothoracic ratio of 0.63, and minimal streaky densities in the right lower lobe signed out as pneumonia. **B**. Repeat Chest X-ray on APL view on the 3rd post-operative day showed decreased CT ratio (0.56), also visualized was the JP-tube within the pericardial space.

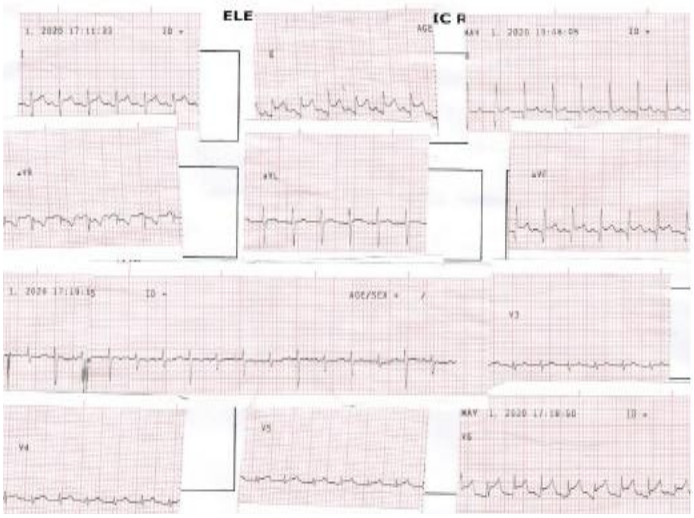

Figure 2: Electrocardiographic changes consistent with pericarditis and pleural effusion in the patient; sinus tachycardia, diffuse concave ST elevation in the lateral and inferior leads; PR depression; alternate beat amplitude of the QRS complex (Electrical Alternans) indicative of pericardial effusion; Low QRS voltage in the precordial leads.

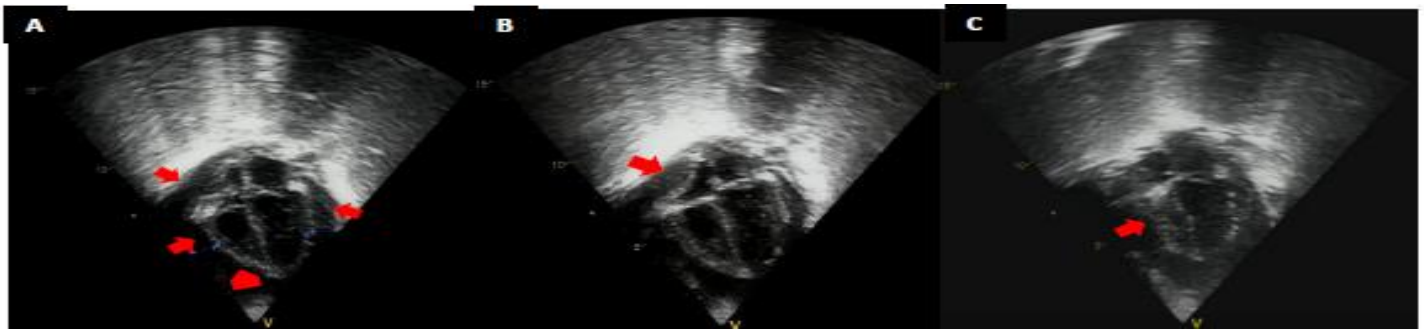

Figure 3: Four chamber view of the patient's echocardiography and tamponade physiology. **A**. Moderate circumferential pericardial effusion (Red arrows). Left ventricle side: 1.52 cm, Right ventricle side: 1.1 cm, Right atrial side: 1 cm. The red arrow in **B** is pointed to a collapse right atrium, while the arrow in **C** is focused on a collapse right ventricle.

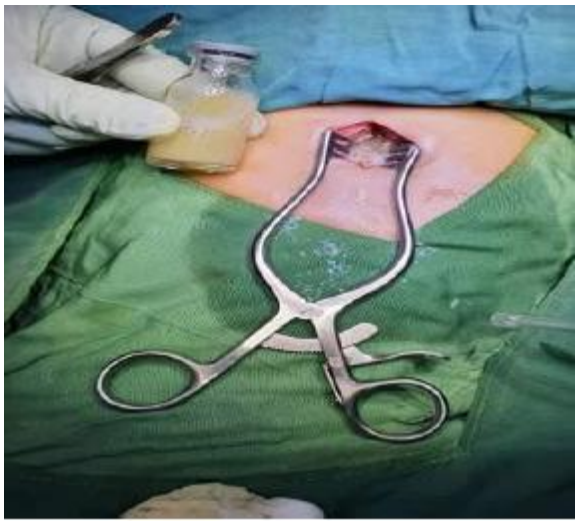

Figure 4: Drainage of a grossly purulent pericardial fluid after pericardiostomy, a JP-tube was placed in the pericardial space for continuous drainage.

Gram stain of the pericardial fluid showed moderate number of gram-positive cocci singly and in clusters and the presence of many wbc/mm3 comprising mostly (82%) of neutrophils, while the pericardial tissue sample demonstrated marked acute and chronic inflammation. Cultures from the pericardial fluid and blood specimens grew methicillin-resistant *Staphylococcus aureus* (MRSA) sensitive to clindamycin, vancomycin, and linezolid. A total of 217 cc of purulent fluid continued to be evacuated from the pericardial drain in the ensuing hospital days. On the 3rd day of admission, cardiac and respiratory rates were down to acceptable limits. A repeat chest x-ray showed a reduction in CT ratio (Fig. 1B), and the electrocardiographic reading was normal; however, the patient remained to have intermittent febrile episodes with temperature of 38-38.6°C. The patient was then referred to the infectious disease service that advised antibiotics be shifted to vancomycin (60 mg/kg/day q6 hours) monotherapy.[9]

The patient was extubated on the 1st postoperative day, weaned from inotrope on 2nd postoperative day, with removal of the pericardial drain on the 12th postoperative day. Repeat laboratory test results on the 12th day of confinement showed a significant reduction in the inflammatory markers – CRP -36.43 mg/L and procalcitonin -0.32 ng/ml, a significantly improved complete blood count, and negative blood cultures. Serial echocardiography demonstrated resolving pericardial effusion, a normal ejection fraction with no chamber compromise (Fig. 5). His course was complicated by healthcare-associated pneumonia (HCAP) with onset on the 24th hospital day manifesting as febrile episodes with recorded maximum temperature of 38.8°C, tachypnea with respiratory rate of 60/min, CRP was elevated at 102.46 mg/L and repeat chest X-ray revealed new bilateral lower lobe infiltrates. HCAP was treated with seven days of cefepime (150 mg/kg/day q8 hours). The patient was discharged after completing four weeks of vancomycin, adhering to the Philippine National Antibiotic Guidelines on treatment of MRSA purulent pericarditis.[9] The parents were advised to adhere to the child's follow-up schedule, update his vaccination and seek immediate medical attention if the patient develops signs and symptoms of constrictive pericarditis. Likewise, observance of proper environmental and personal hygiene and limit exposure to non-family members were instructed. Series of echocardiography done after discharge showed no evidence of constrictive pericarditis. Further follow-up consultations until one year following discharge were devoted for vaccination and well-baby follow-up visits.

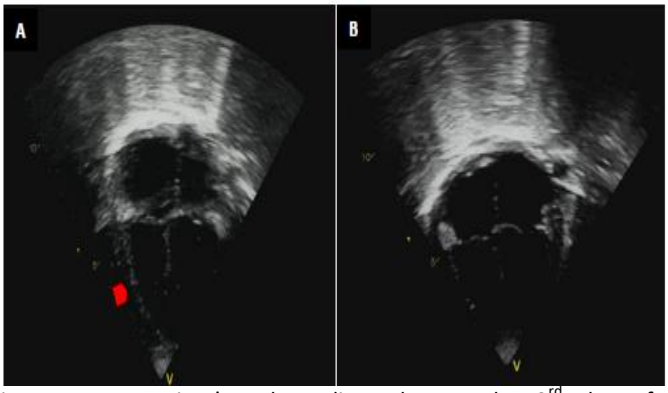

Figure 5: **A.** Patient's echocardiography on the 3rd day after pericardiostomy showing small pericardial effusion (arrow) with no chamber collapse, EF is 68%. **B.** Repeat 2D-echo 25 days post-pericardiostomy demonstrating resolution of pericardial effusion with no evidence of constriction.

DISCUSSION

A diagnosis of pericarditis should be suspected in patients with fever, tachycardia, and tachypnea with evidence of cardiomegaly.[2-3] As in our case, not all tachypnea is secondary to a lung pathology; pericardial disease states should be considered, especially in patients with moderate to severe cardiorespiratory distress with patient's clinical status not proportional to the patient's lung physical examination findings. In infants, purulent pericarditis commonly manifests with signs of sepsis or shock.[11] This condition is often fatal, but if complicated by cardiac tamponade, it becomes a medical emergency, and patient's survival pivots on speedy recognition and initiation of appropriate intervention while anticipating diagnostic test results.[2,6-7] The cardiac tamponade likely occurred in our patient because of an abrupt accumulation of pericardial fluid secondary to the inflamed pericardium. This increases the intrapericardial pressure past the limit of potential pericardial distention causing constraints in ventricular and atrial filling and decreased cardiac output. Pericarditis and cardiac tamponade present atypically among infants, the classic Beck's triad (muffled heart sound, narrow pulse pressure, and distended neck veins) is present only in 35-40% of cases.[2-3] The pericardial friction rub, although a pathognomonic physical examination finding for pericarditis, is often absent in the presence of a significant pericardial effusion.[2,10] The absence of pulsus paradoxus in our patient can be explained by a right-sided tamponade as evidenced by a collapse right atrium and right ventricle, pulmonary hypertension with documented pulmonary artery pressure of 67 mmHg and a left ventricular dysfunction in which the measured ejection fraction was only 38%.[10]

Patients with pericarditis or pericardial effusion may present with normal appearing cardiac shadow while some will have cardiomegaly on chest radiography, and with increasing effusion, the cardiac silhouette may adopt a flask shape, with normal pulmonary vascular markings.[2-6,10] As seen in our patient, electrocardiography usually shows ST segment elevation in the lateral or inferior leads, and PR interval depression.[2-3] The occurrence of low QRS voltages and electrical alternans are observed in the presence of significant effusion.[1] Echocardiography remains to be the most sensitive technique for identifying and locating pericardial effusions while detecting evidence for tamponade, and relatedly, a 2D-echo guided pericardiocentesis is the preferred diagnostic and therapeutic option.[2,10] However, the surgical intervention performed by the team of thoraco-vascular surgery in our patient in draining the effusion is pericardiostomy instead of a pericardiocentesis due to the sizeable circumferential nature of the pericardial effusion with certainty that posteriorly located effusion cannot be approached by the latter. When challenged by a bacterial purulent pericarditis, empiric broad spectrum parenteral antibiotics should be administered immediately and the selection of antibiotics should cover for the potential causative agents including *S. aureus, S. pneumoniae* and other streptococci species*, H. influenzae, N. meningitidis,* and enteric bacilli.[2-3,9] Evidence recommends the use of at least two antibiotics especially when the etiologic organism cannot be identified promptly, however, antibiotics are then directed to the causative organism once culture result is available.[3,9,11] These recommendations were followed in the antibiotic treatment regimen of our patient.

MRSA is frequently linked to hard-to-treat infections. MRSA infections causes significant morbidity, mortality, longer hospital confinement, social and economic burden.[12] Over the past decades, MRSA became an important healthcare-associated pathogen, complicating the care of surgical, chronically ill, and intensive care unit patients. Treatment is challenging, due to its resistance to multiple antibiotics. In the late 1990s, MRSA emerged as a community pathogen, hence the term CA-MRSA.[11-14] It has been found to cause a variety of diseases, ranging from asymptomatic colonization to invasive infections. However, the host and bacterial factors driving these dynamics are poorly understood.[13] CA-MRSA is found in individuals

in the community who are generally healthy. Infections among patients with a MRSA isolate taken less than 48 hours of hospitalization is considered as CA-MRSA.[12] Our patient's infection is community-acquired considering that the cultures grew *S. aureus* taken on admission. Mortality rate associated with this organism is generally from 20-30% and depends largely on the site of infection, however, for untreated purulent pericarditis, fatality is almost certain.[14] Colonization rate for CA-MRSA was reported to be at 3% of all *S. aureus* carriage and mostly identified from the anterior nares, the throat and skin folds.[12-14] We suspect that our patient was already colonized with MRSA and it has gained access into the blood stream via microabrasions from the skin that may have healed and was therefore missed during the physical examination.[8] Confirmation of patient's MRSA carriage was not carried out.

In the current antibiotic era, pediatric purulent pericarditis secondary to MRSA is rare and epidemiologic data is sparse. Once identified, treatment is centered on urgent pericardial drainage and administration of intravenous antibiotics.[9,11] The National Antibiotic Guidelines Committee of the Philippine Department of Health recommends the use of vancomycin at 60 mg/kg/day for 3 to 4 weeks.[9] MRSA purulent pericarditis almost exclusively occurs among individuals with predisposing factors like previous chest trauma, thoracic surgery, intrathoracic focus of infection, and those with immunocompromised states; it is seldom considered in previously healthy children with no risk factors.[2-6,8,11] Our patient did not manifest with signs of overt immunodeficiency. He had an unremarkable past medical, personal, and family histories, and no clues suggestive of a compromised immune function. He had no history of recurrent invasive infections nor hospitalized for any infection, thus considered to be immune competent. He also remained well during the subsequent health visits after he was discharged. Thus, an immunologic investigation was deemed unnecessary. Our case clearly shows the possibility of a drug-resistant organism causing purulent pericarditis in a patient with no predisposing condition in the epoch of antibiotics. To find similar cases, COCHRANE, EBSCO, PUBMED, MEDLINE, HERDIN databases, and locally published journals were searched. Only six similar cases were retrieved; however, of the six, only three children suffered from purulent pericarditis secondary to MRSA with no identifiable risk factors. One of these cases was found in a 2018 case report from the Journal of Pediatric Intensive Care by Sanchez et al. who described an 8-month-old infant presenting with a 10-day history of fever and cardiopulmonary distress and was successfully treated with a 2-week course of vancomycin, 5 days of daptomycin and 4 weeks of oral linezolid, while the other two cases were from an article by Lutmer et al. in the Annals of American Thoracic Society Journal where the authors reported an 8-year old male and a 7-month old female who both presented with fever and cardiopulmonary distress both effectively treated with a combination of vancomycin, gentamicin and rifampin.[4,8] Similar to our patient, pericardial drainage was performed in all three cases, however, a 4-week course of vancomycin proved enough to effectively treat the infection in our case. Unique to our case, however, is the presence of tamponade physiology. The Philippine Pediatric Society (PPS) Disease Registry was also scoured using the ICD for infective pericarditis. The said registry does not provide the specific etiology and type of pericarditis. Since 2006, 106 cases were recorded in the country out of over 4.6 million disease cases registered; from this, only three belong to the less than 1-year old age category.[15] To our knowledge, this could possibly be the first case of purulent pericarditis in a previously healthy child secondary to community-acquired MRSA to be reported in Philippine medical literature.

Our case highlights the virulence of purulent pericarditis caused by community acquired methicillin-resistant *S. aureus*. Our patient did not have any of known risk factors for *S. aureus* infection mentioned in the literatures, he was admitted from the community with a fast onset of shock and cardiopulmonary distress and clinical signs of cardiac tamponade. He survived because of prompt and

uncompromising intervention that included prompt recognition of his condition, appropriate antimicrobial therapy and pericardiostomy. Delay in the diagnosis could have resulted in the patient's demise.

SUMMARY AND CONCLUSION

We presented a case of purulent pericarditis with cardiac tamponade secondary to community-acquired MRSA in a previously healthy 10-month-old male infant presenting with fever and cardiopulmonary distress successfully treated with four-week course of vancomycin and pericardiostomy. Prompt recognition and institution of proper management led to a good outcome.

Purulent pericarditis due to community-acquired MRSA is a lethal disease that can be seen, though rarely even among immunocompetent children without predisposing factors. This highlights the presence and or rise in antibiotic-resistant organisms in the present antibiotic era, thus heralds a clarion call for antimicrobial stewardship. Our case also emphasizes the value of early recognition and prompt initiation of treatment, such as adequate drainage and the use of appropriate antibiotics. A multi-specialty team is also essential to tackle the challenges accompanying such cases.

INFORMED CONSENT

Written consent for publication of this article has been obtained from the mother of our patient.

DISCLOSURE STATEMENT

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

ACCURACY OF NASOPHARYNGEAL ASPIRATE GENEXPERT COMPARED TO GASTRIC ASPIRATE TB CULTURE AND GENEXPERT IN DIAGNOSING PULMONARY TUBERCULOSIS IN PEDIATRIC PATIENTS

Joy L. Morcilla, MD
Ma. Liza Antoinette M. Gonzales, MD
Anna Lisa T. Ong-Lim, MD

University of the Philippines - Philippine General Hospital

Correspondence:
Dr. Joy L. Morcilla
Email: morcilla_joy@yahoo.com

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ABSTRACT

Background: Pulmonary TB in children remains to be a burden in the Philippines. Diagnosis remains to be a challenge for pediatricians due to its paucibacillary nature, difficulty in obtaining specimens, cost of test as well as the varied sensitivity of the different tests available. Gastric aspirate (GA), commonly used for bacteriological diagnosis of pulmonary tuberculosis (PTB) in children, involves an invasive procedure that may cause discomfort and sometimes require admission. Nasopharyngeal aspirate (NPA), on the other hand, can be easily and non-invasively obtained but is currently not a recommended specimen for testing for PTB.

Objectives: This study aims to determine the accuracy of NPA GeneXpert in diagnosing PTB among pediatric patients 0-18 years old with presumptive TB using GA GeneXpert as the initial screening test and GA TB culture as gold standard.

Methodology: This prospective, cross-sectional diagnostic study involved collection of single NPA and GA specimens for GeneXpert and TB culture in 100 patients with presumptive PTB seen at a tertiary government hospital in the Philippines.

Results: Of the one hundred pediatric patients (mean age 6 ± 5.63 years) enrolled, 50 were clinically diagnosed PTB, 16 bacteriologically-confirmed and 34 were not PTB disease. Sensitivity, specificity and predictive values with 95% confidence intervals of the NPA GeneXpert were determined compared to GA GeneXpert and GA culture. Sensitivity, specificity, positive and negative predictive values of the NPA GeneXpert compared to GA GeneXpert were 70%, 96.67%, 70% and 96.67%, respectively. While NPA GeneXpert compared to GA TB culture were 40%, 91.58%, 20% and 96.67%, respectively.

Conclusion: GeneXpert testing on a single NPA specimen is a highly specific and rapid test that can be used to diagnose PTB in pediatric patients, particularly where gastric aspiration or mycobacterial culture is not feasible.

KEYWORDS: *GeneXpert, Gastric Aspirate, Nasopharyngeal Aspirate, TB Culture*

INTRODUCTION

Most cases of tuberculosis (TB) in children occur in TB-endemic countries but the actual burden of childhood TB is unknown. In 2015, the World Health Organization (WHO) estimated that globally, there were 1 million TB cases among children younger than 15 years of age with 210,000 deaths reported as a result of the disease.[1] In 2016, there were approximately 182,200 cases of pulmonary and extrapulmonary TB reported in the Philippines.[2]

The recommended approach in diagnosing pulmonary TB (PTB) in children is based on limited published evidence. A complete history and thorough physical examination remains an important tool. Tuberculin skin testing (TST) and chest X-ray (CXR) are still the most commonly used initial diagnostic examinations and TB culture remains the gold standard. Some of the challenges in the diagnosis of TB in children include the low positivity of cultures due to paucibacillary nature of TB in children, the intricacy of procedures for specimen collection for bacteriologic confirmation of TB (acid fast bacilli (AFB) smear, GeneXpert and TB culture) and high cost of test. The WHO recommends the use of the Xpert MTB/RIF (also known as GeneXpert MTB/RIF or GeneXpert) as the initial test in all children suspected of having pulmonary and extrapulmonary TB.[1,3] The test is useful because of the rapid and early detection of causative mycobacteria in clinical samples.[4]

Gastric aspiration, an invasive procedure that sometimes requires hospital admission, is commonly used for collecting specimens for TB diagnosis in pediatric patients. The gastric aspirate (GA) consists of swallowed respiratory secretions from the stomach. Nasopharyngeal aspiration is a less invasive and an easier method which could be done in the outpatient clinics. In the study of Zar in 2012, NPA GeneXpert had a sensitivity of 56% and specificity of 98.2% compared to culture-positive tuberculosis using either induced sputum or NPA.[5] There are no local data on the use of nasopharyngeal aspiration as a procedure for collection of specimens in children suspected of PTB. Furthermore, there is limited data comparing nasopharyngeal aspirate (NPA) GeneXpert with GA GeneXpert to diagnose PTB in children.

This study aims to determine the diagnostic accuracy of NPA GeneXpert in confirming PTB among pediatric patients compared to GA GeneXpert as the screening test and GA TB culture as the gold standard. The clinico-demographic profile, radiologic and laboratory findings of patients with pulmonary TB were likewise determined.

METHODOLOGY

Study Design and Population

This prospective, cross-sectional study enrolled pediatric patients aged 0-18 years old seen and diagnosed with presumptive TB at the outpatient department (OPD), emergency room (ER) or admitted in the different wards at the University of the Philippines - Philippine General Hospital (UP-PGH) from January 2017 to August 2017. The UP-PGH is a government tertiary hospital with a 1,500-bed capacity. It is the largest government referral center in the country and receives patient referrals from other institutions all over the archipelago.

The Department of Health – National Tuberculosis Control Program (DOH-NTP) guidelines and case definitions were used for the diagnosis of TB.[6] Presumptive TB is diagnosed in any adult or child with clinical signs and/or symptoms suggestive of TB or those with CXR findings suggestive of active TB.[6] In children aged 0-14 years old, a clinical diagnosis of presumptive TB is made when either one of the following is present: (1) at least 3 of the 6 signs and symptoms suggestive of TB (coughing or wheezing for 2 weeks or more, especially if unexplained; unexplained fever of 2 weeks or more, after common causes such as malaria and pneumonia have been excluded; loss of weight/failure to gain weight/weight faltering/loss of appetite; failure to respond to 2 weeks of appropriate antibiotic therapy for lower respiratory tract infection; failure to regain previous state of health 2 weeks after a viral infection or exanthema, and; fatigue, reduced playfulness, or lethargy, or; (2) any one of the above signs and symptoms in a child

who is a close contact of a known TB case. For those 15 years old and above, a presumptive TB case has any one of the of the following: (1) cough of at least 2 weeks duration with or without symptoms (significant and unintentional weight loss, fever, bloody sputum, chest or back pains not referable to any musculoskeletal disorders, easy fatigability or malaise, night sweats, shortness of breath or difficulty of breathing); (2) unexplained cough of any duration in any of the following: close contact of known active TB case or high-risk clinical groups (HIV-AIDS, end-stage renal disease, cancer, connective tissue disease, autoimmune diseases, prolonged systemic steroids). CXR findings suggestive of PTB regardless of age include perihilar or mediastinal lymphadenopathy, parenchymal abnormalities, patchy consolidation, cavitary lesions, fibrotic scarring, atelectasis, calcified nodules and pleural effusion.

Included in the study were newborns suspected to have congenital TB and patients with presumptive PTB with or without associated TB involving other organs (e.g., larynx, pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges). Since gastric aspiration was used as the reference specimen, only those who were unable to expectorate sputum spontaneously were included in the study, regardless of age. Excluded were patients with orofacial abnormalities that prevented the performance of nasopharyngeal aspiration, patients who were hemodynamically unstable, and patients who had received anti-TB treatment for more than 2 weeks except those who have received isoniazid preventive therapy (IPT).

Based on a prevalence of 8% of confirmed TB cases among presumptive pediatric patients and a sensitivity of nasopharyngeal samples in detecting TB at 39.3% with a width of the confidence interval of 0.34, a minimum of 100 subjects were required for this study to achieve 80% power at a level of significance of 5%.[7-9]

Study Procedures

Patient enrolment and Data Collection

Patients diagnosed to have presumptive TB were screened for enrolment using the inclusion and exclusion criteria. General information, demographic data, clinical signs and symptoms, results of the TST, radiographic findings, imaging, and other laboratory findings were recorded in a predesigned data collection form.

Gastric Aspirate and Nasopharyngeal Aspirate Collection

All enrolled patients underwent both gastric aspiration and nasopharyngeal aspiration performed by the primary investigator on the same day using standardized procedures. As recommended by the WHO, only single specimens of GA and NPA were submitted for GeneXpert and TB culture due to unclear advantages in resource-limited settings for testing multiples pecimens.[10] For cases where the specimen was assessed to be inadequate or unsatisfactory, the patient was asked to return for repeat collection and the specimen was submitted and processed using the same method.

Gastric Aspiration. Gastric aspiration was performed in the child early in the morning following an overnight fast of at least 4-6 hours prior to the procedure. With the child kept in recumbent position, the expected distance from nose to stomach was measured and a Fr.8 orogastric tube (OGT) was inserted into the stomach. About 5 to 10ml of gastric contents was aspirated. When the initial attempt to aspirate was unsuccessful, 10 to 20mL of sterile water was instilled and the aspirate was added to the first collection. The minimum acceptable volume of gastric aspirate collected was 10ml. Aspirated gastric fluid was placed in a leak-proof, sterile container and transported to the laboratory at room temperature. When transport will be delayed for more than one hour, the specimen was refrigerated at a temperature of 2-8^{o}C.

Nasopharyngeal aspiration. Nasopharyngeal aspiration was performed using a sterile Fr.8 suction catheter connected to a sterile 10ml syringe. The distance from the tragus of the ear to the tip of the nose was measured and the suction catheter was carefully inserted into the nostril, parallel to the palate. The catheter was rotated gently, and the nasopharyngeal specimen

aspirated while the catheter was slowly withdrawn. This was repeated in the other nostril as necessary. NPA were successfully collected in all subjects. After NPA collection, the suction catheter was flushed with 10ml sterile water and the aspirate was placed in a labelled leak-proof, sterile specimen bottle.[11] In all patients, nasopharyngeal aspiration was performed before gastric aspiration with the two procedures separated by an interval of at least 5 minutes. Patients were monitored during the procedures and for 30 minutes after the procedures were completed for development of any adverse events or complications of the procedure such as intense pain, epistaxis, nasal mucosa trauma, cyanosis or respiratory distress.

Handling, Transport and Processing of Specimens

Both the GA and NPA specimens were sent immediately to the TB-DOTS laboratory in a government infectious disease facility, the San Lazaro Hospital for processing of GeneXpert. Processing of the specimens for GeneXpert was coursed through the UP-PGH Responsive Integrated Multidisciplinary Enhance (UP-PRIME) TB-DOTS where the nurse explained the procedures on specimen transportation to the San Lazaro TB laboratory for GeneXpert. The GA sample for culture was sent to the Microbiology and Infectious Disease Center (MIDC), a private DOH-certified and accredited microbiology laboratory. Both laboratories were not aware of the clinical diagnosis of the patients, and the tests each laboratory performed were interpreted independently of the results of the other's tests.

All GA and NPA specimens were kept in ice and transported in an ice box maintained at 4-8^{o}C temperature to the San Lazaro Hospital TB laboratory for GeneXpert and to the MIDC laboratory for TB culture. All specimens were processed for GeneXpert and culture within 7 days after specimen collection. The adequacy of the specimen was determined by the medical technologist who processed the specimen. In case the specimen was not adequate or unsatisfactory, the primary investigator was informed. The patient was contacted and new specimen was collected. Only one subject was asked to come back for specimen collection due to indeterminate results. TB GeneXpert results were released after 3 days and TB culture results were released after 6-8 weeks.

TB GeneXpert

Xpert MTB/RIF is an automated polymerase chain reaction (PCR) test (that is, a molecular test) utilizing the GeneXpert platform (Cepheid, Sunnyvale, CA, United States). Xpert MTB/RIF is a single test that can detect both *Mycobacterium tuberculosis* complex and rifampicin resistance within 2 hours after starting the assay, with minimal hands-on technical time. PCR amplification and detection of sample are integrated into a single self-enclosed test unit, which is the Xpert MTB/RIF cartridge. Following sample loading, all steps in the assay are automated and contained within the cartridge. In addition, the assay's sample reagent, used to liquefy sputum, is tuberculocidal (that is, it has the ability to kill the TB bacteria) which largely eliminates concerns about biosafety during the test procedures. The test procedure may be used directly on clinical specimens which are obtained after decontamination and concentration. The test material is combined with the reagent, mixed by hand or vortex, and incubated at room temperature for 15 minutes. After incubation, 2ml of the treated sample are transferred to the cartridge, and the run is initiated. Xpert MTB/RIF uses molecular beacon technology to detect rifampicin resistance. The test releases the following results: no TB; TB detected, rifampicin resistance detected; TB detected, no rifampicin resistance detected; TB detected, rifampicin resistance indeterminate; and an invalid result.

In this study, a patient was reported to be a case of bacteriologically diagnosed TB once the specimen tested positive in GeneXpert and/or TB culture. Repeat sample collection and processing of GeneXpert test was done in patients with indeterminate results.[10]

Treatment for TB Disease

All patients with presumptive TB and enrolled in the study were registered with the UP-PRIME TB-DOTS, a DOH accredited center in UP-PGH that facilitates intra- and inter-hospital referral of TB patients for start or continuation of treatment. The patients and their parents or guardians were informed of the GeneXpert and culture results. If the patient was positive for TB on any of the tests, the primary physician or the referring physician was immediately informed for initiation, continuation or revision of treatment. The primary investigator did not interfere with the medical management of the patient. All patients diagnosed to have active TB were referred by the UP PRIME TB-DOTS to the nearest TB-DOTS center upon discharge.

Ethical Consideration

This study underwent approval from the Philippine General Hospital Expanded Hospital Research Office (EHRO) Technical Review Panel and the University of the Philippines Manila Research Ethics Board (UPMREB). The study was conducted in accordance with the principles that have their origin in the Declaration of Helsinki and was consistent with the International Conference on Harmonization Tripartite Guidelines and the Good Clinical Practice Guidelines (ICH-GCP). Written informed consent and assent was obtained by the primary investigator at the OPD clinic for outpatient consults and bedside for admitted patients. Patients enrolled aged 18 years old was asked to sign the informed consent, and 15 to 17 years old was asked to co-sign the informed consent. An assent form was secured for children 12 to 14 years old. Verbal assent was taken for patients 2-7 years old. Parental consent was secured for all patients below 18 years old. The patients and their parents/guardians were informed that they could refuse participation in the study at anytime. All patient information was kept anonymized and confidential. The results of the diagnostic examinations were retrieved by the primary investigator and a copy released only to the parents/guardian of the patient and to the attending physician. Neither the principal investigator nor the co-investigators had any conflicts of interest in relation to the study. This study was partially supported by research funds obtained from the Pediatric Infectious Disease Society of the Philippines (PIDSP), Philippine Pediatric Society (PPS), and UP-PGH–Extended Hospital Research Office (EHRO). The Department of Health-National Center for Disease Prevention and Control (DOH-NTP) and San Lazaro Hospital provided GeneXpert cartridges and processed all specimens for GeneXpert.

Data Analysis

Descriptive statistics were used to summarize the clinical characteristics of all enrolled patients and analyzed using STATA13. Frequencies and proportions were used for nominal variables, mean and SD for interval/ratio variables. Two-way classification table and OpenEpi, a computer application were used to determine and compute the sensitivity, specificity, positive and negative predictive values with 95% confidence intervals (95% CI) of NPA GeneXpert to diagnose PTB using GA GeneXpert results as initial screening test and GA TB culture as the gold standard. Null hypothesis was rejected at 0.05 alpha level of significance.[12]

RESULTS

Demographic Profile

A total of 100 pediatric patients with a mean age of 6 ± 5.63 (SD) years and age range of 0 to 18 years old were enrolled during the study period. Most patients belonged to the age group below 15 years old (86%) and majority were male (64%). Forty-eight (48%) of patients had normal BMI, 29 patients (29%) were severely wasted.

History of TB exposure

Fifty-seven patients (57%) had exposure to known TB, of whom 41 had exposure within the household and 16 patients had exposure outside the household. Among those with household exposure, the primary guardians were the most common TB source whereas outside the household, neighbours were identified as the TB source. Fifty-three (92.98%) patients who had TB exposure were below 15 years old, while 4 (7.02%) patients were 15 years old and above. All 57 patients had daily exposure for more than 3 months duration. Forty-three patients (43%) had no identified TB exposure.

Table 1: Demographic data of Presumptive TB patients

	N=100	%
Sex		
M	64	64.00%
F	36	36.00%
Age		
<15	86	86.00%
>15	14	14.00%
BMI		
Normal	48	48.00%
Wasted	22	22.00%
Severely wasted	29	29.00%
Overweight	1	1.00%
School attended		
None	56	56.00%
Primary	21	21.00%
Secondary	23	23.00%
Tertiary	0	0.00%

Clinical signs and symptoms, TST and CXR of patients 0-14 years old with presumptive PTB

Majority (94.19%) of patients below 15 years of age presented with cough of at least 2 weeks duration. The most common associated symptoms were weight loss (90.70%), followed by unexplained fever of 2 weeks duration or more (77.91%), failure to respond to 2 weeks of appropriate antibiotic therapy for lower respiratory tract infection (30.23%), and fatigue (15.12%). Majority of these patients aged 0-14 years old had perihilar adenopathy (46.51%) on radiologic examination, followed by presence of parenchymal infiltrates (23.26%), pleural effusion (8.13%) and nonspecific pneumonitis (5.81%). Other findings were bronchiectasis, miliary TB and atelectasis. Among these 86 patients, only 26 patients (30%) had positive TST result.

Table 2: Characteristics of Patients 0-14 years old with Presumptive TB

Characteristics	N = 86	%
Clinical Presentation		
Cough 2 weeks or more	81	94.19
Loss of weight/failure to gain/weight faltering/loss of appetite	78	90.70
Fever of 2 weeks or more	67	77.91
Fulfilled 3 out of 6 clinical signs/symptoms suggestive of TB	44	51.16
Failure to respond to 2 weeks of appropriate antibiotic therapy	26	30.23
Fatigue, reduced playfulness, or lethargy	13	15.12
Known TB exposure	53	61.63
Tuberculin skin test		
>10 mm	26	30
<10mm	60	70
Radiologic Chest Findings		
Perihilar adenopathy	40	46.51
Parenchymal infiltrates	20	23.26
Pleural effusion	8	9.30
Pneumonitis	7	8.13
Normal	5	5.81
Bronchiectasis	3	3.49
Miliary	2	2.33
Atelectasis	1	1.16

Clinical signs and symptoms, CXR and exposure of patients >15 years old with presumptive PTB

A total of 14 patients aged 15 years old and above presented with cough of at least 2 weeks duration. The most common associated symptoms were weight loss (78.57%), followed by easy fatigability (71.43%), shortness of breath (71.43%), fever (64.29%) and chest or back pain (64.29%).

None of the enrolled patients presented with bloody sputum. 5 (35.71%) had pleural effusion on the radiologic examination, followed by perihilar adenopathy (21.43%) and presence of parenchymal infiltrates (21.43%). Other findings were nonspecific pneumonitis and pulmonary mass. There were only 4 patients (28.57%) that were identified to have PTB exposure.

Table 3: Characteristics of Patients ≥ 15 years old with Presumptive TB

Characteristics	N=14	%
Clinical Presentation		
Cough 2 weeks or more	14	100
Significant and unintentional weight loss	11	78.57
Easy fatigability or malaise	10	71.43
Shortness of breath or difficulty of breathing	10	71.43
Fever of 2 weeks or more	9	64.29
Chest or back pains	9	64.29
Chest X-ray		
Pleural effusion	5	35.71
Perihilar adenopathy	3	21.43
Cavity	3	21.43
Pneumonitis	1	7.14
Pulmonary mass	1	7.14
Normal	1	7.14
Known TB exposure	4	28.57

Table 4: Final diagnosis of enrolled subjects

Final Diagnosis	<15 years old	≥15 years old	Total
PTB, clinically diagnosed	46 (53%)	4 (29%)	50
PTB, bacteriologically diagnosed	10 (12%)	6 (42%)	16
Not TB disease	30 (35%)	4 (29%)	34
Total	86	14	100

Clinically diagnosed PTB

Fifty patients were clinically diagnosed PTB, of whom46 patients (92%) were below 15 years old and 4 (8%) were 15 years old and above. Of those 46 patients aged below 15 years old, 26 (57%) presented with at least 3 of the 6 clinical signs and symptoms suggestive of TB, had CXR findings compatible with TB and had known TB exposure. Eleven (24%) patients had at least 3 of the 6 clinical signs and symptoms suggestive of TB, had CXR findings compatible with TB, had known TB exposure and a positive TST. Eight (17%) patients had at least 3 of the 6 clinical signs and symptoms suggestive of TB, had CXR compatible with TB and had a positive TST. Only 1 (2%) patient had positive 3 of the 6 clinical signs and symptoms suggestive of TB, known TB exposure, and a positive TST.

All 4 patients 15 years old and above who were clinically diagnosed PTB presented with prolonged cough, 3 (75%) had weight loss and 1 (25%) had extrapulmonary TB (TB osteomyelitis on his foot). All 4 patients had radiologic findings suggestive of PTB but negative GeneXpert and negative TB culture results.

Bacteriologically-confirmed PTB

Out of 100 patients, there were 16 bacteriologically-confirmed TB of which 10 were positive on GeneXpert alone, 3 were positive on TB culture alone, and 3 were positive on both GeneXpert and TB culture.

Of the 13 patients who were positive on GeneXpert, 3 were positive only on NPA GeneXpert, 3 were positive only on GA GeneXpert, while 7 were positive on both NPA and GA GeneXpert.

A total of 6 specimens were positive on TB culture, of which 1 was positive on NPA TB culture but not on GA TB culture, 3 were positive on GA TB culture but not on NPA TB culture and 2 were positive for both GA and NPA TB culture. There were no rifampicin resistant isolates detected on GeneXpert and no drug resistant *Mycobacterium* tuberculosis isolates detected on TB culture. Two patients among these bacteriologically confirmed TB had CNS tuberculosis; one patient had tuberculoma, while another patient had TB meningitis.

Not TB disease

There were 34 patients diagnosed to have no PTB disease, 30 of them were less than 15 years old and 4 were more than 15 years old. These patients were referred back to their referring physician for further work up and management.

Diagnostic accuracy of NPA GeneXpert in detecting PTB

A total of 100 paired NPA and GA specimens were processed for GeneXpert; another 100 GA specimens were submitted for TB culture. Thirteen (13) specimens were positive on GeneXpert and 6 specimens were positive on TB culture. The diagnostic accuracy of NPA GeneXpert compared to GA GeneXpert and GA culture is summarized in Table 5 and Table 6.

The sensitivity of the NPA GeneXpert in detecting PTB compared to GA GeneXpert is 70% (95% CI: 39.68%, 89.22%), specificity is 96.67% (95% CI: 90.65%, 98.86%), positive predictive value is 70% (95% CI: 39.68%, 89.22%) and negative predictive value is 96.67% (95% CI: 90.65%, 98.86%).

Table 5: Diagnostic accuracy of NPA GeneXpert compared to GA GeneXpert as standard screening test

NPA GeneXpert	GA GeneXpert		Total
	Positive	Negative	
Positive	7	3	10
Negative	3	87	90
Total	10	90	100
Sensitivity	70	(95% CI 39.68%, 89.22%)	
Specificity	96.67%	(95% CI 90.65%, 98.86%)	
Positive Predictive Value	70%	(95% CI 39.68%, 89.22%)	
Negative Predictive Value	96.67%	(95% CI 90.65%, 98.86%)	

The sensitivity of the NPA GeneXpert compared to GA TB culture in detecting PTB is 40% (95% CI: 11.76%, 76.93%), specificity is 91.58% (95% CI: 84.25%,95.67%), positive predictive value is 20% (95% CI: 5.668%, 50.98%) and negative predictive value is 96.67% (95% CI: 90.65%,98.86%).

Table 6: Diagnostic accuracy of NPA GeneXpert in diagnosing PTB with GA TB culture as gold standard

NPA GeneXpert	GA Culture		Total
	Positive	Negative	
Positive	2	8	10
Negative	3	87	90
Total	5	95	100
Sensitivity	40%	(95% CI 11.76%, 76.93%)	
Specificity	91.58%	(95% CI 84.25%, 95.67%)	
Positive Predictive Value	20%	(95% CI 5.668%, 50.98%)	
Negative Predictive Value	96.67%	(95% CI 90.65%, 98.86%)	

Safety of Nasopharyngeal aspiration and Gastric aspiration

Both nasopharyngeal aspiration and gastric aspiration procedures were well tolerated. There were no adverse events or complications noted.

DISCUSSION

This study showed that NPA GeneXpert is a rapid and specific test that can be used to diagnose PTB in pediatric patients suspected to have the disease. The GeneXpert test on a single NPA specimen correctly identified majority of the patients with PTB confirmed by either GA GeneXpert or GA TB culture. Nasopharyngeal aspiration is a simple procedure that is easy to perform, is well tolerated and less invasive compared to gastric aspiration. However, since the sensitivity of the NPA GeneXpert remains suboptimal compared with the TB culture results, a negative GeneXpert test cannot be used to rule out TB in children.

PTB in children remains to be a diagnostic dilemma for physician due to its non-specific signs and symptoms and limited laboratory examinations that could establish the diagnosis. This may result in either delayed treatment in those with TB or overtreatment of those who turn out not to have active TB. Bacteriologic tests are important in confirming the diagnosis of PTB in children, however the choice of the respiratory specimen is challenging because of difficulty in collecting sputum in pediatric patients.

Recently, the GeneXpert test has emerged as an important tool in the rapid bacteriologic diagnosis of active TB in both adults and children. This study is the first study conducted among Filipino children with presumptive TB comparing NPA GeneXpert with GA GeneXpert and TB culture.

A total of 100 presumptive TB patients were enrolled and the majority of patients were clinically diagnosed PTB. In this study, presumptive TB patients commonly presented with nonspecific signs and symptoms of fever, chronic cough and inability to gain weight or weight loss. Patients who were clinically diagnosed PTB fulfilled at least 3 out of 6 criteria of clinical signs and symptoms of TB, CXR findings compatible with TB, TB exposure, positive TST and other laboratory tests consistent with TB. Various studies, including a descriptive local study conducted in 2002, support the use of history and clinical features to diagnose childhood TB.[6,13,14] The usefulness of the history of exposure and clinical signs and symptoms suggestive of TB were confirmed in this study. In children living in areas highly endemic for TB, it is important that physicians have a high index of suspicion for the disease. Thorough and comprehensive history and physical examination are necessary to make a correct diagnosis.

The bacteriologic confirmation of PTB in children is challenging due to difficulties in obtaining respiratory samples, particularly in children below 15 years of age. Mycobacterial culture remains to be the gold standard in diagnosing TB, but the procedure has low sensitivity in children. Only about 10 to 50% of tuberculosis cases are culture-proven owing to the paucibacillary nature of the disease and difficulty of specimen collection.[15,16] GeneXpert (Xpert MTB/RIF) may be used rather than conventional microscopy and culture as the initial diagnostic test in all children suspected of having TB.[10] In South Africa, sputum smear microscopy has been replaced with GeneXpert as the initial diagnostic test for tuberculosis.[17] In the Philippines, GeneXpert is currently recommended as the primary diagnostic tool for TB detection in presumptive drug susceptible tuberculosis in children below 15 years old.[18] GeneXpert is useful because of the rapid and early detection of causative mycobacteria in various clinical samples.[4] A systematic review and meta-analysis on the accuracy of GeneXpert to diagnose PTB in children using a variety of respiratory specimens showed that sensitivity varied from 55-90% for samples of expectorated sputum, 40-100% for induced sputum and 40-100% for gastric lavage or aspirate.[10] The reported pooled sensitivity of GeneXpert compared to culture was 66% (95% CI 52-77%) for expectorated or induced sputum and 66% (95% CI 51-81%) for gastric lavage or aspirate. High specificities were reported for all studies using various specimen types, ranging from 93-100%.[10]

Both gastric aspiration and nasopharyngeal aspiration can be performed in children who cannot expectorate sputum, such as infants and young children, patients who are intubated and on mechanical ventilatory support, patients with tracheostomy, and patients with anatomical or structural problems. Compared to gastric aspiration, nasopharyngeal aspiration is less invasive, causes minimal discomfort, and does not require placing patients on 4-6 hours of fasting prior to the procedure. Furthermore, nasopharyngeal aspiration can easily be performed in the outpatient clinic setting, thereby obviating the need for hospitalization and lowering not only healthcare cost but also the risk of acquiring nosocomial infection. This was based on the observation in the institutions where the set up during the study period was admitting patients for gastric aspiration and if unsuccessful the patients need to stay in the hospital for a longer period putting them at risk of acquiring nosocomial infection. Some patients in this study verbalized better tolerance of the NP aspiration.

NPA has been shown to be a useful specimen for the diagnosis of TB in children using GeneXpert. The diagnostic accuracy of the NPA GeneXpert is variable depending on the specimen used as the reference standard. This study showed that NPA GeneXpert had a sensitivity of 70% and specificity of 97% when compared to GA GeneXpert as initial screening test but had a lower sensitivity of 40% and specificity of 91.58% compared to GA culture as the reference standard.

This is the first known study where GeneXpert assay was performed on NPA specimens and compared this with both GeneXpert and TB culture performed on GA specimens. The high specificity found in this study indicates that a positive result on NPA GeneXpert can be used to diagnose PTB; however, the low sensitivity implies that a negative test cannot rule out PTB in

children. In cases of suspected PTB where the GeneXpert is negative, the decision to start anti-TB treatment should still be based on epidemiologic data correlated with findings on clinical history and physical examination.

The lower sensitivity of the GeneXpert compared to TB culture has been reported in several studies.[7,19,20] One possible explanation is the paucibacillary nature of the disease in children.[16,21] When TB culture is used as the reference standard, there is an increased probability that true positive Gene Xpert results will be reported as false positive results, thus underestimating the sensitivity of the GeneXpert test. In this study, 8 patients were positive on NPA GeneXpert but negative on GA culture. These 8 cases were probably true cases of TB since none of the patients received anti-TB treatment in the past, all had clinical manifestations and had chest radiologic findings suggestive of active PTB. Five out of 8 had exposure to a known case of TB. In a study though, four patients with a history of successfully treated TB who presented with lower respiratory tract infections, positive Xpert results, negative liquid TB cultures and clinical improvement without anti- tuberculosis treatment were reported suggesting that the Xpert result was falsely positive.[22]

Other studies have recognized the limitation of TB culture in children and have recommended increasing the number of specimens in order to increase the bacteriologic yield from culture. Zarin found that using 2 specimens of either induced sputum or NPA for TB culture increased positive TB culture results.[7] Nicol et al. reported that using 2 induced sputum samples increased the yield of positive TB cultures in children with smear-negative results.[23] Although studies have shown that processing multiple specimens increases the sensitivity of the GeneXpert assay, the benefit of testing more than 2 specimens has not been shown to consistently increase the diagnostic yield.[24] Due to the additional resources needed for testing multiple specimens with no clear advantages, WHO supports the use of a single specimen for diagnostic testing in resource-limited areas.[10] The use of multiple specimens for GeneXpert and TB culture may be ideal and could increase the diagnostic yield but in resource-limited areas, the decision on the number of specimens to test needs to be guided by the available resources, including considerations for increased transportation costs for the patient.

One limitation of the GeneXpert test for TB is that it cannot be used to detect resistance to all the anti-TB drugs regardless of specimen collected. Hence, it cannot replace TB culture with conventional anti-TB drug susceptibility testing as the gold standard for confirming bacteriologic diagnosis, particularly if data on drug susceptibility is required. However, use of the GeneXpert using NPA may be a more convenient and a more rapid test to diagnose TB in the pediatric population where gastric aspiration is not possible. Another limitation of the GeneXpert may be its variable accuracy in patients with immunosuppression. A meta-analysis to determine the accuracy of GeneXpert in testing samples of expectorated or induced sputum reported sensitivity ranging from 20% to 100% among HIV-positive children and 33% to 100% among HIV-negative children.[5] The wide and overlapping intervals in the results were attributed to the effect of smear positivity status in children regardless of their HIV status. This study was conducted in a low HIV-prevalence country and none of the patients showed clinical manifestations of immunosuppression. Hence, the performance of NPA GeneXpert in the immunosuppressed population cannot be evaluated. In addition, there was only 1 neonate in the study population; this subject was negative for GeneXpert, so it is likewise not possible to draw any conclusions for this age group.

CONCLUSION AND RECOMMENDATIONS

NPA GeneXpert is a highly specific, rapid and early diagnostic test for PTB in children. Compared to GA GeneXpert, NPA GeneXpert had a sensitivity of 70%, specificity of 96.7%, negative predictive value of 96.67% and positive predictive value of 70% while compared to GA TB culture, NPA GeneXpert had a sensitivity of 40%, specificity of 91.58%, positive predictive value of 20%, and negative predictive value of 96.67%. Due to the simplicity and convenience of collection of NPA and more rapid release of results when GeneXpert test is used, a single NPA GeneXpert can be used as an initial screening test to diagnose PTB in pediatric patients. However, a negative GeneXpert test does not exclude a diagnosis of PTB in children. These findings will need to be verified in larger and varied populations including neonates, immuno-compromised patients, and people living with HIV. In order to document the feasibility and acceptability of this test when conducted in a public health setting, NPA GeneXpert test should also be studied in various community and primary care settings.

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ORIGINAL ARTICLE

THE EFFECT OF STORAGE TIME ON THE GROWTH OF MICROORGANISMS IN PASTEURIZED AND UNPASTEURIZED DONOR HUMAN MILK IN A TERTIARY HOSPITAL IN DAVAO CITY: A QUASI-EXPERIMENTAL STUDY

Loradel Marbella S. Calio, MD

Department of Pediatrics, Davao Doctors Hospital

Correspondence:
Dra. Loradel Marbella S. Calio
Email: mdlorabella@gmail.com

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: Donor Human Milk (DHM) is the recommended food of infants whenever mom's own milk (MOM) is not available. However, due to the pathogenic microbiological component of DHM, concerns on the safety of the milk are inevitable.

Objective: To determine the effect of storage time on the microbial growth of pasteurized and unpasteurized Donor Human Milk maintained at a constant temperature of -20°C.

Methodology: This is a Quasi-experimental Research done in the Newborn Care Unit (NCU) and Bacteriology Section of a private tertiary hospital in Davao City. The effect of storage time to the microbial growth of pasteurized and unpasteurized DHM was determined using Friedman Test 2-way Analysis of Variance by Ranks. Pairwise comparison of microbial growth between pasteurized and unpasteurized DHM at different storage times was determined using the Mann-Whitney U test.

Results: Baseline DHM samples had moderately heavy bacterial growth of *Staphylococcus epidermidis*. There was a decrease from moderately heavy to light growth of the same species in the 24-hour storage time for both pasteurized and unpasteurized DHM. Pasteurized DHM did not have any microbial isolates at 48h, 72h, 4w, 8w and 12w while unpasteurized DHM had *Acinetobacter baumanii, Staphylococcus warneri, Kocuria kristinae,* and *Staphylococcus saprophyticus* growths. The analysis revealed that there is a statistically significant difference in the microbial growth in both pasteurized and unpasteurized DHM samples when stored at different times, $\chi^2(6) = 28.457$, $p = 0.00$.

Conclusions: Storage time significantly interacts with the microbial growth on both pasteurized and unpasteurized DHM samples. Therefore, microbial growth in DHM samples may be affected by the length of time stored at a constant temperature of -20°C. Pasteurized DHM samples when stored at -20°C for more than 48 hours resulted to a statistically reduced microbial growth.

KEYWORDS: *Human milk, Pasteurization, Storage time*

INTRODUCTION

Human breastmilk, with its known time-tested benefits, is the most natural perfect food for babies and is highly recommended in the medical field. As proven by clinical and research studies, human breastmilk is the best source of nutrition for the infant because of its compelling advantages in terms of nutrition, immunoprotection, neurodevelopment, psychologic, socio-economic, and environment.[1]

In the Philippines, Expanded Breastfeeding Promotion Act of 2009 states that *"the state shall promote and encourage breastfeeding and provide the specific measures that would present opportunities for mothers to continue expressing their milk and or breastfeeding their infant or young child"* (Republic Act No. 10028). However, if breastfeeding is not possible, international authorities like the World Health Organization (WHO), American Academy of Pediatrics (AAP), European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHN) recommend the use of donated human breastmilk as the first alternative, and pasteurized Donor Human Milk as the most adequate alternative especially for preterm infants.[1,2,6] Nevertheless, the main concern regarding Donor Human Milk is its microbiological safety especially during storage.[3-7] Current official protocols include pasteurization and freezer storage at –20°C to eliminate hazards for newborns and preserve bioactive compounds.[1-5]

Milk pasteurization is a heating process that kills any harmful bacteria or viruses that may be present in the milk. A pasteurization process at 62.5°C for 30 minutes, also known as Holder Pasteurization, is currently recommended in all international guidelines.[3] This process preserves most of the milk's nutrients, immune properties, and other health components.[3-8] Milk is pasteurized to ensure safety from infectious agents potentially contaminating human milk, especially Donor Human Milk.

There are a few guidelines and research studies available regarding the optimum storage time and temperature of pasteurized Donor Human Milk. Thus, evidence-based standard protocol for milk handling and storage pre- and post-pasteurization in hospitals where milk pasteurizers are available is needed. Given the importance of milk storage and handling of expressed human milk to both mother and infant, it is of equal importance to determine and know the potential impact of storage time on Donor Human Milk after pasteurization, hence this study was conducted.

This study intended to determine the effect of storage time on the microbial growth on unpasteurized and pasteurized Donor Human Milk kept at a constant temperature of -20 °C. Specifically, to describe microbiota through bacterial culture of unpasteurized and pasteurized Donor Human Milk (bacteria profiling) and to determine and compare the presence or absence of microorganisms in unpasteurized, pasteurized Donor Human Milk samples when stored at -20°C for 24, 48, and 72 hours and at 4, 8 and 12 weeks.

MATERIALS AND METHODS

Study Design and Setting

This study utilized a quasi-experimental research design and was conducted at the neonatal care unit (NCU) and Bacteriology Section (Laboratory) of a tertiary hospital in Davao City, Philippines. The NCU of the institution does not do milk banking, only milk pasteurization.

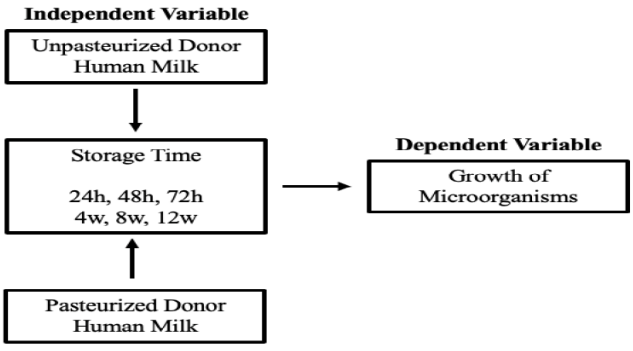

Figure 1. Conceptual Framework on the Effect of Storage Time on the Microbiota of Unpasteurized and Pasteurized Donor Human Breastmilk.

Procedures of the Study

Procurement of permission to conduct study. The primary investigator secured permission from the Medical Director of a tertiary hospital to conduct this study and obtain access to the NCU. This study

followed the Donor Human Milk pasteurization protocol of the hospital, which is adapted from the Philippine Human Milk Banking Manual of Operation (MOO) of the Department of Health (DOH).[1] In addition, this study was submitted and approved by the Institutional Ethics Review Committee of the Davao Doctors Hospital prior to data collection. This study was conducted in accordance with the principles that have their origin in the Declaration of Helsinki and is consistent with the International Conference on Harmonization Tripartite Guidelines and the Good Clinical Practice Guidelines (ICH-GCP). All patient information were anonymized and kept confidential. The primary investigator declares that there was no conflict of interest in the conduct of this study. There was no funding received from any individual nor institution.

Donor recruitment. Milk samples were obtained from mothers who delivered in the said institution. Four mothers who were in their immediate postpartum period consented to participate in this study as milk donors. These donors were interviewed and screened using a standard interview and screening forms which were taken from the Philippine Human Milk Banking MOO of DOH.[1] Physical examination of the breast was done by the primary investigator, and the antenatal test results (e.g., HBsAg, Anti-HBs, VDRL, RPR) at the time of their hospital admission were utilized in this study as an integral part of the screening process. All mothers had negative test results for the above mentioned tests.

Milk collection. Hand washing with soap and water is required upon entry to the NCU. Milk expression was done in the breastfeeding section of the NCU wherein only the primary investigator, NCU nurse, and milk donor were present. The primary investigator instructed the donor to clean her nipple and areola with the use of cotton and water. The recommended and standard method of manual milk expression (Marmet technique) for milk donation prior to pasteurization in the NCU was employed. The milk donor was instructed to use the thumb and forefinger of one hand and to position them one to two centimeters outside the areola as well as to perform the cycle of "Push-Compress-Release" repeatedly for several minutes to stimulate milk ejection. The breast milk was allowed to flow freely to a sterilized wide-mouth container and was made sure not to touch the nipple to avoid contamination. Visual presentations, such as videos and pictures, were used for assistance. After the manual expression, the container was covered with a sterile cap, and both breasts were cleaned with water. Expressed breast milk was stored in a sterile plastic container properly labelled and was frozen within five to ten minutes from milk expression.

Assignment to different treatment groups. Each milk donor was able to express an average of 60-90 ml of breastmilk. A total of 360 ml donor human milk was collected and pooled. This was considered as one milk batch. One milliliter (1 ml) aliquot from the pooled milk was sent immediately to the laboratory for baseline culture, which was done by a registered medical technologist. This milk batch was then distributed equally into two groups, the pasteurized group (Group A) and the unpasteurized group (Group B). Group A underwent the pasteurization process at 62.5^{o}C for 30 minutes (Holder Pasteurization) while Group B remained unpasteurized. Three replicates were made per group at different storage times. Triplicates were done for each batch for validation of observed results. Each replicate was stored in a freezer at -20^{o}C using a Traceable® Jumbo-Display Fridge/Freezer Digital Thermometer for 24h, 48h, 72h, 4w, 8w, and 12w. Bacteriologic testing of each replicate after a designated storage time was done thereafter.

Bacteriologic Testing. The streak plate method using selective and differential culture media were employed to isolate bacteria from the baseline, unpasteurized and pasteurized milk samples at different storage times. Species identity was determined via microscopy and susceptibility testing. To determine whether there is a significant difference in the growth of microorganisms between the unpasteurized and pasteurized milk samples at different storage times, relative to the baseline cultures, a scoring system based on the percent coverage was used and followed. This was based on

standard laboratory interpretations of the plating method as adapted from Bailey and Scott's Diagnostic Microbiology and has been the practice in the laboratory of the institution.[9] If no visible colonies of bacteria were noted, it was labelled as No Growth (NG) and assigned with a score of 0. Visual presentation of bacterial growth based on the percent occupied by the microorganisms on the zone of inoculation of the culture media for Light Growth (LG) was scored as 25, Moderate Growth (MG) as 50, Moderately Heavy Growth (MHG) as 75, and Heavy Growth (HG) as 100 (Figure 2).

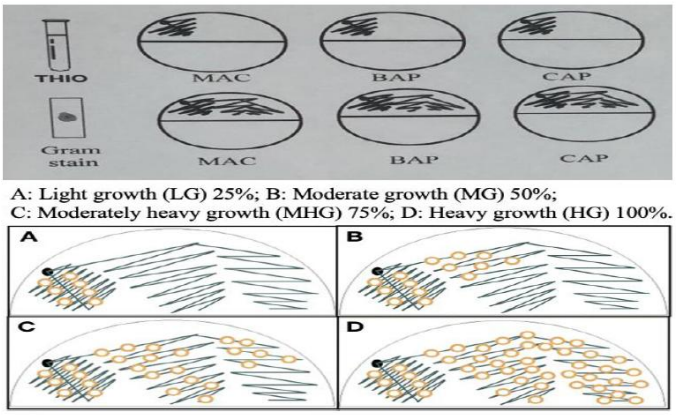

Figure 2: Streak plate method and visual presentation of bacterial growth based on the percent occupied by the microorganisms on the zone of inoculation of the culture media.

The specimen was inoculated on 1/2 blood agar plate, 1/2 chocolate agar plate, and 1/2 MacConkey agar plate; and a smear for gram staining was made. The slide was stained and examined under oil immersion field. After 16 to 24 hours of incubation, the plates were examined for growth.

Microbial growth on MacConkey agar indicates the presence of lactose/non-lactose fermenting type of organisms; and biochemical and susceptibility testing was done thereafter. Those that have microbial growth on the blood agar plate, gram staining and catalase test was done. For those gram-positive cocci, coagulase and susceptibility testing were done. Those with negative catalase test, possible organisms were alpha-hemolytic, hence Optochin and susceptibility testing were done. Otherwise, possible organisms were beta-hemolytic, hence Streptex/Pastorex and susceptibility testing were done. For growth on chocolate agar, gram staining was done to bacterial colony that were translucent. Smears were examined under oil immersion and short rods/pleomorphic microorganisms were identified. Once microorganisms were identified, workup for *Haemophilus influenza*, factors X and V, and susceptibility testing were done. The BIOMERIEUX Vitek® 2 Compact System was used for all identification and susceptibility testing.

If no bacterial growth was observed after 16 to 24 hours of incubation and the thioglycolate medium was clear, the plates was re-incubated up to 48 to 72 hours with daily inspection before releasing as no growth (Figure 3). These processes were done by a licensed senior medical technologist assigned in the bacteriology section of the hospital laboratory who followed standard procedure of accepting specimen for culture.

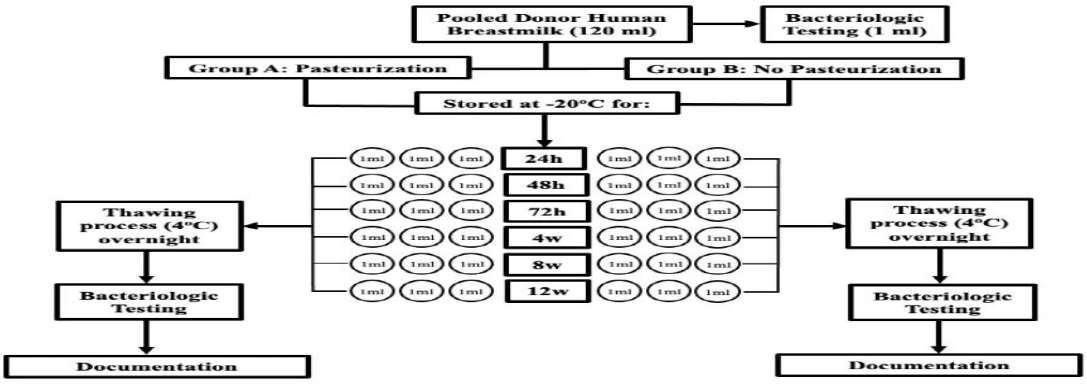

Figure 3: Flowchart of the research procedure.

Data Entry and Statistical analysis

Data collected was entered into a Microsoft Excel for the purpose of coming out with a .csv file type in preparation for the statistical analysis. This study was limited to non-parametric tests due to the qualitative nature of the variable of interest – the ordinal level of measurement of the percentage growth of bacteria. The effect of storage time on the microbial growth of pasteurized and unpasteurized DHM was determined using Friedman Test 2-way Analysis of Variance by Ranks. In addition, pairwise comparison of microbial growth between pasteurized and unpasteurized DHM at different storage times was determined using the Mann-Whitney U test.

RESULTS

Bacterial Isolates from Donor Human Milk (DHM)

Selective and differential culture media were employed to isolate bacteria from all milk samples and species identity was determined via microscopy and susceptibility testing. Table 1 shows the dominant bacterial species isolated from both pasteurized and unpasteurized donor human milk samples at different storage times. The microbial isolates which occupied majority of the section on the zone of inoculation was considered dominant. This has been the practice of releasing culture results in the laboratory.[9]

The baseline DHM samples showed growth of *Staphylococcus epidermidis.* Similarly, among the pasteurized milk samples, only those stored at 24 hours showed growth of the same species as the baseline. For the pasteurized DHM samples, no growth of microorganisms was observed at 48 hours, 72 hours, 4 weeks, 8 weeks, and 12 weeks. On the other hand, unpasteurized DHM samples exhibited a more diverse growth of microorganisms.

Acinetobacter baumanii, a gram-negative, strictly aerobic, non-fermenting and non-fastidious bacterium was isolated at 24 hours of storage time.[10] Two Staphylococcus species were isolated namely *Staphylococcus warneri*, at the 48-hour storage time, and *Staphylococcus saprophyticus*, at the 4-week and 12-week storage times. *Kocuria kristinae* was isolated at 72 hours storage time for unpasteurized DHM samples. At 8 weeks storage time, both unpasteurized and pasteurized DHM samples showed no growth of microorganisms.

Table 1: Dominant bacterial species isolated from pasteurized and unpasteurized donor human milk samples across different storage time.

	Species Identity	
0 hour (baseline)	*Staphylococcus epidermidis*	
Storage Time	Pasteurized	Unpasteurized
24 hours	*Staphylococcus epidermidis*	*Acinetobacter baumanii*
48 hours	none	*Staphylococcus warneri*
72 hours	none	*Kocuria kristinae*
4 weeks	none	*Staphylococcus saprophyticus*
8 weeks	none	none
12 weeks	none	*Staphylococcus saprophyticus*

Effect of Milk Pasteurization and Storage Time on Bacterial Growth

The baseline samples, in all replicates, exhibited a moderately heavy bacterial growth (Figure 4A) of *Staphylococcus epidermidis*, and light growth of the same species was also present in pasteurized samples at 24-hour storage time (Figure 4B). At 48-hours post-pasteurization (Figure 4C), pasteurized DHM samples were found to have no bacterial growth up to 12 weeks of storage time.

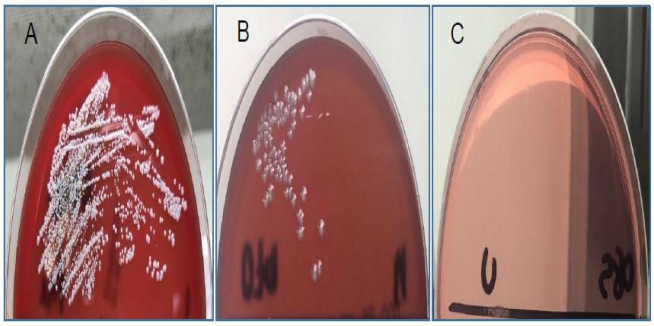

Figure 4: Bacterial cultures at **(A)** baseline samples showing moderately heavy bacterial growth, **(B)** 24-hour storage time of the pasteurized samples showing light bacterial growth, and **(C)** 48-hour storage time of the pasteurized samples showing no bacterial growth.

Unpasteurized and pasteurized DHM samples were compared for bacterial growth at different storage times as shown in Table 2. Baseline DHM samples had moderately heavy bacterial growth of *Staphylococcus epidermidis* and although similar species was isolated at 24 hours storage time for the pasteurized samples, it was noted that there was a decrease from moderately heavy to light growth of the said species. No microbial growth was observed at 48 hours until 12 weeks storage time for the pasteurized DHM samples. This was also noted at 8 weeks storage time for unpasteurized DHM samples. Light growth of *Acinetobacter baumanii*, *Staphylococcus warneri, Kocuria kristinae, and Staphylococcus saprophyticus* were isolated at 24 hours, 48 hours, 72 hours, 4 weeks, and 12weeks of storage time, respectively, for unpasteurized DHM samples.

Microbial Growth Comparison among Treatment

The effect of storage time on the microbial growth of pasteurized and unpasteurized donor human milk was determined using Friedman Test two-way Analysis of Variance by Ranks. The analysis revealed that there is a statistically significant difference in the microbial growth in both pasteurized and unpasteurized DHM samples when stored at different times, $\chi^2(6) = 28.457$, $p = 0.00$ (Figure 5).

This result signifies the interaction of storage time with the microbial growth on both pasteurized and unpasteurized DHM; thus, microbial growth in DHM samples may be affected by the length of time stored in a freezer at a constant temperature of -20°C.

Table 2: Comparison of bacterial growth between pasteurized and unpasteurized donor human milk (DHM) samples at different storage times.

Storage Time	Pasteurized Milk			Unpasteurized Milk		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
0 hour (Baseline)	MHG	MHG	MHG	MHG	MHG	MHG
24 hours	NG	LG	LG	LG	LG	LG
48 hours	NG	NG	NG	NG	LG	LG
72 hours	NG	NG	NG	LG	LG	LG
4 weeks	NG	NG	NG	LG	LG	LG
8 weeks	NG	NG	NG	NG	NG	NG
12 weeks	NG	NG	NG	LG	LG	LG

Legend: NG - No growth, LG - Light growth, MG - Moderate growth, MHG - Moderately Heavy growth, HG - Heavy growth.

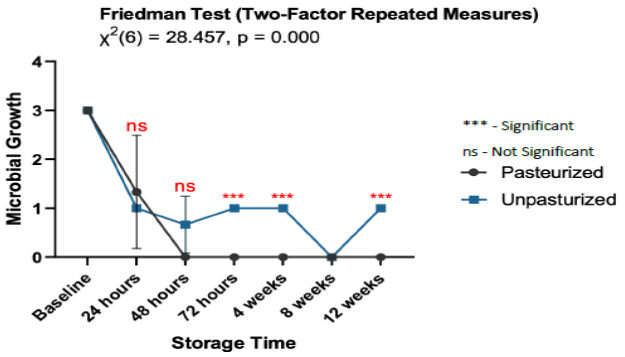

Figure 5: Interaction of Storage Time with Microbial Growth on Pasteurized and Unpasteurized Donor Human Milk

Despite the significant interaction between the storage time and microbial growth, the multiple comparison analysis (Table 3) of related samples between the baseline microbial growth and the succeeding measurements in varying storage times revealed that there is not enough evidence to support statistically significant differences ($p>0.05$) in the microbial growth from the baseline culture samples with other samples stored at different times. In other words, for example, although there was a reduction in the [light] bacterial growth of the unpasteurized DHM samples stored at -20°C for 24 hours compared to the [moderately heavy bacterial growth of] baseline DHM samples, this was not statistically different from each other. This may warrant further investigation using additional test DHM samples.

Table 3: Multiple Comparison of Microbial Growth in Pasteurized and Unpasteurized Donor Human Milk Stored in Various Storage Times.

Storage Time	Pasteurized		Unpasteurized	
Baseline vs.	Z	Asymptotic Sig. (2-tailed)	Z	Asymptotic Sig. (2-tailed)
24 hours	-1.633	0.102	-1.732	0.083
48 hours	-1.732	0.083	-1.633	0.102
72 hours	-1.732	0.083	-1.732	0.083
4 weeks	-1.732	0.083	-1.732	0.083
8 weeks	-1.732	0.083	-1.732	0.083
12 weeks	-1.732	0.083	-1.732	0.083

In addition, the Mann-Whitney U pairwise comparison test (Table 4) revealed that microbial growth in pasteurized and unpasteurized DHM samples stored at 24 hours and 48 hours do not significantly differ ($p>0.05$) from each other while those milk samples stored in 72 hours, 4 weeks, and 12 weeks, has statistically significant difference ($p<0.05$). The analysis implies that pasteurized DHM stored in longer duration may result to reduced microbial growth. Therefore, pasteurized DHM poses greater advantage over unpasteurized DHM in terms of microbial growth after storage. Furthermore, this analysis also supports that storage time may have an interaction to pasteurization that may have resulted to reduced microbial growth.

Table 4: Mann-Whitney U Pairwise Comparison of Microbial Growth between Pasteurized and Unpasteurized Donor Human Milk at Different Storage Times.

Storage Time	Mann-Whitney U	Z statistic	Asymptotic Sig. (2-tailed)
Baseline	4.50	0.00	1.00
24 hours	3.00	-1.00	0.317^{ns}
48 hours	1.50	-1.58	0.114^{ns}
72 hours	0.00	-2.24	0.025*
4 weeks	0.00	-2.24	0.025*
8 weeks	4.50	0.00	1.00
12 weeks	0.00	-2.24	0.025*

* Significant ($p<0.05$), ns-not significant ($p>0.05$)

DISCUSSION

Human milk has high nutritional content which includes proteins, fats, carbohydrates, vitamins, minerals, and essential amino acids that can support a rich microbiota.[11, 12] It may, however, also be a vehicle for microorganisms derived from the mother or the environment during its collection, storage, and handling. Freshly collected breast milk is rarely sterile and normally contains bacteria originating from the maternal skin and nipple duct microflora, but it also sometimes contains potential pathogens. Although it is questioned whether it is possible to aseptically collect human milk, culture-dependent methods have confirmed the presence of bacteria in assumed aseptically collected milk. Bacterial contamination in milk can originate through a variety of sources which includes teat apex, milking equipment, air, and other environment by which it is exposed.[13-15] These microorganisms are known to play several roles such as facilitating dairy fermentations (e.g., *Lactococcus* and *Lactobacillus*), causing spoilage (e.g., *Bacillus a*nd *Clostridium*), promoting health (e.g., *Lactobacilli* and *Bifidobacteria*) or causing diseases (e.g., *Listeria, Salmonella, Escherichia coli* and *Campylobacter*).[12] One of the most commonly isolated bacterial species from human milk include *Staphylococcus epidermidis*, an emerging leading cause of subacute mastitis in both women and veterinary medicine. The baseline DHM samples in this study, which came from a single pooled milk

batch from four donors, demonstrated a moderately heavy growth of *Staphylococcus epidermidis*, suggesting that one or more of the milk donors could have the said condition. Furthermore, the same species was isolated in the pasteurized milk samples at 24 hours storage time. This species is an opportunistic human pathogen which is one of the leading causes of nosocomial infections and are also commonly associated with hospital-acquired medical device infections.[16,17] Its capacity to form biofilms, exopolymers, and various defense mechanisms give it protection from antibiotics and host defenses, making it difficult to eradicate.[18] The biofilms of such species are temperature-sensitive but can still maintain some level of cell viability even after exposure at 60^{o}C for 1 hour.[17] This could partly explain why Holder pasteurization was not able to fully eradicate *S. epidermidis* from the samples used in this study. Its growth seemed to be inhibited beginning at 48 hours post-pasteurization, possibly due to the preserved bactericidal properties in the breastmilk. Another possibility is that pasteurization may not have fully eradicated this organism within the 24-hour mark, but the heating process was enough to make the organisms incapable to proliferate further. Coagulase-negative Staphylococci (CoNS) have traditionally been part of the normal skin microbiota. It represents a regular part of the microbiota of the skin and mucous membranes of humans and animals.[19] The differences in skin thickness and folds as well as hair follicles and glands densities define distinct differing microbiota including CoNS. In humans, *S. epidermidis* is the most frequently recovered CoNS species.[19] While the virulence of these organisms is relatively low, their opportunistic behavior can cause clinically significant infections of the bloodstream and other tissue sites.

Unpasteurized DHM samples were observed to have a consistent light growth of microorganisms from 24 hours until 12 weeks of storage time, except for the cultures at two months storage time which showed no growth at all. Microorganisms grown in closed culture follow a reproducible growth pattern referred to as the growth curve that consists of four phases namely the lag, exponential, stationary, and death phases. It could be possible that microorganisms found in unpasteurized DHM when stored at -20^{o}C for two months may be in transition between the stationary and the death phases of the growth curve, meaning that at this point, microbes may begin to decrease in number of living bacterial cells. Four microbial isolates were identified in the unpasteurized DHM samples, namely, *Acinetobacter baumanii, Staphylococcus warneri, Kocuria kristinae,* and *Staphylococcus saprophyticus*. These were either typical skin commensals, part of the normal flora of the human oropharynx, or species that are ubiquitous in nature.

Acinetobacter baumanii, isolated from the unpasteurized milk samples at 24 hours of storage time, is a gram-negative, strictly aerobic, non-fermenting and non-fastidious bacteria that is usually pathogenic. The possibility that this isolated microorganism could be a contaminant is highly favored since the processes of milk collection until milk culture inoculation were done in a hospital setting, specifically the NICU and Bacteriology Section of the Laboratory. Another possibility, though least probable, is that one of the milk donors belong to the few percentages of the population that harbor the rare species as part of their natural skin microbial flora. Two Staphylococcus species were also isolated from the unpasteurized DHM samples namely *Staphylococcus warneri*, at the 48-hour storage time, and *Staphylococcus saprophyticus*, both at 4 weeks and 12 weeks of storage time. Staphylococcus species are gram-positive cocci that form clumps and are traditionally divided based on their coagulase reaction. *Staphylococcus aureus* and *Staphylococcus intermedius* are the only known coagulase positive Staphylococci while the rest are known to be coagulase negative. Many Coagulase Negative Staphylococci (CoNS) such as *S. warneri* and *S. saprophyticus* are common commensals on the skin and membrane linings, although several species are known to cause infections in both

humans and animals.[20] *S. warneri* is a gram-positive skin commensal which rarely causes disease, but also occasionally cause infections particularly in immunocompromised patients.[21] It has already been reported as an emerging pathogen although there is still a lack of scientific data on the pathogenesis and epidemiology of the species.[22] Previous studies reported the clinical significance of *S. warneri* in orthopedic infections, pediatric and adult bacteremia, septicemia, endocarditis, urinary tract infections, as well as its pathogenicity in neonates being a predominant CNS isolated from the hands of nurses.[22-26] On the other hand, *S. saprophyticus* has been widely documented as one of the leading causes of urinary tract infections (UTI), second to *Escherichia coli*, and is commonly found in the gastrointestinal tract particularly exhibiting rectal, vaginal and urethral colonization.[27,28] Furthermore, the bacteria have been found to contaminate various food samples in a study conducted in Sweden with high prevalence in raw beef and pork and has been isolated from rectal swabs from cattle and pigs.[29,30] Among the more severe complications that it can cause includes acute pyelonephritis, septicemia, nephrolithiasis and endocarditis.[31-34] Another flora found is the *Kocuria kristinae,* a gram-positive coccus, which is also a natural skin and mucous membrane commensal and usually non-pathogenic was isolated from the unpasteurized samples at 72 hours storage time.[35] Members of the genus *Kocuria* are responsible for different types of infections, mostly in immunocompromised host with serious underlying conditions.[36,37] Opportunistic infections caused by this species in patients with malignancy has also been reported.[38] Furthermore, it has also been reported to cause infections in premature babies and immunocompromised pediatric patients which highlights its expanding infection spectrum.[39] There is still limited information on the epidemiology and virulence of Kocuria species, but the formation of biofilms has been suggested to mediate adhesion, colonization and subsequent infection.[40]

Current official protocols for donor human milk include pasteurization and freezer storage at -20^{o}C to eliminate hazards for newborns and preserve bioactive compounds. In order to strike a balance between microbiological and immunological safety, Low Temperature Long Time (LTLT) milk pasteurization is usually employed to reduce microbial load and viable pathogenic bacteria, to limit the number of spoilage microorganisms that can cause foodborne diseases, and to ensure safety for human consumption.[8] Holder pasteurization has been proven to effectively remove any detectable bacteria from samples in a previous study using routine bacterial cultures for Staphylococcus, Streptococcus and Enterococcus species.[4] This method is also often simulated in small aliquots rather than being performed in compliance with Human Milk Bank (HMB)-implemented protocols, which are causing huge variability of results in many published studies using the same technique.[3] Although pasteurization assures the microbiological safety of human milk, the mechanism of thermal inactivation of bacteria is detrimental to the bioactivity of the milk since most of the proteins will denature when exposed to heat.[41] Several studies have shown that Holder pasteurization reduces, to some extent, the activity of important immunomodulating components. Hence, thermal treatment may not only impair the beneficial antibacterial properties of human milk but may also increase its susceptibility to subsequent bacterial contamination.[42] However, in this study, the pasteurized DHM samples showed no microbial growth from 48 hours to three months storage time. Despite such result, there is still not enough basis to attribute this effect to pasteurization alone as light bacterial growth was observed at 24-hour storage time post-pasteurization, like that of the unpasteurized DHM samples. Holder pasteurization significantly reduces (50–70%) the bactericidal effect, but if the pasteurized donor milk is kept refrigerated, the residual bactericidal capacity remains stable for up to 72 hours.[3,43] This suggests that other processes are contributing to the decline of the microbial flora between the 24-hour and 48-

hour mark, and that this was sustained up to three months of storage time.

The evidence for the benefits of pasteurized donor milk is limited, and the exact effect of frozen storage time after pasteurization on human milk composition is not clear. There is yet no complete agreement about storage times, however it is always preferable to store milk for as short a time as possible to ensure minimal growth of bacteria and minimal loss of antibodies and nutrients. Milk is typically stored at colder temperatures that reduce the growth of most bacteria, except for cryo-tolerant microorganisms that can proliferate under these conditions and become a major cause of milk spoilage.[13] Freezing breast milk at --20^{o}C for up to three months has been recommended as optimal. This 12-week time frame is within the recommended storage guidelines for milk by the National Institute for Health and Care Excellence, which is 6 months after expression, and to pasteurize within 3 months after expression.[44] Human milk has been known to have a natural bactericidal capacity which provides defensive factors against many disease-causing microorganisms, although this property can be altered during the storage of milk and post-processing events such as pasteurization.[42,45]

Literatures on the effect of storage duration on the bactericidal capacity of milk are limited. Refrigeration for less than 48 hours does not modify the bactericidal capacity of human milk, thus the protective properties for the nursing infant remain intact. However, if storage is extended beyond this time, then bactericidal capacity decreases, and the loss of this protection is very significant statistically after 72 hours. This property of maternal milk is lost over a period in which other components remain stable and within the limits advised by usual protocols. When frozen storage is employed, just like in this study, milk stability is prolonged. Frozen storage is an option when longer storage periods are needed.

Results of this study showed that storage time significantly interacts with the microbial growth on both pasteurized and unpasteurized DHM samples as determined by using the Friedman Test two-way Analysis of Variance by Ranks. Therefore, microbial growth in DHM samples may be affected by the length of time stored in a freezer at a constant temperature of -20^{o}C. Despite this, the multiple comparison analysis of related samples between the baseline microbial growth and the succeeding measurements in varying storage times revealed that there is not enough evidence to support statistically significant differences ($p>0.05$) in the microbial growth from the baseline culture samples with other samples when stored at different times. This means that among the unpasteurized DHM samples, for example, the microbial growth is relatively the same at different storage times. The absence of considerable differences of bacterial growth among these samples can be attributed to the pooling of milk samples from different donors, and the qualitative nature of the data obtained from bacterial culture techniques, all of which could possibly limit the explanatory power of the independent variables being tested. The Mann-Whitney U pairwise comparison test revealed that microbial growth in pasteurized and unpasteurized DHM samples stored at 24 hours and 48 hours do not significantly differ ($p>0.05$) from each other while those milk samples stored in 72 hours, 4 weeks, and 12 weeks, in contrast, has statistically significant difference ($p<0.05$). The analysis implies that pasteurized DHM stored in longer duration may result to reduced microbial growth. It is hypothesized therefore that this was because of a possible positive feedback to the bactericidal capacity after most bacterial contaminants were eradicated post-pasteurization. The innate bactericidal properties that are still preserved after pasteurization were able to enhance their function in regulating the remaining microbiota during the prolonged storage period at low temperature condition (-20^{o}C). Previous studies have reported that bactericidal activity of human milk is better preserved by means of holder pasteurization compared to other pasteurization methods, and that its microbiological quality can be maintained when properly handled and refrigerated at 4-

6°C.[44,46] It is therefore worth investigating in future studies whether the changes in biochemical and microbial composition after pasteurization enhances the bactericidal capacity of and if this enhanced effect is only applicable for certain types of bacteria. Therefore, pasteurized DHM poses greater advantage over unpasteurized DHM in terms of microbial growth after storage. Furthermore, this analysis also supports that storage time may have an interaction to pasteurization that may have resulted to reduced microbial growth.

Nevertheless, this study highlighted the importance of pasteurization in preventing the growth of several bacterial species such as *Acinetobacter baumanii, Staphylococcus warneri, Staphylococcus saprophyticus* and *Kocuria kristinae*. The absence of these bacteria in the pasteurized DHM samples highlights the benefit that pasteurization can provide in terms of reducing microbial populations which have the potential to cause infections and put consumers such as newborns at risk. Furthermore, this benefit can be enhanced with the proper storage at low temperatures to extend its shelf life while maintaining the essential nutrients present and keeping it safe for consumption.

CONCLUSION AND RECOMMENDATION

In conclusion, storage time significantly interacts with the microbial growth on both pasteurized and unpasteurized DHM samples as determined by the Friedman Test Two-way Analysis of Variance By Ranks. Therefore, microbial growth in DHM samples may be affected by the length of time stored at a constant temperature of -20°C. In addition, the Mann-Whitney U pairwise comparison test revealed that pasteurized DHM samples when stored at -20°C for more than 48 hours resulted to a statistically significant reduced microbial growth. Furthermore, this analysis supports the result that storage time may have an interaction to pasteurization which have resulted to a reduction in the growth of microorganisms.

It is recommended to continue the practice of pasteurizing donor human milk in the NICU and to store them in a freezer at a constant temperature of -20°C as this will pave the way for the institution to develop and establish a local milk bank which will be very useful for the community. Also, it is worth investigating whether the changes in biochemical and microbial composition of Donor Human Milk (DHM) after pasteurization enhances the bactericidal capacity components of breastmilk and if this enhanced effect is only applicable for certain types of bacteria, as well as the viability to reproduce the recalcitrant microbes that grew. Furthermore, an equally spaced time intervals is suggested in data gathering to better capture the events of microbial growth within the duration of a study. It is also worth exploring what would happen to the quality of pasteurized DHM, in terms of its nutritional aspect, beyond 3 months of storage.

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ORIGINAL ARTICLE

MATERNAL AND NEONATAL CLINICO-DEMOGRAPHIC PROFILE AND OUTCOMES DURING THE COVID-19 PANDEMIC AT THE CHINESE GENERAL HOSPITAL AND MEDICAL CENTER

Maria Ronallaine d.L. Bello, MD[1]
Shirley Kwong-Buizon, MD, MDH, FPPS, FPSAAI[1,2]

[1] Chinese General Hospital and Medical Center
[2] Research Committee Chairman, Department of Pediatrics, Chinese General Hospital and Medical Center

Correspondence:
Dr. Maria Ronallaine d.L. Bello
Email: ronallainebello@gmail.com

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: COVID-19 is an ongoing health concern that hospitals have struggled to keep up with, given its increasing burden with the passage of time. Considerations for the management of COVID-19 should be made especially for pregnant patients and their neonates.

Objectives: To determine COVID-19 prevalence and the clinical profile of mothers admitted for childbirth at Chinese General Hospital and Medical Center from May 2020 to July 2020. The profile and outcomes of neonates born to these mothers were likewise studied.

Materials and Method: A descriptive cross-sectional study was done that included mothers admitted for childbirth who had SARS-CoV-2 RT PCR swab test and their neonates. A total of 408 medical records of mother and neonate dyads were reviewed. Relevant variables such as the patients' demographic profile, clinical characteristics, co-morbidities and the maternal and neonatal outcomes were obtained. Frequency distributions were made to assess the prevalence of COVID-19 among the patients, as well as maternal and neonatal outcomes.

Results: Twenty-two (5.39%) mothers tested positive for COVID-19, while all neonates (n = 22) that underwent RT-PCR swab at the 24th hour of life had negative results. Of the 22 COVID-19 positive mothers, 2 (9.09%) were symptomatic upon admission while 20 (90.09%) were asymptomatic. The following were the key trends among those mothers who tested positive for COVID-19: (1) 81.82% were from ages 20-39 years old, (2) 72.73% were multigravida mothers, (3) 54.55% had normal spontaneous delivery, (4) diabetes mellitus was the only noted comorbidity. Key findings on the neonatal outcomes observed in the study population of both COVID-19 positive and negative cases, include: (1) majority of neonates had an APGAR score of greater than 7 at 1st and 5th minute of life; (2) higher frequency of neonates with Ballard's score of more than 37 weeks AOG; (3) more male neonates as compared to female neonates; (4) a normal birth weight for majority of cases; (5) 45.45% of neonates born to COVID positive mothers had a length of stay of <48 hours as compared to 72.8% of neonates born to COVID negative mothers; and (6) neonatal pneumonia as the most common comorbid condition in both cases.

Conclusion: This study noted a prevalence of 5.39% COVID-19 positive mothers. SARS-CoV-2 virus was not detected in all of the neonates born to COVID-19 affected mothers. Neonates delivered to COVID-19 positive mothers had similar trends in the neonatal outcomes when compared to neonates delivered to mother who were COVID-19 negative.

KEYWORDS: *COVID-19, Neonatal Outcomes, Maternal Outcomes, COVID-19 Pregnant Women*

INTRODUCTION

On December 31, 2019, a pneumonia case with an unknown etiology was discovered in Wuhan, China, and was reported to the WHO Country Office in China. Then known as the novel coronavirus (nCOV), an increase in the cases of this coronavirus prompted the WHO to declare this outbreak a Public Health Emergency of International Concern on January 30, 2020. Furthermore, the disease was finally named as COVID-19 on February 11, 2020. In the Philippines, there has been a total of approximately 1 million cases recorded as of April 2021, of which, approximately 70,000 are active cases, 925,000 have recovered from the disease and approximately 17,000 deaths were recorded.[1] Of the 1 million COVID-19 positive cases, there has been approximately 95,000 recorded cases among the pediatric population. Since the recording of the first case in the country in January 30, 2020, the government has imposed community quarantine protocols akin to the lockdown protocols established by other countries in hopes to curb further spread of the virus. As of this time, these community quarantine protocols have been eased to accommodate the growth of a vital part of the Philippine economy, but the cases have not decreased.

There are some studies that reported on how COVID-19 presents in relatively vulnerable population group. A systematic review by de Rose et al. shows that cases in the pediatric population subgroup have been relatively few and seem to have a more favorable clinical course as compared with other age groups. It is further noted that there are a fewer cases reported in neonates. In the same study by de Rose et al, it has been cited that clinical features of COVID-19 in newborns and infants can be non-specific and may include acute respiratory distress syndrome, temperature instability, gastrointestinal, and cardiovascular dysfunction as some of its more common signs and symptoms.[2] None of the neonates included in the study population were reported to have severe complications. Zimmerman et al. had the same findings in that COVID-19 presents a milder disease course in the pediatric population but suggests that children are similarly likely to develop infection as with the predisposition in adults. There were some data in the study that pertained to mothers being admitted to the intensive care unit for contracting COVID-19 during their pregnancy, and a subset of data also reported that there were neonatal complications of COVID-19 infection. Maternal complications described in the study by Zimmerman et al. include premature rupture of membranes, pre-eclampsia, gestational hypertension, and gestational diabetes while neonatal complications described were respiratory distress or pneumonia, low birth weight, disseminated intravascular coagulation, asphyxia and perinatal death.[3]

Although the primary focus has been on vulnerable groups, particularly the elderly and individuals with underlying medical conditions, pregnant women and newborns are also at higher risk for COVID-19 complications. To date, there were several studies on the clinical features of COVID-19 in the pediatric and neonatal population. A local study by Po described the outcomes of infants born to mothers with SARS-CoV-2 Infection in a public tertiary hospital that included 47 neonates. Among the 47 neonates, 72.3% neonates had no symptoms and were sent home immediately with a reliable caregiver while 27.7% were symptomatic and the predominant causes were feeding intolerance, neonatal pneumonia and transient tachypnea of the newborn.[4]

However, there is still limited data published in the Philippine setting, hence there is a need for these data in the local setting so that there will be a deeper understanding of the COVID-19 situation in the country, as well as to determine features that will help a pediatrician conduct evidence-based decision making for an accurate and sound treatment for a vulnerable population group for this disease. This study aims to determine COVID-19 prevalence among mothers admitted for childbirth and their neonates, clinical profile of mothers admitted for childbirth and the outcomes of neonates delivered at Chinese General Hospital and Medical Center from May 2020 to July 2020.

Specifically, it aims to describe the mothers admitted for childbirth in terms of: age; parity; mode of delivery; presence of maternal comorbidities, presence of COVID symptoms, SARS-CoV-2 RT-PCR swab result and to determine the clinical profile and outcomes of neonates delivered in terms of: APGAR Score; gender; birth weight; Ballard's Score; SARS-CoV-2 RT PCR Swab Result; length of stay and neonatal morbidity.

MATERIALS AND METHODS

Study Design: Descriptive Cross-Sectional Design

Chinese General Hospital (CGH) is a 600-bed capacity private tertiary hospital located in Manila and is delegated by the national government to be one of the healthcare facilities to provide COVID-19 diagnostic and admission services.

Study Population: Mothers admitted for childbirth and neonates delivered at Chinese General Hospital and Medical Center from May 2020 to July 2020

Inclusion Criteria:

- All mothers who were considered Non-COVID, COVID Suspect, COVID Probable or COVID Positive admitted for childbirth who underwent COVID-19 RT PCR swab test.
- All neonates delivered whose mother underwent COVID-19 RT PCR swab test

Exclusion Criteria:

- Mothers with RT-PCR not done at Chinese General Hospital
- Mothers with invalid or indeterminate RT-PCR results
- Mothers and neonates with incomplete charts

Sample size: Based on the total number of mothers admitted for childbirth from May 2020 to July 2020 of 416 patients, with a confidence limit of 5%, a confidence interval of 95%, and hypothesized frequency of outcome factor of 50%, the sample size computed for this study is 200.

Data Collection Methods: Medical records from May 2020 to July 2020 of all mothers admitted for childbirth and all neonates delivered were obtained and reviewed. Data were collected by the researcher using a data collection form. The following data were obtained from the medical records:

I. Maternal Data: age of mother, gravida, mode of delivery, maternal comorbidity, presence of COVID symptoms, SARS-CoV-2 RT-PCR test result
II. Neonatal Data: APGAR score, gender, Ballard's score, birthweight, length of stay, SARS-CoV-2 RT-PCR test result, neonatal morbidity

Statistical Analysis: For the analysis of data, SPSS version 25 was used. As a statistical software, this generated the frequency distributions of the different variables pertaining to the clinical profile and neonatal outcomes of the study population. The prevalence of COVID-19 from this study population was also calculated by this software. Microsoft Excel was used to form graphs of the result.

Ethical Considerations: The protocol of this study adhered to the ethical consideration and ethical principles set out in relevant guidelines, including the Declaration of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, Data Privacy Act of 2012, and National Ethics Guidelines for Health Research.

Data Safety, Privacy, and Confidentiality. Subject information was kept confidential. All identifiable information and data was given a code number. A master list linking the code number and subject identity was kept separately from the research data. The investigator and all key personnel have completed the Good Clinical Practice (GCP) training on the responsible conduct of research with human data. Pursuant to the Data Privacy Act of 2012, the gathering, storage, and eventual disposal of the data used in this study will minimize, if not eliminate, any risk for the revelation of any personal information pertaining to the patient records that was retrieved. Necessary procedures for the procurement of informed consent from the patients were also observed, though it was not necessary since the study only involved the review of medical records. Nonetheless, this study fully complied with any additional measure the ethics review committee gave regarding the

ethical conduct of this study and its relevant data collection procedures.

The study commenced upon the approval of the Research Ethics Review Board of Chinese General Hospital and Medical Center. As the RERB gave the period of ethical clearance of the study, the proponent saw to it that the study protocol and all its related procedures was done before the expiry date of the ethical clearance given to the study, and filed the necessary documentation to the RERB once the study has ended. The authors of this study certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this study.

RESULTS

From May to July 2020, a total of 416 pregnant mothers were admitted for childbirth at the study site, 8 mothers did not undergo RT-PCR swab test hence a total of 408 records of mothers and neonates were included. 22 out of the 408 mothers tested positive in the RT-PCR test, resulting in a COVID-19 prevalence of 5.39% in the study population. The 22 neonates born to COVID-19 positive mothers were tested with COVD-19 RT-PCR at 24th hour of life and all 22 neonates had negative results. Table 1 shows the clinical profile of the mothers admitted for childbirth. The following key trends among those mothers who tested positive for COVID-19 in the study population were seen: (1) a more notable frequency of cases from 20-39 years (81.82% of all COVID-19 positive mothers); (2) a higher frequency of multigravida mothers (72.73%) as compared to primigravid mothers (27.27%); (3) a slightly higher frequency of patients underwent normal spontaneous delivery (54.55%); and (4) diabetes being the only noted comorbidity.

For the COVID-19 negative mothers, almost the same trends were observed, though the counts are higher in terms of the comorbidities recorded by these patients. Urinary Tract Infection (10.36%) was the most frequent comorbid condition recorded among COVID-19 negative mothers, followed by hypertension (8.55%), and diabetes (7.51%).

Table 1: Profile of Mothers Admitted for Childbirth at CGH from May 2020-July 2020 as to SARS-CoV-2 RT PCR test result (N=408)

Clinical Parameters	SARS-CoV-2 PCR Result of Mother			
	Positive (n= 22)	%	Negative (n= 386)	%
Mother's Age (years)				
15-19	0	0	9	2.33
20-24	4	18.18	39	10.10
25-29	6	27.27	119	30.83
30-34	5	22.73	122	31.61
35-39	7	31.82	77	19.95
40-44	0	0	19	4.92
>44	0	0	1	0.26
Gravida				
Primigravid	6	27.27	157	40.67
Multigravid	16	72.73	229	59.33
Mode of Delivery				
Normal Spontaneous Delivery	12	54.55	185	47.93
Cesarean Delivery	10	45.45	201	52.07
Maternal Comorbidity				
Hypertension	0	0	33	8.55
Diabetes	1	4.55	29	7.51
Cardiovascular Disease	0	0	2	0.52
Thyroid Disorders	0	0	9	2.33
Urinary Tract Infection	0	0	40	10.36
Upper Respiratory Tract Infection	0	0	4	1.04
Bronchial Asthma	0	0	10	2.59
*Others	0	0	9	2.33

**Other Maternal Comorbidities: Acute Gastroenteritis, Bell's Palsy, Rubella Infection, Gingivitis, Vaginitis, Antiphospholipid Antibody Syndrome, Hyperemesis Gravidarum, Autoimmune Urticaria*

Key findings on the neonatal outcomes and variables observed in the study population of both COVID-19 positive and negative mothers, include: (1) majority of neonates had an APGAR score of greater than 7 at 1st and 5th minute of life; (2) higher frequency of neonates with Ballard's score of more than 37 weeks AOG; (3) more male neonates as compared to female neonates; (4) a normal birth weight for majority of cases; (5) 45.45% of neonates born to COVID positive mothers had a length of stay of <48 hours as compared to 72.8% of neonates born to COVID negative mothers; and (6) neonatal pneumonia was the most common comorbid condition in both cases. However, the trend is slightly different for the second most commonly reported comorbid condition-prematurity for COVID-19 positive mothers (18.18%), and neonatal sepsis for COVID-19 negative mothers (13.21%).

Table 2: Clinico-demographic Profile and Outcomes of Neonates Born to Mothers that Delivered at CGH from May 2020-July 2020 as to Mother's SARS-CoV-2 PCR Test Results (N=408)

Clinical Parameters	SARS-CoV-2 RT PCR Result of Mother			
	Positive (n=22)	%	Negative (n= 386)	%
APGAR Score				
> 7 at 1st minute of life	22	100	370	95.85
<7 at 5th minute of life	22	100	383	99.22
Ballard's Score				
< 37 weeks AOG	4	18.18	24	6.22
> 37 weeks AOG	18	81.82	362	93.78
Gender				
Male	15	68.18	210	54.4
Female	7	31.82	176	45.6
Birthweight (grams)				
<2500	5	22.73	38	9.84
2500-3499	17	77.27	297	76.94
3500 and more	0	0	51	13.21
Neonatal SARS-CoV-2 RT-PCR Swab Result				
Positive	0	0	Not tested	Not tested
Negative	22	100	Not tested	Not tested
Length of Stay				
<48 hours	10	45.45	281	72.8
>48 hours	12	54.54	105	27.2
Neonatal Morbidity				
Neonatal Pneumonia	10	45.45	63	16.32
Respiratory Distress Syndrome	0	0	3	0.78
Prematurity	4	18.18	24	6.22
Neonatal Sepsis	3	13.64	51	13.21
Neonatal Jaundice	2	9.09	14	3.63
Congenital Heart Disease	0	0	3	0.78
**Others	0	0	2	0.52
Neonatal Mortality	0	0	1	0.26

***Others: Persistent Pulmonary Hypertension, Spina Bifida*

The prevalence of asymptomatic COVID-19 positive mothers was 90.91% (20/22 mothers) compared to symptomatic positive mothers at 9.09% (2/22 mothers) among all mothers who tested positive for COVID-19 infection. The overall prevalence of positive test result among asymptomatic patients was 5.1% (20/392 asymptomatic mothers) as compared to the prevalence of positive test among symptomatic patients at 33% (2/6 symptomatic mothers). See Table 3. No asymptomatic mother who tested negative developed further symptoms or required further testing during their hospital stay.

Table 3: SARS-CoV-2 RT-PCR Results of Mothers Admitted for Childbirth, Stratified as to the Presence of COVID Symptoms (N=408)

Screening Characteristic	SARS-CoV-2 PCR Result	Mothers screened (n = 408)	
		Total	%
Asymptomatic Mothers on Admission	Positive	20	5.1%
	Negative	382	94.9%
Symptomatic Mothers on Admission	Positive	2	33%
	Negative	4	73%

DISCUSSION

The study findings revealed a wealth of data on the maternal and neonatal outcomes of mothers and neonates who were screened for COVID-19. The prevalence of COVID-19 among pregnant females noted in this study is 5.39% which is lower than the reported prevalence in New York City, USA, which had a prevalence of COVID-19 positive pregnant patients at 38%.[5]

This study also did not record any neonatal COVID-19 infection in the study population, in contrast to findings by Patil et al. where out of the 45 neonates that they tested for COVID-19 using RT-PCR swab, 42 neonates tested negative while 3 neonates tested positive.[5] In their study, RT-PCR nasopharyngeal swab were done after birth following their first bath. The 3 neonates who tested positive were monitored in their NICU, until 2 consecutive tests obtained at least 24 hours apart were negative and all of them remained asymptomatic, thus suggesting transient colonization. A systematic review and meta-analysis of 27 studies conducted last July 2020 by Dubey et al. also noted a low prevalence of COVID-19 infected neonates at only 1%, noting a rare transmission of infection to babies from mothers, although, more research is needed to examine the long-term outcomes of maternal infection and its interactions with neonates born to COVID-19 positive mothers.[6] Another study published last July 2020 by Salvatore et al. studied 120 neonates delivered to COVID-19 positive mothers.[7] Of the 120 neonates, all tested negative for SARS-CoV-2 by RT PCR nasopharyngeal swabs taken at 24 hours, 5-7 days and 14 days of life. These findings are the same with the results shown in our study although we only tested the neonates once at 24th hour of life. In contrast to a systematic review done last March 2020 by Chi et al. where they noted that 8.8% of the tested neonates were positive for SARS-CoV-2, indicating that the risk of vertical transmission should be considered.[8] The same literature reported that 8 out of the 14 studies included found no evidence of vertical transmission and could not detect the virus in amniotic fluid, cord blood, breast milk, serum, feces, placenta, nasopharyngeal, rectal or vaginal swabs of neonates that were tested. Wang et al. also reported that there was no positive RT-PCR result in neonate specimens obtained within 24 hours post-birth, implying no virologic evidence for congenital infection.[9] However, the serologic characteristics of infants reported showed 3 neonates with elevated IgM antibodies to SARS-CoV-2 born to mothers with COVID-19, suggesting a possible vertical transmission of SARS-CoV-2 from mother to newborn. They suggested that this inconsistency may be due to the disruption of the placenta or amniotic barrier caused by the inflammatory mediators from mothers that, induced by SARS-CoV-2, facilitates the transfer of IgG and IgM. Virologic evidence for supporting the in-utero transmission should be diagnosed based on RT-PCR test results of the samples from neonates but not IgM detection due to a high incidence of false-positive and false negative results. Further studies are warranted to draw a definite conclusion as to the transmission of infection from mothers to neonates.

Most of the COVID-19 positive mothers in our study population were between the ages of 20-39 (cumulative prevalence of 81.82%). A retrospective study done by Ayed et al. reported a maternal median age of 31 years old, with an age range of 22-40 years old for those diagnosed with COVID-19. Besides this being the noted age where most pregnancies occur, this is also the reported age by WHO where most COVID-19 cases occur.[1,10] These age groups are the most common members of the workforce, and being required or necessitated to be out of their homes for work signifies that they will be exposed more significantly to the virus as compared to the other age ranges. Studies done by Dubey et al., Chi et al. and Chamseddine et al. noted a higher rate of cesarean section among mothers who were COVID-19 positive, in contrast to our study where 54.55% of COVID-19 positive mothers had vaginal delivery.[6,8,11]

This study reported a prevalence of asymptomatic COVID-19 positive mothers at 90.09%, which supports the findings of Patil et al. where they noted that the majority of the SARS-CoV-2 positive

mothers (n = 27, 60%), were asymptomatic.[5] This was also similar to the study of Wang et al. where universal SARS-CoV-2 screening for women admitted for delivery found that all women with positive test results were asymptomatic at the time of testing.[9] The same study also noted that the clinical characteristics of COVID-19 infection in pregnancy were similar to those reported for non-pregnant adults with COVID-19 infection. In brief, Wang et al. reported the typical symptoms in positive pregnant women, includes fever, cough, myalgia, malaise and sore throat, and none of them developed severe COVID-19 pneumonia or died.[9] Salvatore et al. also noted 18 of the 22 mothers who were symptomatic within 7 days of delivery, that 18 (82%) had cough, 9 of whom also reported a fever.[7] These findings were similar to our study wherein 2 out of the 22 COVID-19 positive mothers presented with mild symptoms, both had cough as a presenting symptom upon admission.

One of the COVID-19 positive mothers had diabetes as comorbidity. According to one systematic review and meta-analysis by Allotey et al. that determined clinical manifestations, risk factors, and maternal and perinatal outcomes of COVID-19, they reported that maternal risk factors associated with severe COVID-19 were increasing age, high body mass index, chronic hypertension and pre-existing diabetes, which are known risk factors in the general population.[12] In another systematic review by Chamseddine et al., they also noted that the rates of gestational diabetes, hypertensive disorders of pregnancy and pre-eclampsia did not appear to be higher in pregnant women with COVID-19 compared to pregnant women without.[11] Fortunately, the mother in our study did not develop any complications of COVID 19.

Although this study did not report any COVID-19 infection among the neonates included, it is still important to know the neonatal outcomes of neonates born to COVID-19 positive mothers. All of our neonates born to COVID-19 positive mothers had an APGAR Score of >7 at their 1st and 5th minute of life, which is similar to the findings of the systematic review by Juan et al. that reported an APGAR score of 7 to 10 at 1st and 5th minute of life of neonates born to COVID-19 positive mothers.[13] Ayed et al. and Chamseddine et al. noted a prevalence of 35.3% (71/201 neonates) and 26.8% of preterm delivery, whereas, our study reported a prevalence of 18.18% of preterm delivery, however, those studies were not consistently clear whether early delivery was induced in light of obstetric complications or maternal SARS-CoV-2 infection.[10,11] Majority of the neonates in this study had a normal birth weight (77.27%), which is the same with other published literatures.[7,10]

This study showed that 45.45% (10/22) of neonates born to COVID-19 positive mothers were symptomatic at birth (presenting with tachypnea, subcostal retractions) and were treated as cases of neonatal pneumonia. All 10 neonates tested negative for SARS-CoV-2 using RT-PCR at 24th hour of life, chest x-ray showed signs of pneumonia, most required oxygen supplementation via nasal cannula or oxygen hood, and none required mechanical ventilation. Unfortunately, this population only had one swab done. 54.54% of neonates in this study required to be admitted for >48 hours in the isolation area/NICU for further management of comorbidities, but more importantly all neonates were eventually discharged improved. For the most part, some of the findings in this study were similar to those already reviewed in literature, including the presentation of neonatal pneumonia as a comorbid condition in 45.45% of neonates in this study and 26.5% in the study by Yoon et al.[14] The same study also reported other neonatal morbidities such as low birth weight (15.65%), small for gestational age (8.3%), respiratory distress syndrome (6.4%). However, all reported cases were discharged healthy or were still hospitalized in stable condition. A study by Flaherman et al. which included 263 neonates reported that adverse outcome, including preterm birth, NICU admission, and respiratory disease, did not differ between those born to mothers testing positive for SARS-CoV-2 and those born to mothers testing negative.[15]

CONCLUSION

In conclusion, this study noted a prevalence of 5.39% COVID-19 positive mothers in the study population. From the study findings, it could be observed that pregnant patients with COVID-19 display similar trends in the clinical features and maternal outcomes as with those who do not have the infection. SARS-CoV-2 virus was not detected in all of the neonates born to COVID-19 affected mothers. This study also noted that the neonates delivered to COVID-19 positive mothers had similar trends in the neonatal outcomes when compared to neonates delivered to mother who were COVID-19 negative. Mothers and care givers should be taught on proper isolation precautions, safe distancing, personal protective equipment use, safe breastfeeding and pumping of breastmilk to reduce the risk of transmission of COVID-19 to the newborn.

RECOMMENDATIONS

This study has several limitations. Since the study is a retrospective chart review, we were not able to follow up the patients after discharge to assess the mother and child for their well-being, presence of symptoms or any repeat testing done. A larger sample size and a follow up period with repeat testing might be needed to confirm that perinatal transmission is unlikely to occur if correct protective strategies are used. Further studies that would explore which maternal variables and neonatal outcomes that may be significantly associated with the risk of COVID-19 infection, which would then enable the determination of any significant predictors from these factors. This would greatly help clinical practice in the treatment and management of COVID-19 cases especially in this population and would also be a significant contribution to experts in the field of epidemiology, to be able to formulate preventive strategies based on any significant predictors that may be noted in future studies.

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ORIGINAL ARTICLE

RAPID DETECTION OF RESPIRATORY PATHOGENS USING A MULTIPLEX PCR ASSAY AMONG HOSPITALIZED CHILDREN WITH ACUTE RESPIRATORY INFECTION

Katherine B. Javier, MD
Josephine Anne Navoa-Ng, MD, FPPS, FPIDSP
Nikki Cotoco-Chu, MD, FPPS

Department of Pediatrics
St. Luke's Medical Center – Quezon City

Correspondence:
Dr. Katherine B. Javier
Email: jav.katherine@gmail.com

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: Acute respiratory infection (ARI) is a major cause of morbidity and mortality among children worldwide however, local data on the etiologic diagnosis of ARI are limited.

Objectives: To determine the prevalence and the most commonly detected respiratory pathogens using a multiplex PCR assay, known as the Respiratory Panel, among hospitalized children with ARI and compare their clinical and laboratory differences.

Methods: This is a cross sectional study of children with ARI who were tested with a multiplex PCR assay. Retrospective chart review was done on these patients admitted from January 2018 to February 2020.

Results: There were 47 charts reviewed, mean age was 4.2 years old. Out of 47 patients, 36 (76.6%) tested positive for a pathogen. Respiratory syncytial virus (RSV) being the most common followed by Influenza A/H1-2009 and Human metapneumovirus (hMPV). Two patients had viral co-infections and no bacteria were detected on all subjects. 61.7% patients were started on antibiotics on admission. Fever and cough were the most common sign and symptom, respectively. Normal WBC (68% with neutrophilic predominance) and platelet were detected in 72.3% and 70.2% of patients, respectively; 50% of patients had normal CRP and 60.5% had abnormal findings on chest x-ray. Only the presence of chest x-ray findings was found to have a higher probability of yielding a positive Respiratory Panel p=0.27.

Conclusion: Among admitted patients with ARI, 76.6% tested positive for a respiratory pathogen. All were caused by viruses presenting as nonspecific manifestations - fever and cough. Clinical manifestations, CBC and CRP showed no association with the Respiratory Panel result while abnormal chest x-ray had a higher probability of yielding a positive Respiratory Panel result.

KEYWORDS: *Acute respiratory infection, Respiratory Panel, Multiplex PCR assay*

INTRODUCTION

Bacteria and viruses have been reported as the main causes of acute respiratory infections (ARIs). An estimated 50% of all illnesses in all age groups, and approximately 75% of illnesses in young children are viral upper respiratory tract infections (URTI).[1] In children under 5 years, ARIs are mainly due to viruses -- RSV, parainfluenza viruses, influenza virus A and B, and hMPVs are the most common viruses isolated.[2] In most developing countries like the Philippines, data on the etiologic diagnosis are limited, primarily because of difficulties in obtaining adequate samples and the low sensitivity of presently available diagnostic methods, such as blood culture and serological tests. Viral etiology studies likewise are uncommon.[3]

A community-based ARI research named Philippine Control of Acute Respiratory Infections program (Phil-CARI) was established in 1981. By 1989, the community-based research collaboration between the Research Institute for Tropical Medicine (RITM) and the Australian government generated about 7 years' worth of valuable local data on baseline ARI incidence and risk factors. They confirmed the high incidence of ARI, at 6.1 episodes per child-year, among Filipino children less than 5 years old in depressed urban communities in Metro Manila, with a peak age incidence between 6 and 23 months. The incidence of acute lower respiratory tract infection (LRTI) was also found to be exceedingly high, at 0.5 episode per child-year.[4] In 1984, an outpatient clinic and hospital study was conducted among Filipino children 5 years old and below living in a periurban slum and a middle-class neighborhood. A total of 198 viral infections was confirmed in 162 patients (51.9%), 42.3% with single viral infection and 9.6% with mixed (two or more) infection. The infections were measles (21.4%), influenza A (15.9%), parainfluenza types 1, 2, and 3 (8.8%), RSV (7.1%), influenza B (5.8%), enteroviruses (5.1%), adenoviruses (39%), herpes simplex virus (1.6%), and cytomegalovirus (1.3%).[5] From April 1990 to December 1992, another local study on the etiology of acute LRTI was identified in 119 (36.9%) of 317 hospitalized children below 5 years of age. A higher proportion of respiratory viruses (27.2%) than bacterial agents (10.7%) were identified through blood culture, nasopharyngeal aspirate culture and immunofluorescence technique. Viral agents (adenovirus, RSV, parainfluenza 3, influenza A and influenza B) and bacterial agents (mainly *Haemophilus influenzae* and *Streptococcus pneumoniae*) are the pathogenic agents involved in acute LRTI among Filipino children less than 5 years old.[3]

After the discovery of polymerase chain reaction (PCR), there are many important milestones in the evolution of diagnostic molecular tests. A multiplex point-of-care diagnostic technology (MPOCT) can test the presence of multiple infectious pathogens within a specimen. The test results can be obtained within 15 minutes to several hours. The development of new molecular panel diagnostics that can provide results this fast would provide both clinical and economic benefits. Analysis of the results provides the clinician with an opportunity to administer directed therapies in a short time. The set of tests on a multiplex technology is known as a test panel. Syndromic test panels are designed to test for multiple diseases associated with a similar set of symptoms, or a syndrome and these panels help in the evaluation of the etiology of the disease at the point of care. Respiratory panels and gastrointestinal panels are two examples of syndromic panels.[5] MPOCT has been utilized in other countries and is now being used as rapid diagnostic tests in many parts of the world. St. Luke's Medical Center, Quezon City, a tertiary private hospital, acquired a Biofire® FilmArray® Multiplex PCR system (mPCR) last January 2018. This mPCR system, commonly known as the Respiratory Panel, provides a rapid and accurate identification of causative agents of respiratory tract infections. Thus, we would like to determine the prevalence of the respiratory pathogens and to determine the most common pathogens involved among admitted children with ARI. We would also like to determine the correlation of the presence and absence of a respiratory pathogen with the clinical manifestations, laboratory and chest x-ray findings of admitted children with ARI.

MATERIALS AND METHODS

Study design and participants

This is a cross-sectional study, using retrospective chart review of pediatric patients admitted in St. Luke's Medical Center – Quezon City who were tested with Biofire® Filmarray® Respiratory Panel for respiratory pathogens via mPCR from January 2018 to February 2020.

Inclusion and exclusion criteria for subject selection

This study enrolled consecutive pediatric patients (below 19 years old) admitted for ARI and underwent Biofire® Filmarray® Respiratory Panel (with positive or negative results). An ARI is defined as the presence of any symptom and/or signs such as cough, difficulty of breathing, sore throat or rhinorrhea. Patients with chronic medical conditions as defined by ICD-9 code were excluded from the study.[6]

Description of the study procedure

Between January 2018 to February 2020, records of pediatric patients with acute respiratory infection who were tested with Biofire® Filmarray® Respiratory Panel were included. The Respiratory Panel is a multiplex PCR system that tests for the presence of Adenovirus (AdV), Coronavirus HKU1, Coronavirus NL63, Coronavirus 229E, Coronavirus OC43, hMPV, Human RhV/ Enterovirus, Influenza A, Influenza A/H1, Influenza A/H1-2009, Influenza A/H3, Influenza B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, RSV, *Bordetella pertussis, Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. The test is manufactured in Salt Lake City, Utah. Sensitivity ranges from 94.6-100% for most of the viruses, with the lowest sensitivity of 90% for influenza A. Specificity is 98.3-100% for most of the viruses in the test with 94.6% for hMPV and 89.1% sensitivity for RSV. A nasopharyngeal swab is obtained and placed on a Filmarray® reagent pouch. This pouch stores all the necessary reagents for sample preparation, reverse transcription, PCR and detection then placed on the Filmarray® machine. The Filmarray® software automatically generates a result for each target in a single report within an hour. A Respiratory Panel test roughly costs 23,000 in Philippine pesos.

Characteristics and variables such as age, sex, clinical presentations, CBC, CRP, chest x-ray results and management on admission were determined in this study. The prevalence of respiratory pathogens, the most commonly detected pathogens and the signs and symptoms associated with either Respiratory Panel positive or negative were analyzed.

Sample size estimation

Sample size was calculated based on the population proportion estimation. Sample size as calculated based on the proportion of patients positive for RSV (it having the highest sample size calculation possible of all respiratory microorganisms). Assuming that the proportion of patients with RSV is 48% with a maximum allowable error of 7.5-10%, and a reliability of 80%, sample size required is 42-74.[7]

Mode of data analysis

Determination of Respiratory Panel result, prevalence and identification of respiratory pathogens, signs and symptoms of ARI, laboratory and chest x-ray findings were determined using frequency and percentage. Association of the clinical characteristics and laboratory and chest x-ray findings with the Respiratory Panel result was determined using Fisher's exact test. Level of significance was set at $p<0.05$.

Ethical considerations

The study upholds the highest ethical standard of confidentiality, transparency and integrity in processing personal information. The study abides by the Principles of the Declaration of Helsinki (2013) and was conducted along the Guidelines of the International Conference on Harmonization-Good Clinical Practice (ICH-GCP). The clinical protocol and all relevant documents were reviewed and approved by the SLMC Institutional Ethics Review Committee on October 14, 2019. Patient confidentiality was respected by ensuring anonymity of patient records. Each patient document is coded and did not contain any identifying information in order to ensure confidentiality. The chart review was done by the author and was done in the hospital premises. All study data were recorded and investigators were responsible for the integrity of the data.

The manner of disseminating and communicating the study results guarantee the protection of the confidentiality of patient's data. All study-related documents such as the all versions of the protocol, ethical clearance, data collection forms, hard copies of source documents, are kept and stored by the principal investigator in strict confidentiality; after which they will be shredded. Data collection commenced upon approval of the research protocol by the Institutional Review Board and Institutional Ethics Review Committee. The study was self-funded, one of the authors though was a lecturer for Biofire® Filmarray® Respiratory Panel in 2020. The authors deny any other conflicts of interest.

RESULTS

Overall, 65 Respiratory Panel tests were done in children within the study period. All tested patients presented with signs and symptoms of ARI but 5 were done as outpatient. 13 patient records were found to have chronic illnesses like SLE, severe pneumonia and septic shock with multi-organ dysfunction syndrome, acute flaccid myelitis, SMA type 1, Down's syndrome and acute lymphoblastic leukemia, chronic lung disease and were excluded in the study while 1 patient had 4 Respiratory Panel tests done within the study period. A total of 47 patient records met the inclusion and exclusion criteria.

Table 1 shows the characteristics of admitted patients with ARIs included in this study. Majority of the patients were females comprising 53.2% and most of which belong to the age group of 1-5 years old. On admission, 61.7% were started on antibiotics. Among those who tested positive for Respiratory panel, 58.3% were started on antibiotic. After the positive Respiratory Panel result was relayed, 23.8% discontinued the antibiotic. Among 11 patients who tested negative for Respiratory Panel, 72.7% were started on antibiotic. After the negative Respiratory Panel result was relayed, 14.3% discontinued the antibiotic.

Table 1: Demographic Profile of patients with ARI who underwent Respiratory Panel

		***n* = 47(%)**
Gender	Male	22 (46.8)
	Female	25 (53.2)
Age (in years)	<1	8 (17)
	1-5	26 (55.3)
	6-12	10 (21.3)
	13-18	3 (6.4)
Management on admission	Supportive	18 (38.3)
	Antibiotic	29 (61.7)

At least 1 pathogen was detected on 76.6% of patients, all of which were viruses. Single viral infection was detected in 34 (72.3%) and viral co-infections in 2 (4.3%).

All respiratory pathogens detected were viruses, RSV being the most prevalent followed by influenza A/H1-2009 and hMPV. Co-infection of viruses were detected in two patients: RSV-RhV/enterovirus and coronavirus 229E-hMPV. No viral-bacterial and bacterial co-infections were detected.

Table 2: Respiratory pathogens in children with ARI who underwent Respiratory Panel

Pathogen	***n* = 47(%)**
None detected	11 (23.4)
RSV	10 (21.3)
Influenza A/H1-2009	8 (17)
hMPV	5 (10.6)
Influenza B	3 (6.4)
Human RhV/Enterovirus	3 (6.4)
Parainfluenza Virus 4	2 (4.3)
Influenza A/H3	2 (4.3)
Parainfluenza Virus 2	1 (2.1)
Influenza A/H1	1 (2.1)
Coronavirus NL63	1 (2.1)
Coronavirus 229E	1 (2.1)
Adenovirus	1 (2.1)
Parainfluenza Virus 1	0
Parainfluenza Virus 3	0
Influenza A	0
Coronavirus OC43	0
Coronavirus HKU1	0
Bordetella pertussis	0
Chlamydophila pneumoniae	0
Mycoplasma pneumoniae	0

In children 5 years old and below, ARIs were caused mainly by RSV (21.3%, n = 10), Influenza A/H1-2009 (12.8%, n = 6) and hMPV (10.6%, n = 5). All children positive for RSV were noted to be less than 5 years old (see Table 3).

Table 3: Respiratory pathogens per age group in children with ARI

Pathogen	**Age (in years), *n*= 47 (%)**			
	<1	**1-5**	**6-12**	**13-18**
None detected	1 (2.1)	5 (10.6)	3 (6.4)	2 (4.3)
RSV	6 (12.8)	4 (8.5)	0	0
Influenza A/H1-2009	0	6 (12.8)	1 (2.1)	1 (2.1)
Human Metapneumovirus	0	5 (10.6)	0	0
Influenza B	0	1 (2.1)	2 (4.3)	0
Human Rhinovirus/ Enterovirus	1 (2.1)	1 (2.1)	1 (2.1)	0
Parainfluenza Virus 4	0	1 (2.1)	1 (2.1)	0
Influenza A/H3	0	2 (4.3)	0	0
Parainfluenza Virus 2	0	0	1(2.1)	0
Influenza A/H1	1(2.1)	0	0	0
Coronavirus NL63	0	0	1(2.1)	0
Coronavirus 229E	0	0	1(2.1)	0
Adenovirus	0	1 (2.1)	0	0
TOTAL	**9 (19.2)**	**26 (55.3)**	**11 (23.4)**	**3 (6.4)**

Table 4 shows that the most common physical examination finding was fever having a mean maximum temperature of 39.2°C, followed by retractions and nasal obstruction or discharge. Cough was the most common symptom followed by rhinorrhea. When positive and negative Respiratory Panel results were compared, a respiratory pathogen is more likely to be detected with the presence of fever, retractions, tachypnea, wheeze, crackles, cough and difficulty of breathing (see Table 5). No statistically significant relationship was noted on all variables measured.

Table 4: Clinical manifestations of children with ARI who underwent Respiratory Panel

Signs	*n =47* (%)
Fever	43 (91.5)
Nasal obstruction/discharge	36 (76.6)
Retractions	37 (78.7)
Tachypnea	15 (31.9)
Wheeze	18 (38.3)
Crackles	23 (48.9)
Symptoms	
Rhinorrhea	37 (78.7)
Sore throat	5 (10.6)
Cough	42 (89.4)
Difficulty of breathing	14 (29.8)

Table 5: Comparison of signs and symptoms of ARI between positive and negative Respiratory Panel

Variable		**Positive (*n*=36) *n* (%)**	**Negative (*n*=11) *n* (%)**	***p-value***
SIGNS				
Fever	Present	34 (79.1)	9 (20.9)	0.229
	Absent	2 (50)	2 (50)	
Nasal obstruction/ discharge	Present	27 (75)	9 (25)	0.492
	Absent	9 (81.8)	2 (18.2)	
Retractions	Present	16 (88.9)	2 (11.1)	0.111
	Absent	20 (69)	9 (31)	
Tachypnea	Present	12 (80)	3 (20)	0.507
	Absent	24 (75)	8 (25)	
Wheezes	Present	15 (83.3)	3 (16.7)	0.312
	Absent	21 (72.4)	8 (27.6)	
Crackles	Present	20 (87)	3 (13)	0.101
	Absent	16 (66.7)	8 (33.3)	
SYMPTOMS				
Rhinorrhea	Present	28 (75.7)	9 (24.3)	0.570
	Absent	8 (80)	2 (20)	
Sore throat	Present	2 (40)	3 (60)	0.076
	Absent	34 (81)	8 (19)	
Cough	Present	33 (78.6)	9 (21.4)	0.332
	Absent	3 (60)	2 (40)	
Difficulty of breathing	Present	12 (85.7)	2 (14.3)	0.287
	Absent	24 (72.7)	9 (27.3)	

Table 6 summarizes the laboratory and radiologic findings gathered. Leukopenia and thrombocytopenia were detected in 14.9% and 4.3% of patients, respectively. Mean WBC was 10.08 x 10^9/L and stabs were seen on 12.8% of patients and 68% had neutrophilic predominance. Mean platelet count was 307 x 10^9/L. CRP was done on 24 patients, half of patients had abnormal CRP, 20.8% had equivocal CRP and 29.2% had high CRP. Chest x-ray was done on 38 patients, 60.5% were radiologically diagnosed with LRTI. On Table 7, positive and negative Respiratory Panel results were compared and the presence of x-ray findings had a higher probability of yielding a positive Respiratory Panel (p = 0.027).

Table 6: Laboratory and radiologic findings of children with ARI who underwent Respiratory Panel

Laboratory findings	***n* (%)**
WBC	*n=47*
Normal	34 (72.3)
Low	7 (14.9)
High	6 (12.8)
Predominance of differential count	*n=47*
Neutrophilic	27 (57.4)
Neutrophilic + stabs	5 (10.6)
Lymphocytic	14 (29.8)
Lymphocytic + stabs	1 (2.1)
Platelet	*n=47*
Normal	33 (70.2)
Low	2 (4.3)
High	12 (25.5)
CRP	*n=24*
Normal	12 (50)
Equivocal	5 (20.8)
High	7 (29.2)
Findings on chest x-ray	*n=38*
With radiographic findings	23 (60.5)
Normal	15 (39.5)
X-ray findings	*n=24**
Streaky densities	7 (29.2)
Linear/Hazy opacities	7 (29.2)
Interstitial lung findings	7 (29.2)
Consolidation	2 (8.4)
Increased peribronchial markings	1 (4.2)

*One patient had more than 1 CXR finding.

Table 7: Comparison of laboratory and chest x-ray findings between positive and negative Respiratory Panel

Variable		**Positive (*n*=36) *n* (%)**	**Negative (*n*=11) *n* (%)**	***p-value***
WBC	Normal	25 (73.5)	9 (26.5)	0.266
	Low	7 (100)	0 (0)	
	High	4 (66.7)	2 (33.3)	
Predominance of differential count	Neutrophilic	20 (74.1)	7 25.9)	0.927
	Neutrophilic + Stabs	4 (80)	1 (20)	
	Lymphocytic	11 (78.6)	3 (21.4)	
	Lymphocytic + Stabs	1 (100)	0 (0)	
Platelet	Normal	26 (78.8)	7 (21.2)	0.507
	Low	2 (100)	0 (0)	
	High	8 (66.7)	4 (33.3)	
CRP	Results	N=19	N=5	0.826
	Normal	10 (83.3)	2 (16.7)	
	Equivocal	4 (80)	1 (20)	
	High	5 (71.4)	2 (28.6)	
X-ray	Results	N=32	N=6	0.027
	Normal	10 (66.7)	5 (33.3)	
	With radiographic findings	22 (95.7)	1 (4.3)	

DISCUSSION

Our retrospective study of admitted children with ARI tested with Respiratory Panel revealed a 76.6% prevalence rate of respiratory viruses. This finding is not surprising since most pediatric ARIs are of viral origin and the risk of concurrent (or subsequent) bacterial infection has been reported to be low in children over three months of age. As the agents of ARIs, viruses have constantly been shown to predominate.[8]

All of our subjects who tested positive for Respiratory Panel were due to viruses and no bacteria were detected. Our study showed similar findings of previous local study on acute LRTI in children that identified a higher proportion of viruses (27.2%) than bacterial agents (10.7%).[3] This is same as the study done among hospitalized children with ARI in China, wherein at least 1 virus was detected on 74.7% hospitalized children with ARI and only 22.2% had at least 1 bacteria detected.[9] Other studies regarding hospitalized children with ARIs had viral detection rates of 35-95%.[1,7] Possible explanations for the wide differences in detection rates in the literature include heterogeneity in study populations, differences in presenting respiratory symptoms, number of respiratory pathogens tested, method used for detection and genetic variability between populations.[1]

Children 5 years old and below comprise 72% of the total population for this study and most of the respiratory pathogens were detected in this age group. All RSV positive patients belong to this age group. RSV is responsible for more than 50% of cases of bronchiolitis and may also cause pneumonia especially in children less than 2 years old.[11] A local study done in 1989 supported the high incidence of ARI among Filipino children less than 5 years old in depressed urban communities in Metro Manila.[10] The higher detection rate of respiratory pathogens among infants and young children has been ascribed to a higher infection rate, lower viral clearance rate due to underdeveloped immune system and incomplete vaccinations. Furthermore, parents of younger children may seek healthcare earlier in the course of disease due to parental anxiety.[1]

Using the Respiratory Panel, RSV was the most frequent respiratory virus detected. In a local study, RSV was the second most frequently detected virus out of all ARI cases however, RSV was the most frequently detected among hospitalized patients.[12] The results of this study is comparable to several studies done abroad.[2,7,13,14] When categorized into upper and lower respiratory tract infections, the leading viral etiology of LRTI morbidity and mortality globally is RSV while RhV for URTI.[2,15] Symptoms of ARI include cough or difficulty of breathing, other signs and symptoms including fever, nasal obstruction/discharge, retractions, tachypnea, crackles, wheezes, rhinorrhea and sore throat. Our subjects presented mainly with LRTI reflected by the combination of cough and presence of retractions as the most common clinical manifestations. Acute cough in children is mostly caused by URTI but may also be a manifestation of serious conditions like bronchiectasis and the presence of chest wall indrawings identifies more severe disease like bronchiolitis and pneumonia which are LRTIs.[15,16] These findings may also be related to RSV being the most commonly detected respiratory pathogen in this study and that 60.5% of patients with chest x-ray had abnormal findings.

The presence of a sign or symptom of ARI did not show a significant relationship with the presence of a respiratory pathogen.[17] In cases of dual infections, a study found that fever and cough were the two main significant predictors for virus co-infection. Fever alone was a significant predictor for bacteria co-infection. Fever, cough, and sputum were significantly more frequent in virus and bacteria co-infection cases than monodetected.[9] Only 2 cases out of 36 patients with detected pathogens have viral co-detection in our study. Both Human Rhinovirus/Enterovirus and RSV were detected in one patient while Coronavirus 229E and hMPV in another. There were no viral-bacterial and bacterial co-infections. Among these 4 viruses, only Coronavirus 229E was not detected as a single pathogen for a patient with ARI. One study compared the clinical manifestations and laboratory tests for patients with negative detection, single infection, and co-infections. No statistically significant differences was found in terms of CRP level and CBC counts.[18]

Whether the detected pathogens are actually the cause of the respiratory symptoms or are simply colonizing the respiratory tract during symptomatic episodes remains unclear. It can be speculated that not every infection with a pathogen leads to respiratory symptoms and that pathogenicity might depend on host or environmental factors.[17] Primary infections with viral pathogens can predispose to secondary bacterial infections.[7] On the other hand,

vaccinations provide protection from vaccine preventable diseases (VPD) which in turn decrease the spread of related diseases, and improve child survival prospects (as children, particularly those under five years old, are more likely than adults to die from VPDs).[19] VPDs caused by *Bordetella pertussis, S. pneumoniae, H. influenzae type B, Corynebacterium diphtheriae*, measles virus and influenza virus frequently cause respiratory tract diseases.[20] The vaccination history of our study subjects was not looked into but this is an important aspect that should be explored as this may explain as to why no bacterial pathogen was detected in our subjects.

The possibility of detecting multiple targets in a single sample is particularly important when multiple different pathogens can cause the same clinical presentation. mPCR assays enable detection of an array of viruses with higher specificity, sensitivity, and faster turn-around time than previous testing using immunoassays or cultures.[21] Potential advantages also include conserving and optimizing analysis of other samples, simplify ordering algorithm as only one test needs to be requested, potential saving in reagents by testing multiple organism at once compared to testing each pathogen separately and standardized testing.[22] However, these assays also have their own limitations. Until now, implementation of multiplex molecular tests in clinical laboratories has been hindered by the high cost of the kits. Other disadvantages include false positive results due to cross reactivity or unspecific amplification caused by multiple primers/targets present in the reaction, false negative results due to use of preferential amplification of one target over the other, negative internal control due to exhaustion of reagents in samples with a high amount of one particular target.[22] Despite of these limitations, mPCR assays are being adopted rapidly in clinical practice.

The use of mPCR testing for respiratory viruses among hospitalized patients was significantly associated with decreased healthcare resource utilization including decreased use of chest radiographs.[21] Unlike CBC and CRP, a chest x-ray cannot differentiate between a viral or bacterial pathogen but an abnormal finding would mean a LRTI exists. This study showed that there is a higher probability of having a positive Respiratory Panel result when chest x-ray is positive however, 4.3% of patients will have a negative Respiratory Panel. A negative Respiratory Panel might be due to other bacterial pathogens not included in the panel. The prevalence of respiratory viruses in this study could enable approximation on the local epidemiology of respiratory infections and will influence physicians to decide on the management. Immunization status is also relevant because children fully immunized against *H. influenzae type b* and *S. pneumoniae* are less likely to have VPDs caused by these pathogens. However, in cases of diagnostic dilemmas like worsening of a disease severity despite of proper management and inconclusive laboratory and ancillary findings, Respiratory Panel is highly suggested. The utility of chest x-ray as a diagnostic tool for respiratory pathogens has not been established since there is limited evidence to support its routine use in distinguishing between viral and bacterial infections.[23] CRP, on the other hand, is one of the most frequently evaluated and indicative biomarkers for identifying bacterial infections in children because their levels are higher in bacterial infections than in viral infections.[24] In this study though, equivocal and high CRP were detected in half of the patients tested. This figure almost match the high CRP values in a study wherein 66.5% of patients with ARI was positive for a viral pathogen using a mPCR that detects 14 respiratory viruses and 59% of them had abnormal CRP.[8] Respiratory Panel can detect *Bordetella pertussis, Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae* which are causes of atypical pneumonia. These organisms do not respond to penicillin derivatives, which may cause failure of antibiotic empirical therapy. Moreover, these fastidious bacteria are difficult to identify by culture or serology, and therefore often remain undetected. Thus, rapid and accurate identification of bacterial pathogens causing atypical pneumonia is crucial and need to be treated with macrolides.[25] However, the cost of a Respiratory Panel is still quite prohibitive compared to antibiotics and laboratory tests necessary for the

diagnosis and management of an atypical pneumonia and other ARIs.

The impact of mPCR on physician antimicrobial prescription practices remains unclear. In this study, we observed that antibiotics are prescribed too often. Among patients with ARI, 58.3% was already started on antibiotic and after the positive viral pathogen was relayed, 23.8% discontinued the antibiotic and 87.5% of patients with a negative Respiratory Panel result discontinued the antibiotic. In a study by Mcculloh, physicians started antibiotics more often in children with a negative Respiratory Panel result and occasionally discontinued antibiotics in children diagnosed with a viral pathogen.[26] These results suggest that Respiratory Panel testing may enhance physician decision-making when prescribing antimicrobials in children hospitalized with ARI. In another study, mPCR testing on admission was associated with less use of antibiotics compared with testing with non-mPCR based methods.[21] In a multicenter pediatric study, interviewing medical doctors on fictitious ARI cases, RT-PCR decreased antibiotic use. However, in real life, the same physicians did not alter their antibiotic prescriptions based on the results of RT-PCR.[27] The difference in findings could indicate that provider decisions for antibiotic use in the emergency department or ambulatory setting may be more impacted by clinical factors like physical examination or past medical history and less impacted by mPCR test results. In addition, in these settings, decisions are made within shorter time frames, and despite the relatively rapid turn-around time for mPCR testing, results may still not be timely enough to impact decision making.[21]

Most viral infections are supportively managed, hence rapid viral detection may help to make appropriate decisions and decrease unnecessary antibiotic use.[18] The precise diagnosis of certain viruses may contribute to timely antiviral agent treatment as well, like oseltamivir against influenza infection. In this study, Influenza A/H12009 is the most commonly detected pathogen in children 1-5 years old and the 2nd most commonly detected pathogen in all children admitted with ARI, but if all influenza virus variants will be combined, it turns out to be the most common virus in all ages. The Influenza A and B viral antigen rapid test has a sensitivity of 94.7% and specificity of 94% for influenza A, while a sensitivity of 91.7% and specificity of 97.5% for influenza B. This is almost the same as the Respiratory Panel having a sensitivity of 90-100% and specificity of 100% for influenza A, and 100% sensitivity and specificity for influenza B. Rapid diagnosis of influenza viruses and early treatment with oseltamivir is crucial.[28] Therefore, influenza rapid testing may be done instead for Respiratory Panel in detecting Influenza virus. It is a highly sensitive and specific test and relatively cheaper than the Respiratory Panel. The Centers for Disease Control and Prevention (CDC) also recommended that those patients who present with a syndrome consistent with influenza and have a negative rapid antigen test result should either receive a confirmatory RT-PCR test or be treated as if they have influenza.[29]

The guidelines of the Royal College of Paediatrics and Child Health (RCPCH) and the European Society of Paediatric Infectious Diseases (ESPID) recognize that RT-PCR is increasingly replacing immunofluorescence and serology, but they have not given recommendations when to use it and what the consequences are of the results when they become available.[27] One of the major drawbacks of the Respiratory Panel is its very high cost and despite the clinical impact of respiratory virus infections, its cost-effectiveness is incompletely understood.[29] Respiratory Panel mainly detects viruses and detects 3 bacterial species only, other common pathogens like *Streptococcus pneumoniae* and *Hemophilus influenzae* are not included in the panel hence single bacterial infections and co-infections that may modify the impact of respiratory pathogens on symptom could have not been detected. Hence, the researchers also looked on how the most common laboratory tests and the prevalence of respiratory pathogens may serve as a substitute for the Respiratory Panel.

CONCLUSION AND RECOMMENDATIONS

In conclusion, the prevalence of respiratory pathogens among admitted children with ARI in our institution during the study period showed 76.6% of children tested positive for Respiratory Panel and all of which were viruses. RSV was the most prevalent virus detected followed by Influenza A/H1-2009 and hMPV. Viral co-infections were detected in 4.3%. Due to similarities of viral and bacterial ARIs, empiric antibiotic may lead to antibiotic misuse. Respiratory Panel, as an emerging multiplex POCT system for the simultaneous detection of different pathogens, provides rapid and high-yield results which can guide diagnosis, therapy and infection control measures.[5]

This study was done in a comparatively small sample size and may be continued on a larger scale. The lack of statistical significance on some of the findings might improve with increase in number of subjects. Another limitation is that patients involved were admitted patients, implying that clinical manifestations are relatively more severe. Further studies may also include patients seen on an outpatient basis.

Some studies found high susceptibility for ARIs after natural viral infections. The detection of pathogens does not always mean that it is the cause of the current ARI but might be due to a previous or co-existing viral illness that could have predisposed to the current ARI. A better understanding of the etiological role of viral infections and the risk for subsequent ARIs is also needed for the prevention and management of childhood ARIs.

Lastly, future prospective studies to further assess the impact of Respiratory Panel on outcomes including arriving at a correct diagnosis, time to diagnosis, use or misuse of antibiotics, minimizing other diagnostic tests, length of hospital stay and clinical course is recommended.

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ORIGINAL ARTICLE

CLINICAL CHARACTERISTICS AND PATIENT SYMPTOMS ASSOCIATED WITH POOR OUTCOMES AMONG CHILDREN WITH COVID-19: A RAPID REVIEW

Krista Maye D. Catibog, MD[1]
Ian Theodore G. Cabaluna, RPh, MD, GDip[2,3]
Anna Lisa T. Ong-Lim, MD[1]
Chrizarah A. San Juan, MD[1]
Maria Angela M. Villa, MD[1]
Leonila F. Dans, MD, MSc[1]

[1] Department of Pediatrics, University of the Philippines-Philippine General Hospital, Manila
[2] Department of Clinical Epidemiology, University of the Philippines, Manila
[3] Pacific Center for Evidence Based Healthcare, Manila

Correspondence:
Dra. Krista Maye D. Catibog
Email: kmdcatibog@gmail.com

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

Objective: To identify specific clinical characteristics and patient signs and symptoms that increase the risk of developing severe/critical COVID-19 disease or death in the pediatric population, and identify strength of these associations

Methodology: A systematic search was done in PubMed, Science Direct, Cochrane Library and grey literature databases focusing on severe and critical COVID-19 disease in the zero to eighteen year old age group until August 26, 2020. Data regarding patient characteristics, signs and symptoms on admission and disease severity were extracted. Outcomes measured were severe or critical COVID-19, Multisystem Inflammatory Syndrome in Children (MIS-C) or death. Results were pooled and meta-analyzed.

Results: Four eligible studies with a total of 292 pediatric patients with COVID-19 were examined. Older children (MD=6.62, 95%CI=4.23 to 9.00, p-value<0.00001, I^2=33%) significantly present with a higher percentage of severe disease. Shortness of breath (OR=8.14, 95%CI=2.33 to 28.47, p-value=0.001, I^2=42%) was also found to be associated with severe COVID-19 disease. The presence of a pre-existing medical condition (OR=4.02, 95%CI=1.55 to 10.43, p-value=0.004, I^2=0%), especially cardiac disease (OR=6.40, 95%CI=1.45 to 28.38, p-value=0.01, I^2=13%) and diabetes (OR=7.01, 95%CI=1.54 to 31.95, p-value=0.01, I^2=0%) was noted to be a risk factor for severe disease.

Conclusion: Based on poor quality observational studies, older age group, shortness of breath, and a pre-existing medical condition, especially cardiac disease or diabetes were found to be associated with poor outcomes in children with COVID-19.

KEYWORDS: *COVID-19, Pediatrics, Disease severity*

INTRODUCTION

The 2019 Novel Coronavirus Disease, now known as Coronavirus Disease 2019 (COVID-19) presents with a wide clinical disease spectrum. Mild disease with fever, cough and fatigue are typically seen, but severe respiratory symptoms associated with other systemic disorders have also been reported.[1] COVID-19 has been a cause of multiple fatalities. More than 23 million cases have already been documented globally as of August 2020, with the pediatric population accounting for 1-5% of cases.[2,3]

Since the start of the pandemic, cases of COVID-19 reported among children have mostly been mild with good prognosis.[3-5]However little is known about the other side of the disease spectrum, and as more individuals are diagnosed, the number of severe and critical cases among children have increased as well. In a nationwide study conducted in China between January 16 to February 8, 2020 among 2,135 pediatric patients with confirmed and suspected COVID-19, more than 80% had mild to moderate disease, 5.2% presented with severe illness, while 0.6% were critical.[6] In another study from a tertiary medical center in New York City, 28% (N=67) of children with positive COVID-19 results necessitated admission to an intensive care unit, with 1 death reported.[7] As of April 2020, there have been reports of a new phenotype of severe to critical pediatric cases occurring weeks after an outbreak of SARS-CoV-2. Disease symptomatologies likened to Kawasaki disease and other hyperinflammatory illnesses were reported with a significant increase in incidence (OR 184, p<0.00001).[8,9] In a report from the United Kingdom, a mortality was attributed to a large cerebrovascular infarction, while another patient developed a giant aneurysm after being discharged from the intensive care unit.[10] This condition is now referred to as Multisystem Inflammatory Syndrome in Children (MIS-C).[9]

It is important to identify the pediatric population at risk of developing severe or critical disease, as delayed recognition may lead to development of complications or death. The objectives of this review are to (1) identify specific clinical characteristics and patient signs and symptoms that increase the risk of developing severe/critical COVID-19 disease or death in the pediatric population, and (2) identify strength of these associations.

MATERIALS AND METHODS

Databases (PubMed, Cochrane Library and Science Direct) and grey literature database (Google Search, ChinaXiv, Medrxiv, Biorxiv) were searched for relevant studies on August 26, 2020 and published studies from December 01, 2019 to August 26, 2020 were reviewed. The pre-defined keywords “Coronavirus Infections", "Coronavirus", “novel coronavirus”, “NCOV”, "COVID-19", “COVID-2019”, "severe acute respiratory syndrome coronavirus 2", “SARS-COV2”, “death”, “mortality”, “critical”, “severe”, “outcomes”, “intensive care unit”, “prognosis”, “signs”, “symptoms”, “pediatric”, “neonates”, “children”, and “adolescents”, with Medical Subject Headings (MeSH terms) and Boolean operators were used for a comprehensive and organized search strategy. Reference lists of studies were reviewed for inclusion. A total of 3,622 articles came up in the initial search. Three review authors (KDC, IGC, CSJ) screened and appraised the articles based on the inclusion criteria:

1. Type of participants: Patients diagnosed with COVID-19 aged 18 years old and below
2. Types of exposure: Epidemiology, clinical characteristics, Signs & symptoms
3. Types of outcome measures: Severe COVID-19, critical COVID-19, MIS-C or death
4. Types of study designs: Systematic reviews and meta-analyses, observational studies

Duplicate articles and studies which did not satisfy the inclusion criteria were removed, as well as reports which only contained abstracts or had insufficient data to be analyzed. From these, 4 studies were considered valid and included in the review. Disagreements among reviewers were discussed and settled through a consensus.

The following items were extracted from each study, if available: journal title, author, date of publication, study design, country in study, time

period, population, epidemiologic data, clinical symptoms, outcomes and risk factor for severe outcomes. If multiple studies were noted to include the same cohort, the most comprehensive and reliable study was selected. The data collated were entered into the Cochrane Collaboration Review Manager Version 5.4 statistical software for data analysis and assessment of level of heterogeneity.[11] Random effects model was utilized during analysis and odds ratios with their 95% confidence intervals were applied to present pooled effect sizes.

RESULTS

Literature Search Yield

After a detailed search for eligible studies, 116 articles were initially considered. Duplicates were removed and records were further screened. Forty-six full-text articles were retrieved and 4 studies were finally deemed eligible. Reasons for exclusion are the following: 1) majority of the sample population are adults (n=7); 2) reported cases are either only non-severe/critical (n=20) or only severe/critical (n=1); 3) no raw data available (n=12); 4) editorial (n=1); and 5) projection study (n=1). An article by Bellino et al. which studied risk factors for disease severity was not included due to lack of available raw data on proportions of severe and critical pediatric cases.[12]

Characteristics of Included Studies

As of August 26, 2020, we included 4 articles, all of which were retrospective cohort studies, with data collated from February to early May 2020 among participants from the United States of America. The sample size of the studies ranged from 19-177, with a total of 292 individuals.[7,13-15]

Three out of 4 studies have a sample population of pediatric individuals with mild to critical COVID-19. One cohort study included children and young adults aged 19 to 34 (n=12), which comprised 6.8% of the sample population.[14] All studies analyzed the epidemiologic and clinical characteristics of individuals. Outcomes noted were severe/critical, and need for mechanical ventilation and admission to pediatric intensive care unit as an indirect measure of the desired outcome.

Critical Appraisal

Studies were assessed using the prognosis guide questions from the book Painless Evidence-Based Medicine.[16] Risk for bias was noted, including: (a) lack of or unclear objective definition of the desired outcome, and where no admission criteria for intensive care was mentioned, or admission to an intensive care unit was based on physician's decision; (b) study population also included young adults, which constituted 4 out of 9 critically ill patients in one study; (c) incomplete follow-up of patients, where outcomes reported were only up to the time of manuscript writing and possible progression of disease was not considered or not mentioned.[7,14-15] Given the appraisal done and possible biases listed, the overall validity assessment of the included studies is moderate.

Prognostic Outcomes[11,16]

Severe/Critical Disease versus Non-severe/critical

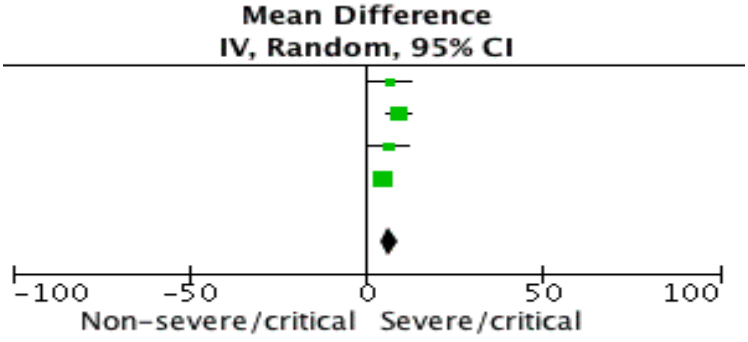
I. Age (4 studies)

Patients with severe/critical disease were significantly older than those with non-severe disease(MD=6.62, 95%CI=4.23 to 9.00, p-value<0.00001) as seen in the 4 pooled studies, with a low heterogeneity of 33%(Table 1).[7,13-15]

Table 1: Age in association with severe/critical COVID-19 disease.

	Severe/critical			Non-severe/critical				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Bhumbra 2020	12	6.1	7	5.4	6.1	12	14.3%	6.60 [0.91, 12.29]
Chao 2020	12.8	5.4	13	3.6	4.3	33	31.1%	9.20 [5.92, 12.48]
De Biasi 2020	15.1	8.5	9	8.7	7.2	168	14.4%	6.40 [0.74, 12.06]
Zachariah 2020	13.7	3.1	9	9	5.2	41	40.2%	4.70 [2.12, 7.28]
Total (95% CI)			**38**			**254**	**100.0%**	**6.62 [4.23, 9.00]**

Heterogeneity: $Tau^2 = 1.95$; $Chi^2 = 4.47$, df = 3 (P = 0.21); $I^2 = 33\%$
Test for overall effect: Z = 5.44 (P < 0.00001)



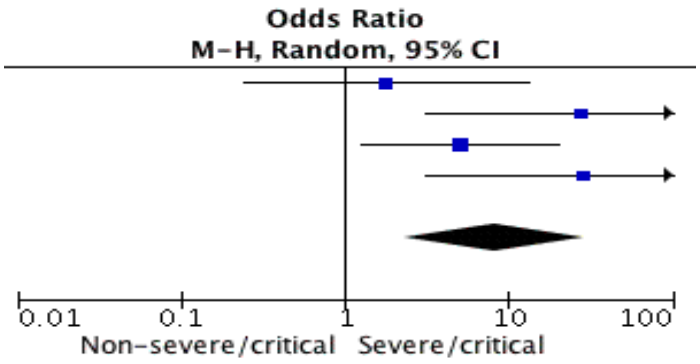
II. Symptoms (3-4 pooled studies)

The forest plot showed that shortness of breath was significantly associated with severe disease (OR=8.14, 95%CI=2.33 to 28.47, p-value=0.001, I^2=42%, n= 4 studies) (Table 2).[7,13-15] Symptoms not associated with severe disease in the pooled studies are fever (OR=2.24, 95%CI=0.71 to 7.07, p-value=0.17, I^2=0%), sore throat (OR=0.87, 95%CI=0.12 to 6.30, p-value=0.89, I^2=57%), cough (OR=1.54, 95%CI=0.71 to 3.32, p-value=0.27, I^2=2%), chest pain (OR=2.77, 95%CI=0.98 to 7.79, p-value=0.05, I^2=0%), and gastrointestinal symptoms, including diarrhea and vomiting (OR=2.77, 95%CI=0.66 to 11.67, p-value=0.17, I^2=40%).[7,13-15]

Table 2: Shortness of breath in association with severe/critical COVID-19 disease

	Severe/critical		Non-severe/critical			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Bhumbra 2020	5	7	7	12	23.6%	1.79 [0.24, 13.21]
Chao 2020	12	13	10	33	21.3%	27.60 [3.15, 241.94]
De Biasi 2020	4	9	23	168	34.3%	5.04 [1.26, 20.18]
Zachariah 2020	8	9	9	41	20.9%	28.44 [3.13, 258.38]
Total (95% CI)		**38**		**254**	**100.0%**	**8.14 [2.33, 28.47]**
Total events	29		49			

Heterogeneity: Tau² = 0.69; Chi² = 5.21, df = 3 (P = 0.16); I² = 42%
Test for overall effect: Z = 3.28 (P = 0.001)



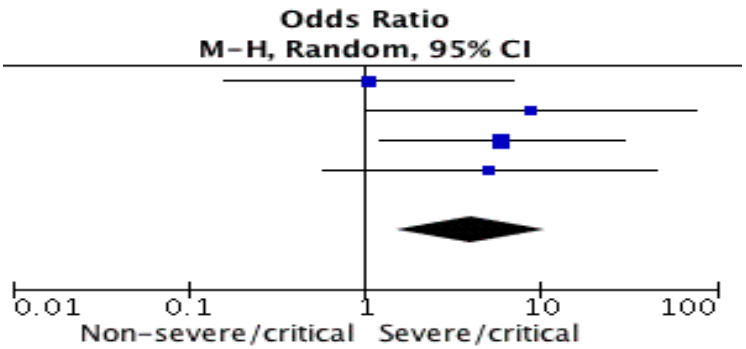
III. Presence of Pre-existing Medical Condition (4 pooled studies)

In the pooled studies of Chao et al., Bhumbra et al., De Biasi et al. and Zachariah et al., presence of any comorbidity was significantly associated with severe disease (OR=4.02, 95%CI=1.55 to 10.43, p-value=0.004) with no heterogeneity (I^2=0%)(Table 3).

Table 3: Impact of a pre-existing medical condition to COVID-19 disease severity

	Severe/critical		Non-severe/critical			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Bhumbra 2020	3	7	5	12	25.6%	1.05 [0.16, 6.92]
Chao 2020	12	13	19	33	19.6%	8.84 [1.03, 76.18]
De Biasi 2020	7	9	62	168	35.5%	5.98 [1.21, 29.71]
Zachariah 2020	8	9	25	41	19.3%	5.12 [0.58, 44.91]
Total (95% CI)		**38**		**254**	**100.0%**	**4.02 [1.55, 10.43]**
Total events	30		111			

Heterogeneity: Tau² = 0.00; Chi² = 2.77, df = 3 (P = 0.43); I² = 0%
Test for overall effect: Z = 2.86 (P = 0.004)



Two specific underlying medical conditions were found to increase the risk of having severe COVID-19 disease. Cardiac disease (OR=6.40, 95%CI=1.45 to 28.38, p-value=0.01, I^2=13%, n=3 studies) and diabetes (OR=7.01, 95%CI=1.54 to 31.95, p-value=0.01, I^2=0%, n=3 studies) were significantly associated with severe disease.[7,13-15] Both showed low to no heterogeneity in their results (Table 4,5). On the other hand, several co-morbidities are not associated with severe disease. The following are:

- Asthma (OR=1.11, 95%CI=0.45 to 2.77, p-value=0.82, I^2=0%)[7,13-15]
- Neurologic condition (OR=4.50, 95%CI=0.83 to 24.39, p-value=0.08, I^2=52%)[7,14,15]
- Hematologic disease (OR=1.35, 95%CI=0.24 to 7.59, p-value=0.73, I^2=0%)[13-15]
- Oncologic disease (OR=5.20, 95%CI=0.55 to 49.36, p-value=0.15, I^2=0%)[7,14]
- Immunosuppression (OR=1.07, 95%CI=0.18 to 6.18, p-value=0.94, I^2=0%)[7,15]
- Obesity (OR=3.46, 95%CI=0.74 to 16.22, p-value=0.12, I^2=57%)[7,13-15]

Table 4: Cardiac disease in association with severe/critical COVID-19 disease

	Severe/critical		Non-severe/critical			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Chao 2020	1	13	0	33	19.1%	8.04 [0.31, 210.67]
De Biasi 2020	2	9	3	168	47.3%	15.71 [2.25, 109.62]
Zachariah 2020	1	9	3	41	33.5%	1.58 [0.15, 17.25]
Total (95% CI)		31		242	100.0%	6.40 [1.45, 28.38]
Total events	4		6			

Heterogeneity: $Tau^2 = 0.24$; $Chi^2 = 2.30$, df = 2 (P = 0.32); $I^2 = 13\%$
Test for overall effect: Z = 2.44 (P = 0.01)

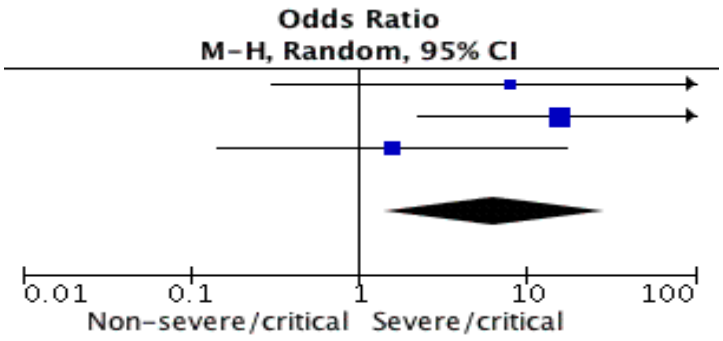
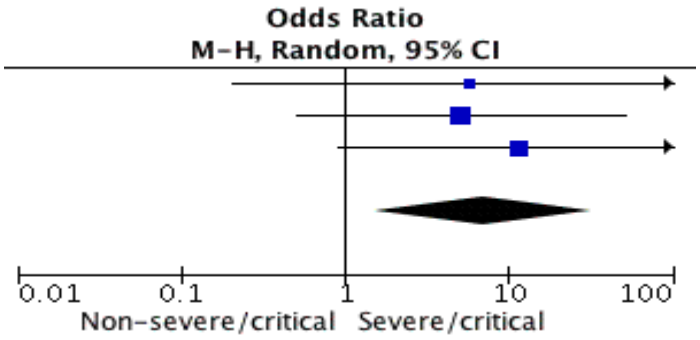


Table 5: Diabetes in association with severe/critical COVID-19 disease

	Severe/critical		Non-severe/critical			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Bhumbra 2020	1	7	0	12	20.7%	5.77 [0.20, 162.48]
De Biasi 2020	1	9	4	168	43.4%	5.13 [0.51, 51.29]
Zachariah 2020	2	9	1	41	35.9%	11.43 [0.91, 143.64]
Total (95% CI)		25		221	100.0%	7.01 [1.54, 31.95]
Total events	4		5			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.23$, df = 2 (P = 0.89); $I^2 = 0\%$
Test for overall effect: Z = 2.51 (P = 0.01)



DISCUSSION

Since COVID-19 is a new disease with the pediatric population contributing only a fraction of cases among affected patients, little is still known of the effect of an individual's profile on severity of disease. In this review, 4 articles were examined to estimate the current available evidence on risk factors for poor outcomes in children afflicted with COVID-19. Three factors including age, symptoms and comorbidities were selected for analysis, and the pooled results revealed significant associations with COVID-19 disease severity in the pediatric population. As of the time of writing, correlation between these factors to COVID-19 prognosis in children has not yet been studied.

This study showed that older age is statistically associated with a poor outcome in children with COVID-19. This is consistent with a study by Sun et al. in China, where 3 (37%) out of 8 severe or critically ill pediatric patients were aged 13–15 years old.[17] In contrast, another study done in France presented severe/critical cases of COVID-19 with a median age of 6 years old (range 0.2-17.8 years old, N=27). However, no available raw data on age was presented. Of the 27 affected children, 5 deaths were reported, with 3 belonging to the adolescent age group (16-17 years old).[18]

Statistically significant moderate heterogeneity was noted in the pooled results of shortness of breath/dyspnea as a risk factor. This may be because dyspnea is a subjective report that an individual can quantify which can point to disease severity.[19] However, in the included studies, signs and symptoms are counted as present or not. On the other hand, fever, sore throat, cough, chest pain and gastrointestinal symptoms showed no statistical difference between the severe/critical and non-severe/critical groups.

Presence of any underlying medical condition increases a child's risk for severe/critical COVID-19. These findings are similar to reports of adult patients from China, where presence of co-morbidities such as cardiovascular disease, hypertension and respiratory system disease may be risk factors for severe disease.[20]

However, in our study, only cardiac disease and diabetes were noted to be risk factors. Focusing on immunosuppression, our results were consistent with a study in a cancer center in New York City, where none of 20 pediatric patients who tested positive for COVID-19 required critical care.[21]

Limitations of this study include 1) studies originated from a single country, and 2) small sample sizes of included pooled articles. Thus, further research is needed to define risk factors for having poorer outcomes in pediatric patients with COVID-19.

Recommendations from Other Guidelines

Presence of an underlying medical condition (serious genetic, metabolic, neurologic disorders, congenital heart disease, obesity, diabetes, asthma, chronic lung disease, or immunosuppression) might increase the risk of having severe COVID-19 disease in children as stated by the Centers for Disease Control and Prevention.[22]

CONCLUSION

Based on poor quality observational studies, older age group, shortness of breath, and a pre-existing medical condition, especially cardiac disease or diabetes were found to be associated with poor outcomes in children with COVID-19. These findings can provide some aid in prognostication and disease management. Further studies with larger sample sizes are recommended.

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ORIGINAL ARTICLE

EFFECTS OF RAPID INFLUENZA ANTIGEN TEST ON ANTIMICROBIAL MANAGEMENT OF PEDIATRIC PATIENTS WITH INFLUENZA-LIKE ILLNESS IN THE EMERGENCY ROOM

Dianne Alexis C. Millado-Riambon, MD
Elizabeth E. Gallardo, MD, FPPS, FPIDSP
Aaron G. Tulay, MD

Saint Louis University Hospital of the Sacred Heart, Baguio City

Correspondence:
Dr. Dianne Alexis C. Millado-Riambon
Email: dialexmillado@gmail.com

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: Influenza is a commonly encountered respiratory tract infection and diagnosis remains to be a challenge. Use of a rapid antigen test may influence decisions on treatment in the emergency room (ER).

Objectives: This research aims to determine the effects of rapid influenza antigen test (RIAT) on antimicrobial management of influenza-like illness (ILI) in the ER, determine the clinical profile of pediatric patients with ILI and look into the relationship between RIAT result, symptomatology, and immunization status.

Methods: This is a cross-sectional study which involved review of charts of 195 pediatric patients with ILI who underwent RIAT (Klintec™) through a nasopharyngeal swab in the ER of a tertiary hospital from September 2019 to February 2020. Chi-square, Fischer exact test and likelihood ratio were used for data analysis.

Results: Most pediatric patients were 7–12 years old males. Majority presented with fever, cough, and colds and underwent RIAT at 2–4 days from onset of illness. About 73.33% of study participants did not receive their yearly influenza vaccine and 70.7% of patients with positive RIAT had no influenza vaccine. There is a lower percentage of vaccinated children who developed cough (86.5% vs. 89.5%) and colds (80.8% vs. 83.2%) when compared with unvaccinated children. RIAT result significantly affected management in terms of antimicrobial prescribing to patients with ILI.

Conclusion: Influenza presents with non-specific symptoms and vaccination remains a major preventive measure against the disease. The result of RIAT facilitates targeted treatment for influenza and decreases unnecessary antibacterial use, but this should be done with careful thought and interpretation.

KEYWORDS: *Influenza, Influenza-like illness, Rapid influenza antigen test*

INTRODUCTION

Influenza-like Illness (ILI) is defined by the World Health Organization (WHO) as an acute respiratory illness with fever of ≥38°C and cough with onset within the past 10 days.[1] Although not exclusively caused by the influenza virus, the rise in cases of ILI correlated well with the seasonal levels of transmission of influenza virus in the community. ILI affects millions of people worldwide and results in high morbidity and mortality in children. Symptoms shared by both ILI and influenza include myalgia, malaise, chills, headache, anorexia, coryza, pharyngitis, abdominal pain, vomiting and diarrhea but also present rarely as acute respiratory distress syndrome, encephalitis, and myocarditis.[2] These symptoms prompt parents to seek medical attention in the emergency room.[3] In the Philippines, influenza season starts from June to November but may extend during colder months.[4] According to the Department of Health Influenza/SARI Monthly Surveillance Reports, there were 68,091 ILI cases in the country from January 1 to June 29, 2019 and 48,329 belonged to the pediatric age group.[5] Also, out of 68,091 cases, only 4% were subjected to influenza diagnostic tests. Our locality reported 382 out of 3,664 ILI cases in our region in the first 6 months of 2019.[6] Influenza may be underreported because of the challenge in differentiating it from other viral and bacterial infections owing to the overlapping symptomatology. There are also respiratory tract infections with mixed bacterial and viral etiologies.[7] The diagnosis of influenza is difficult to ascertain based solely on clinical grounds and repercussions of misdiagnosis could result to irrational use of antimicrobial agents. Often times, clinicians start antibacterial treatment at the first instance they encounter patients with respiratory infections which may be caused by viruses. Overuse of antimicrobials can lead to resurgence of resistant organisms, surge of chronic infections, longer hospital stays if admitted, higher health care costs and even death.[8]

Diagnostic tools such as the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and viral culture for influenza are very costly, not readily accessible and require a longer turn-around time.[9,10] The use of Rapid Influenza Antigen Test (RIAT) is an alternative diagnostic modality that is relatively inexpensive, readily available and practical especially in fast-paced clinical settings like the emergency department.[9,10] RIAT is an immunoassay that can detect influenza A and B nucleoprotein antigens from respiratory specimens. On the average, the sensitivity and specificity of RIAT is 50-95% and 90-95%, respectively. One study showed that influenza diagnosed through RIAT led to a more guided treatment using antibacterial and antiviral agents.[11] Increased detection of influenza virus and timely availability of results from specimen collection resulted in lower antibacterial prescriptions and more directed use of antiviral agents.[11,12] The clinical benefits with the administration of the recommended first line antiviral agent Oseltamivir for suspected and confirmed influenza cases can also be maximized if given within 48 hours from symptom onset.[13]

RIAT became available in our institution last September 2019. The increased cases of influenza diagnosed through RIAT from children with ILI prompted the investigator to explore the effects of the said diagnostic test in the antimicrobial management of patients with ILI consulting in the emergency room; determine the clinical profile of pediatric patients presenting with ILI seen at the ER as to age and gender, signs and symptoms, history of influenza vaccination, and duration of signs and symptoms before testing; determine the association between history of influenza vaccination and RIAT result; determine the association between the symptomatology of ILI and vaccination history, and determine the association between RIAT and management in the emergency room in terms of antimicrobial prescriptions (antiviral alone, antiviral and antibacterial, and antibacterial alone). To the best of our knowledge, no similar study has been done and published locally.

MATERIALS AND METHODS

Study Design, Setting and Participants

This is a cross-sectional study that utilized total population sampling in a tertiary hospital. Data were collected from charts of pediatric patients seen in the emergency department from September 2019 to February 2020.

Inclusion and Exclusion Criteria

The study enrolled patients aged 0 to 18 years old diagnosed with ILI (as per WHO criteria) who were seen at the emergency department and underwent RIAT through a nasopharyngeal swab.

Excluded in the study were patients who did not undergo RIAT, patients with incomplete medical records, those requiring hospital admission, patients who were tested with RIAT ≥ 5 days from onset of symptoms, patients with Severe Acute Respiratory Infection (SARI) and those with other respiratory tract infections (pulmonary tuberculosis, pneumonia, empyema, pulmonary abscess) and comorbidities like chronic lung diseases.

Study Size

The sample size was computed using OpenEpi version 3 (2013) based on a prior local data on the prevalence of ILI which was 382. A total of 192 was derived at 95% confidence interval, and sets the minimum population required for the study. A total of 221 patient records were screened and from this, 195 were included in the final study.

Methods

Retrospective chart review and data extraction were done by two research assistants who were oriented on the inclusion and exclusion criteria. A standardized Data Collection Tool (DCT) was also utilized to ensure consistency. This study was conducted during the influenza season, between September 2019 to February 2020. Medical records of enrolled participants who met the eligibility criteria were examined. RIAT (Klintec^{TM}) was performed at the discretion of the ER physicians to patients with ILI during the study period. The test kit was manufactured by Zhejiang Orient Gene Biotech Co., LTD. It can indicate the type of influenza virus as either type A, B or both. It has a 92.6% sensitivity and 96.4% specificity for influenza A virus and 90% sensitivity and 95.8% specificity for influenza B virus for nasopharyngeal swab specimens. Its positive and negative predictive values were highly dependent on influenza prevalence. The test turn-around time is 10–15 minutes. There were no cross-reactions with adenovirus, coronavirus, Coxsackie virus, HHV, rhinovirus, measles, mumps, Sendai virus and parainfluenza virus.[14]

Through the DCT, the demographics and clinical profile were documented according to age group (divided according to age and stages, i.e., infancy, toddler, preschool, school age and adolescence), gender, influenza vaccination status, signs and symptoms, time of onset of signs and symptoms before RIAT, RIAT result, and the treatment prescribed.

All DCTs were submitted to the researcher who tallied each categorical data with their corresponding codes. The data was recorded using a password-protected Microsoft Excel sheet on a password-protected laptop. Double data entry was done to ensure data recording accuracy and frequencies were done to check for consistency.

Outcome Measures

The primary outcome measure is the effect of RIAT result in the management of pediatric patients with ILI in the emergency department in terms of antimicrobial prescription whether it be an antiviral alone, a combination of antiviral and antibacterial or an antibacterial alone.

Statistical Analysis

Demographics, clinical profile and the association between influenza vaccination history and RIAT result were presented as frequencies and percentages, and were analyzed using Chi-square test. The analysis of the association of the time of testing for RIAT from illness onset and RIAT result, the association of administration of antiviral and day of illness, and the association between RIAT result and the management of influenza with antimicrobials also made use of chi-square, Fisher exact test and likelihood ratio. Data were analyzed using SPSS Statistics version 21. The level of significance was set at $p < 0.05$.

Ethical Considerations

The study was approved by the institution's Research Ethics Committee (REC). The consent process was not applicable as this study utilized a retrospective chart review. However, permission was sought through informed and/or written consent from the attending pediatricians as recommended by the REC.

No data was divulged to comply with the Data Privacy Act of 2012. Chart review for data collection was only done at the Records Division of the hospital by the research assistants. Patient confidentiality was respected by ensuring the anonymity of patient records. All documents (electronic and printed copies) that were related to the study (i.e., data collection forms, consent forms, letters etc.) were kept and stored safely by the researcher.

RESULTS

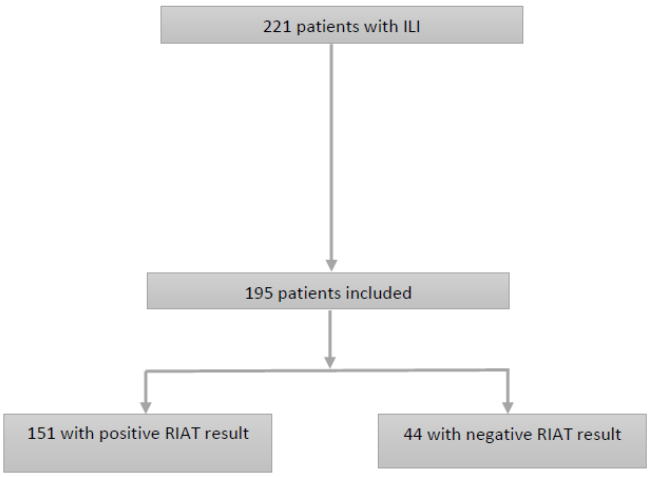

Figure 1: Schematic flowchart of patients included in the study; ILI, Influenza-like illness; RIAT, rapid influenza antigen test

A total of 221 patients with ILI were screened, 26 of whom were excluded for failing some of the eligibility criteria, reducing the total study population to 195 (Fig. 1). Among excluded patients, 7 were not tested for RIAT, 8 required hospital admission, 3 had co-morbidities, 3 were tested with RIAT ≥ 5 days from illness onset and 5 had incomplete medical records. Of 195 patients, 151 had a positive RIAT.

The demographic profile and vaccination status of 195 study participants are shown in Table 1. Majority are males (57.43%, n=112) and the modal age group is 7–12 years old (28.21%). About 73.33% (n=143) of the study population did not receive an influenza vaccine.

Table 1: Demographic and Influenza Vaccine Profile of Study Patients (N=195)

Age group	**With Vaccine (n=52)**	**Without Vaccine (n=143)**	**Chi-square (p <0.05)**
<12months	5 (2.56)	9 (4.62)	0.52
1-3 years	9 (4.62)	32 (16.41)	
4-6 years	8 (4.10)	34 (17.44)	
7-12 years	16 (8.21)	39 (20)	
13-18 years	14 (7.18)	29 (14.87)	
Chi-square value = 3.2275			
Gender	**With Vaccine (n=52)**	**Without Vaccine (n=143)**	**Chi-square (p <0.05)**
Male	33 (16.92)	79 (40.51)	0.30
Female	19 (9.74)	64 (32.82)	

Table 2 shows that the most common reported symptoms throughout all age groups were fever, cough, and colds. There is a higher proportion of patients belonging to the younger age group who manifested with loose bowel movement, vomiting, and seizures compared to older children. There is also a higher proportion of older children who presented with chills, body malaise, sore throat, ear pain, abdominal pain, headache, and anorexia (χ^2= 123.682, p value = 0.00001).

Table 2: Signs and Symptoms of Patients at the Emergency Room

Age Groups				Chi-Square (p <0.05)
0 – 3 years old (n=55)		4 – 18 years old (n=140)		
Symptom	**n (%)**	**Symptom**	**n (%)**	
Fever	55 (100)	Fever	140 (100)	0.00001
Cough	49 (89.1)	Cough	124 (88.6)	
Colds	49 (89.1)	Colds	112 (80.0)	
Loose bowel movement	19 (34.5)	Sore throat	55 (39.3)	
Vomiting	17 (30.9)	Vomiting	35 (25.0)	
Seizures	2 (3.6)	Body malaise	34 (24.3)	
Chills	0 (0)	Headache	32 (22.9)	
Body malaise	0 (0)	Abdominal pain	27 (19.3)	
Sore throat	0 (0)	Chills	23 (16.4)	
Ear pain	0 (0)	Ear pain	22 (15.7)	
Abdominal pain	0 (0)	Loose bowel movement	14 (10.0)	
Headache	0 (0)	Anorexia	12 (8.6)	
Anorexia	0 (0)	Seizures	0 (0)	

There is a significantly higher proportion of patients with positive RIAT who did not receive the influenza vaccine while 20% of patients with negative RIAT were vaccinated for influenza (χ^2 = 111.5832, p value = 0.00001) (Table 3).

Table 3: Influenza Vaccination History and RIAT result

Vaccination Status	Rapid Influenza Antigen Test Result		TOTAL n (%)	Chi-Square (p <0.05)
	RIAT Positive n (%)	RIAT Negative n (%)		
With influenza vaccine	13 (6.7)	39 (20)	52 (26.7)	0.00001
Without influenza vaccine	138 (70.7)	5 (2.6)	143 (73.3%)	
TOTAL	151 (77.4)	44 (22.6)		

RIAT: rapid influenza antigen test

Table 4 shows the difference in symptomatology of pediatric patients with ILI in relation to vaccination history. Irrespective of vaccination status, the top presenting symptoms are fever, cough, and colds and symptoms of ILI are independent of vaccination history (χ^2 = 5.208, p value = 0.970).

Table 4: Symptomatology of ILI and Vaccination History

Symptom	Without Vaccination n (%)	With Vaccination n (%)	TOTAL
Fever	143 (100)	52 (100)	195
Cough	128 (89.5)	45 (86.5)	173
Colds	119 (83.2)	42 (80.8)	161
Chills	16 (11.2)	7 (13.5)	23
Loose bowel movement	24 (16.8)	9 (17.3)	33
Vomiting	37 (25.9)	15 (28.8)	52
Seizures	2 (1.4)	0 (0)	2
Body malaise	23 (16.1)	11 (21.2)	34
Sore throat	41 (28.7)	14 (26.9)	55
Ear pain	19 (13.3)	3 (5.8)	22
Abdominal pain	20 (14.0)	7 (13.5)	27
Headache	24 (16.8)	8 (15.4)	32
Anorexia	10 (7.0)	2 (3.8)	12
TOTAL			195

Chi-square value = 5.208*; p value = 0.970
ILI: Influenza-like Illness
*Significant at 0.05 level

The duration of signs and symptoms of influenza prior to testing are shown in Table 5. There is a significantly higher proportion of patients who were positive for either influenza A or B or both antigens when RIAT was done at 2–4 days from illness onset (χ^2 = 8.043, p value = 0.039).

Table 5: Duration of Signs and Symptoms before RIAT

RIAT Positive (n=151)					RIAT Negative (n=44)	TOTAL n (%)
Onset of Symptoms	*Influenza A*	*Influenza B*	*Both*	*Total n (%)*		
≥ 12-24 hours	43	22	1	66 (33.85)	29 (14.87)	95 (48.72)
2-4 days	51	30	4	85 (43.59)	15 (7.69)	100 (51.28)
TOTAL	94	52	5	151 (77.44)	44 (22.56)	195

Chi-Square value = 8.043*; p value = 0.039
Fisher Exact Test value = 7.864; p value = 0.042
RIAT: rapid influenza antigen test
*Significant at 0.05 level

Table 6 shows a significantly higher proportion of patients with ILI who were prescribed by ER physicians with an antiviral alone (n=84) by the 2nd to 4th day of illness (χ^2 =5.677, p value = 0.059).

Table 6: Medications Administered and Day of Illness

Management	Day of Illness		TOTAL n (%)
	1st day, n (%)	2nd to 4th day, n (%)	
Antiviral Alone	73 (37.4)	84 (43.1)	157 (80.5)
Antiviral + Antibacterial	17 (8.7)	16 (8.2)	33 (16.9)
Antibacterial Alone	0 (0)	5 (2.6)	5 (2.6)
TOTAL	90 (46.1)	105 (53.9)	195

Chi-square value: 5.677*; p value = 0.059
Likelihood ratio: 7.605; p value = 0.022
*Significant at 0.05 level

There is a significantly higher proportion of patients who tested positive for influenza antigen and were prescribed with an antiviral agent alone (64.10%, n=125) while 13.33% were prescribed with both an antiviral and antibacterial. No patient with positive RIAT was given an antibacterial agent alone while 16.41% (n=32) of patients with negative RIAT were still prescribed with an antiviral (χ^2 = 17.621, p value = 0.001) (Table 7).

Table 7: Results of RIAT and Prescribed Antimicrobials

RIAT result	Antiviral Alone n (%)	Antiviral + Antibacterial n (%)	Antibacterial Alone n (%)	TOTAL n (%)
Positive	125 (64.10)	26 (13.33)	0 (0)	151 (77.44)
Negative	32 (16.41)	7 (3.59)	5 (2.56)	44 (22.56)
TOTAL	157 (80.51)	33 (16.92)	5 (2.56)	195 (100)

Chi-square value = 17.621*; p value = 0.001
Fisher Exact Test value = 13.567; p value = 0.001
RIAT: rapid influenza antigen test
*Significant at 0.05 level

DISCUSSION

Influenza is a common and highly contagious respiratory disease that causes a wide array of clinical symptoms in children. Features may vary with age, immunization status, and presence of co-morbidities.[2] The demographic data presented in this study varies from what was presented by Reyes et al. where majority of children with ILI were females with a mean age of 5–9 years old. Our findings of fever, cough, and colds were the most common presenting manifestation in patients with ILI in the pediatric population and were congruent with those of Reyes et al.[15] Our study also showed that loose bowel movement, vomiting, and seizures were commonly manifested by the younger age group which suggest that influenza may be less distinct in younger children.[2,16] Non-specific symptoms such as chills, body malaise, sore throat, ear pain, abdominal pain, headache, and anorexia were prominent in the older age group and are also consistent with the clinical manifestations of influenza infection.[2]

Children are more likely to be infected by influenza and have a higher viral load compared to adults thus harboring and actively transmitting the virus for a prolonged period.[17] About 73.33% (n=143) of our study patients did not receive their yearly influenza vaccine and 70.7% (n=138, p value = 0.00001) of patients with positive RIAT results were unvaccinated for influenza. Lack of vaccine coverage can be due to geographical factors, financial constraints, personal belief and lack of education on vaccination.[15] Our findings also indicate that there is

no relation in symptoms of pediatric patients with ILI with vaccination history (p value = 0.970). One hundred percent of vaccinated and unvaccinated children experienced fever. However, there is a lesser percentage of vaccinated children who experienced cough and colds as opposed to those who were unvaccinated. Children who do not receive the annual influenza vaccine are more likely to develop influenza-related illness.

During the 2009 influenza A (H1N1) pandemic, CDC reported that among the pediatric deaths registered, about 80% were unvaccinated.[18] Increasing vaccination coverage strengthens the control of viral spread in the community and confers a degree of protection against influenza.[17] Despite the findings that symptoms of ILI are independent of vaccination history, children who received the influenza vaccine have reduced influenza-related signs and symptoms. They have less severe infection and a shorter duration of illness compared to unimmunized children.[19] Moreover, vaccines are effective in decreasing flu-associated complications and healthcare burden.[17,19] At every opportunity, physicians should ensure the availability of vaccines, educate on the importance of vaccination, and actively encourage them to receive influenza vaccine yearly.

The diagnosis of influenza imposes a challenge to physicians because its presentation overlaps with other respiratory viruses and possible bacterial co-infections. Furthermore, clinical assessment alone may be insufficient especially in younger children who may not present with the classic findings of influenza.[20] The most definitive diagnostic tool for influenza requires a significant amount of time which is impractical in a fast-paced setting like the emergency department. An alternative diagnostic technique is RIAT which offers a fast and convenient evaluation of children with ILI.[21] The duration of signs and symptoms of ILI prior to testing can affect the results of RIAT.[10,22] Our study showed that RIAT was more likely to detect influenza viral antigen when the time of testing was done at 2–4 days from symptom onset (43.59%, p value = 0.039). The American Academy of Pediatrics (AAP) and Centers for Disease Control and Prevention (CDC) state that specimen collected and tested within 2–4 days from onset of illness will more likely yield a positive result.[9-10] The likelihood of an accurate diagnosis of influenza in conjunction with RIAT is further strengthened when the clinical symptoms are consistent with influenza and when its activity is high in a population.[9,10,22] Symptoms consistent with influenza increase the pre-test probability of an influenza viral infection, consequently increasing the reliability of a positive RIAT result.[10] Patients with ILI were tested with RIAT under the ER physician's discretion during the peak season of influenza. These findings suggest that ER physicians should subject patients to undergo RIAT during the influenza peak season within 2–4 days from onset of illness if the symptoms are consistent with influenza.

Patients on the 2nd to 4th day of illness were more likely prescribed with an antiviral alone (43.1%, p value = 0.059). Oseltamivir, one of the most commonly prescribed antiviral agents for influenza, is best started within 48 hours of illness onset. However, patients would still benefit from this antiviral if started by the 3rd and 4th day of illness if the clinical presentation is compatible with influenza.[13] ER physicians should not delay or defer prescribing an antiviral agent even after 48 hours of symptom onset especially if there is a high index of suspicion for influenza infection.

This study revealed that the results of RIAT affect decision-making of ER physicians in the management of ILI. Of 195 patients, 125 (64.10%) who tested positive were prescribed with an antiviral alone. Similar studies by Blaschke et al. and Jennings et al. stated that the most common antiviral agent prescribed was Oseltamivir when the diagnosis of influenza was confirmed through RIAT.[11,23] These findings suggest that the decision to initiate this antiviral by the physician was linked with a higher level of confidence and provided diagnostic certainty once influenza was confirmed through RIAT along with clinical assessment.[23] Likewise, RIAT facilitates the physician's decision-making for targeted treatment by prescribing an antiviral to patients with positive results. The physician's decision for use of

an antiviral may itself limit antibacterial prescriptions. About 13.33% (n=26) of study participants positive for influenza antigen test were prescribed with both an antiviral and antibacterial and no patient with positive RIAT was given an antibacterial alone. Jennings et al. and Bonner et al. observed that some physicians would prescribe both an antiviral and antibacterial to patients who have a positive influenza antigen test result.[23-24] On the other hand, both studies also stated that physicians were more willing to withhold antibacterial treatment once the diagnosis of influenza was supported by RIAT.[23] These decisions on treatment were made based on the physician's clinical evaluation, experience, and discretion that certain patients with influenza might have bacterial co-infections that warrant antibacterial use along with an antiviral.[11] Additionally, physicians would rarely start antibacterial treatment alone if the RIAT result is positive, limiting inappropriate antibacterial use.[23,25] About 16.41% (n=32) of our patients received an antiviral despite negative RIAT results. A negative RIAT result does not exclude influenza infection and nor should it affect the physician's decision in prescribing an antiviral especially when clinical suspicion for influenza is high. Physicians should not rely solely on RIAT results in their decision to initiate antiviral therapy.[21] ER physicians must take caution when interpreting results of RIAT. This diagnostic test has its advantages, but it should be interpreted in conjunction with careful clinical examination. In settings where RIAT or other means of testing for influenza are unavailable, physicians should still commence treatment with an antiviral especially if the patient's clinical manifestations are consistent with influenza infection to ensure timely treatment and avoidance of unnecessary antibacterial prescriptions which will help prevent the emergence of resistant bacteria.

CONCLUSION

In conclusion, this study showed that majority of pediatric patients with ILI belong to the 7–12 year old age group and were predominantly male. Fever, cough, and colds were the most common manifestations and majority were tested positive for RIAT at 2–4 days from symptom onset. A significant proportion of study participants did not receive an influenza vaccine and were positive for influenza antigen test. Vaccinated children have lesser probability of developing influenza-related symptoms compared to children who were unimmunized. RIAT in the diagnosis of influenza significantly affects decision making of ER physicians in terms of appropriate antimicrobial prescriptions. A positive influenza antigen test result led to a targeted treatment using the recommended antiviral thus preventing unnecessary antibacterial prescriptions.

LIMITATIONS AND RECOMMENDATIONS

This study was done in a single influenza season and in one tertiary hospital with a relatively few number of study patients. Furthermore, selection bias might be present in this study since it only included pediatric patients who underwent RIAT under the ER physician's discretion. Other factors such as physical examination and diagnostic tests (i.e., complete blood count, chest radiographs) that may also influence the ER physician's decision making for antimicrobial prescriptions were not investigated. Future studies may opt to explore other testing modalities for influenza and involve admitted patients or those seen in the outpatient department. A multi-center study done through multiple influenza seasons to make more conclusive findings is also suggested.

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ORIGINAL ARTICLE

CLINICO-DEMOGRAPHIC PROFILE AND OUTCOME OF PEDIATRIC HIV/AIDS PATIENTS IN WESTERN VISAYAS MEDICAL CENTER

Mae Anne Rizalyn J. Allam, MD, DPPS
Mary Jane Dolores E. Ayson, MD, DPPS, DPIDSP

Department of Pediatrics
Western Visayas Medical Center

Correspondence:
Dr. Mae Anne Rizalyn J. Allam
Email: margjava_md@yahoo.com

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4TH PLACE 2021 PIDSP RESEARCH CONTEST

ABSTRACT

Objective: Pediatric HIV is a national health concern that has grown exponentially in the past 5 years. This study aimed to determine the clinico-demographic profile and outcome of pediatric HIV/AIDS patients 0-18 years old seen at the Western Visayas Medical Center (WVMC) HIV/AIDS Treatment Hub from March 2006 to September 2018.

Methods: Medical chart records of all pediatric HIV/AIDS patients seen at the treatment hub during the study period were reviewed. Data on clinical and demographic profile and outcomes were gathered and descriptive statistics was used to analyze data.

Results: A total of 30 children 0-18 years old were registered consisting of 29 (97%) males and 1 (3%) female. A sudden increase in pediatric HIV patients was noted in the past 3 years, mostly among male adolescents engaged in male-to-male sexual contact (MSM). Majority (73%) were symptomatic at diagnosis with flu-like symptoms, fever and vomiting. Common physical exam findings were lymphadenopathy and rashes. HIV-related infections were tuberculosis and pneumonia. About 60% of study participants had severe immunodeficiency. Two-year mortality rate was 38%. Correlation of age and baseline CD4 count with outcome did not show any significant results.

Conclusion: Pediatric HIV/AIDS patients were symptomatic, male adolescents who engaged in male to male sexual contact. Co-infections with pneumonia and tuberculosis were common and severe immunodeficiency was present at diagnosis. Thirty-eight percent of patients had poor outcomes 2 years after diagnosis

KEYWORDS: *Pediatric HIV/AIDS, Clinico-demographic Profile*

INTRODUCTION

The global emergence of Human Immunodeficiency Virus (HIV) has a substantial impact on public health as well as on the lives of affected patients and their families. Children with HIV suffer from medical, socioeconomic, cultural, psychosocial, and treatment consequences of the disease.

The Joint United Nations Programme on HIV/AIDS (UNAIDS 2018) reports that HIV affects an estimated 36.9 million people worldwide, resulting to 940,000 Acquired Immunodeficiency Syndrome (AIDS) related deaths in 2017. About 53% of cases are in sub-Saharan Africa, followed by the Asia Pacific region which accounts for 14% of cases. Among children, approximately 5 million have died of AIDS, with an estimated 110, 000 pediatric AIDS-related deaths in 2017 alone. Despite recent global reductions in HIV/AIDS statistics, there are still 1.8 million children less than 15 years living with HIV. UNAIDS reports an alarming 180,000 new infections in 2017 and an average of 400 new pediatric cases diagnosed everyday.[1] In developing countries such as the Philippines, the prevalence of HIV in children continues to increase as a result of rising numbers of HIV-infected women and inefficient measures to prevent perinatal transmission.[2] Furthermore, infection among older children and adolescents are increasing steadily due to early, unprotected sex.[3]

A thorough understanding of the epidemiology of pediatric HIV infection and course of disease may provide opportunities to minimize or possibly eradicate transmission, especially in resource poor settings. Early identification of infected children will enable adequate and prompt treatment and significantly improve quality of life. Lastly, knowledge of clinical, laboratory and epidemiologic profile and outcome of HIV-infected children will aid clinicians to formulate diagnostic and management guidelines to effectively address the special challenges presented by HIV-infected children.

The study aimed to describe the socio-demographic profile of HIV/AIDS patients as to: age, sex, residence, educational attainment, occupation; describe the clinical profile of HIV/AIDS pediatric patients as to mode of transmission, presenting signs and symptoms at consult or admission, co-morbid illness, CD4 count at diagnosis, opportunistic infections and HIV-related conditions; determine the outcome of HIV/AIDS pediatric patients at 1 month, 1 year and ≥2 years after diagnosis whether living, dead, or lost to follow-up; and determine the correlation between age of onset of illness and outcome and CD4 count at diagnosis and outcome.

MATERIALS AND METHODS

This was a retrospective, descriptive medical chart review of all HIV/AIDS pediatric patients enrolled at the Western Visayas Medical Center (WVMC) HIV/AIDS Treatment Hub.

Data collection was conducted from April 2018 to November 2018. Medical records of all pediatric HIV/AIDS patients 0-18 years old registered at the Western Visayas Medical Center HIV/AIDS Treatment Hub from March 2006 to September 2018 were included.

The study commenced after approvals from the Research Ethics Review Committee and the Technical Review Committee were obtained. Data was collected using paper Case Report Forms to record demographic information (age, sex, residence, educational attainment and occupation), clinical profile (mode of transmission, presenting signs and symptoms at the time of consult, co-morbid illness and CD4 count at time of diagnosis) and outcome (living, died or lost to follow-up).

The study was restricted to the review of existing medical records and the researcher did not have any direct contact with subjects. Confidentiality of patient information was maintained throughout the duration of the research by use of study codes. The medical charts of all pediatric HIV/AIDS patients in the WVMC Treatment Hub did not contain any identifying personal information and were labelled using a case number. To further protect patient data

and maintain anonymity of subjects, each case report form was assigned a 6-digit study identification (ID) number. The first four digits of the ID number consisted of the year the patient was diagnosed and the last two digits was the patient number. The case report form only contained the study ID number. On a separate document, a master list was created which contained patient case numbers along with their assigned unique study ID numbers. This file was kept separately from other documents and stored in a secure password-protected drive where only the primary investigator had access. This master list was deleted after data collection.

Data collection was conducted solely by the researcher in a designated room within the WVMC Treatment Hub. Medical records were prepared by authorized treatment hub personnel. Information from charts was recorded by the investigator.

Access to data files encoded in the computer was done with the use of a security password. Collected data were summarized and encrypted.

Information collected was used strictly for this research. Case report forms and computerized data were kept and stored securely in a locked container for the duration of the study. All printed data will be destroyed within 5 years and encoded data will be deleted permanently from the computer after 3 years.

Data collection was strictly limited to information that existed and was available in the patients' medical records. Most of these records only contained data during the patients' first and subsequent outpatient visits and medical records during admissions in the hospital, if any, were not available. Similarly, some of the data required in this study were not found in the patient's records, and lacking data were noted.

Data was analyzed using Epi Info 7 software. Descriptive data were presented as frequencies and percentages. Fisher's exact test was used to test for the significance of association of age and CD4 count at the time of diagnosis with outcomes of HIV/AIDS patients. P-value less than 0.05 was considered significant.

RESULTS

As of September 2018, the Western Visayas Medical Center HIV/AIDS Treatment Hub has catered to a total of 1,363 HIV-confirmed cases since 2006 and a total of 30 (2.2%) pediatric patients aged 0-18 years old were registered. All 30 of these were included in the study.

Figure 1 shows that there were no pediatric patients enrolled during the first six years of the WVMC Treatment Hub. The first 3 confirmed cases of HIV in children were registered in 2013. There was only one new case in 2014 and no new patients in 2015. This was followed by a surge in the number of new patients recorded starting 2016, with 9 new cases. The spike continued in 2017, where 8 new cases were reported. From January to September 2018, a total of 9 new pediatric HIV cases were diagnosed.

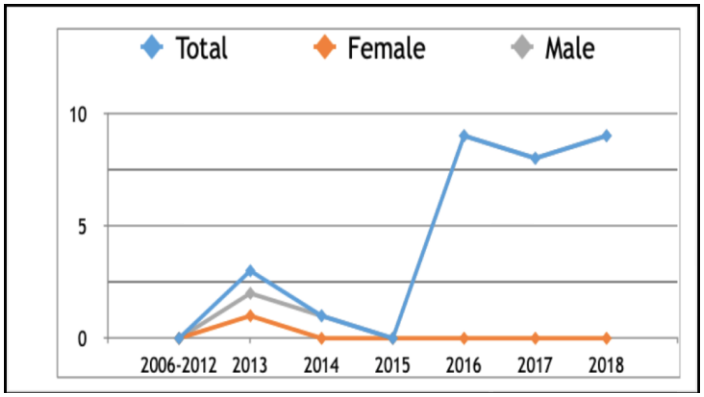

Figure 1: Number of diagnosed pediatric HIV cases per year

Figure 2 shows the profile of pediatric HIV patients as to age and sex. Out of 30 patients, 29 (97%) were males and only 1 (3%) was a female. Majority of patients (90%) belonged to the adolescent (12-18 years old) age group. The average age at diagnosis was 16.2 years old. For those in the younger age groups (<12 years old), one patient was an infant, a preschooler and a school age child. The only female patient was diagnosed in 2013 at 4 years old.

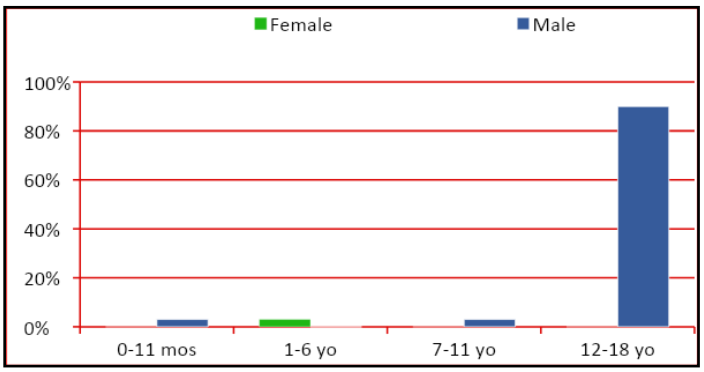

Figure 2: Distribution of patients based on age and sex

Almost half of the HIV-positive pediatric patients came from Iloilo Province (47%), followed by Iloilo City (40%), while the rest came from Capiz (7%), Antique (3%) and Guimaras (3%).

Fifty percent of pediatric HIV patients are students coming from all levels, but mostly belonging to high school and college. Four (13%) patients were employed (Table 1).

Table 1: Profile of patients as to educational attainment and occupation

Educational Attainment	N	%
Elementary	2	7
High School	9	30
College Undergraduate	8	26
Data not available/ not applicable	11	33
Occupation	**N**	**%**
Student	15	50
Employee	3	10
Farm Laborer	1	3
Data not available/ not applicable	11	33

The most common mode of transmission among patients is male-to-male sexual contact (MSM) in 27 patients (90%). Mother-to-child (MTC) transmission accounts for 10% of cases. The children recorded to have been infected vertically all belonged to the younger age group (<12 years old), the first case being a four year old female from Capiz who presented with skin lesions in 2013. This was followed in 2016 by an 8-year old male with fever who was born to HIV-positive parents. The last documented case of MTC was in 2018 in an 8 month old male infant who had recurrent cough.

Table 2 shows that majority (66%) of patients who acquired HIV through sexual transmission had multiple partners. Among these patients, 47% had 2-5 sexual contacts, 9% had 6-10 sexual partners and 14% had more than 10 sexual partners. Five (18%) patients regularly engaged in commercial sex. There were 7 (23%) patients who had sexually transmitted infections: one patient had syphilis, 3 had Hepatitis B and 3 had Human Papilloma Virus infection.

Table 2: Risk factors for sexual transmission of pediatric HIV

Risk Factors	N	%
Single partner	1	3
Multiple partners	20	66
Accepts payment for sex	5	18
Sexually Transmitted Infection	7	23

Majority (73%) of HIV-infected patients were symptomatic upon diagnosis. The most common manifestations were flu-like symptoms, such as cough and nasal catarrh seen in half (50%) of patients, followed by fever (37%) (Table 3).

Table 3: Common presenting symptoms of pediatric HIV

Presenting Symptoms	N	%
Flu-like Symptoms (cough/nasal catarrh)	15	50
Fever	11	37
Vomiting	4	13
Myalgia/ Muscle Pain	3	10
Anorexia	2	7
Nausea	2	7
Dizziness	1	3
Headache	1	3
Confusion	1	3
Fatigue	1	3
ASYMPTOMATIC	8	27

Table 4 shows that the most frequently reported clinical signs at diagnosis were lymphadenopathy (27%), rash or skin lesions (20%), weight loss (13%) and oral lesions (10%). Less common signs were pharyngitis and genital ulcers.

Table 4: Common presenting signs of pediatric HIV

Common Presenting Signs at Diagnosis	N	%
Lymphadenopathy	8	27
Rash	6	20
Weight Loss	4	13
Mouth Ulcers	3	10
Abscess	2	7
Genital Ulcers/ warts	1	3
Pharyngitis	1	3

Co-morbidities among registered pediatric patients were rare, with only 4 out of 30 (13%) children having known food and drug allergies, and 2 (7%) children having bronchial asthma.

Table 5 summarizes the most common HIV-related conditions reported among pediatric patients. Pneumonia with radiologic findings was the most frequently associated condition and accounted for almost half (47%) of cases. Tuberculosis (TB) ranked second with 10 (33%) patients diagnosed clinically with active pulmonary TB. One patient had multi-drug resistant (MDR) tuberculosis. Six (20%) patients suffered from oral candidiasis. The other opportunistic infections were staphylococcal skin lesions/abscesses (7%) and dermatophytosis (7%). One patient presented with confusion and was diagnosed with cryptococcal meningitis.

Table 5: Common opportunistic infections and HIV related conditions

Opportunistic Infections and HIV Related Conditions	N	%
Pneumonia	14	47
Tuberculosis	10	33
Oral Candidiasis	6	20
Staphylococcal Skin Infection	2	7
Dermatophytosis	2	7
Cryptococcal Meningitis	1	3

In terms of laboratory profile, most (60%) patients had an actual CD4 count of less than 200 cells/uL at the time of diagnosis. Four patients (14%) had a CD4 count of 200-499 cells/uL while only 3 (10%) patients had values >500 cells/uL. The median CD4 count of patients was 154 cells/uL (Table 6).

Table 6: CD4 count at time of diagnosis

CD4 Count (cells/uL)	N	%
<200	18	60
200-499	4	14
>500	3	10
No Data	5	17

Table 7 illustrates the outcome of pediatric HIV patients. The first mortality was recorded in 2013, and he was an 18-year old male diagnosed with multi-organ failure, cryptococcal meningitis and MDR-TB. Along with an increase in the number of enrolled cases in 2016 was a peak in mortality rate, one of which was an 18-year old male with poor history who died at the emergency room and later on tested positive for HIV. Another mortality was an 18-year old male who was HIV-positive for 2 years before he received antiretroviral treatment. He succumbed to AIDS 6 months later. The third mortality in 2016 was an 8-year old male infected through vertical transmission. There were no deaths among the 8 cases enrolled in 2017. An 8-month old male was also infected via mother-to-child transmission and was a mortality in 2018.

Table 7: Yearly outcome of pediatric HIV

Year	Total Number of Cases (N)	Living		Died		Lost to follow up	
		N	%	N	%	N	%
2013	3	2	66	1	33	0	0
2014	1	1	100	0	0	0	0
2015	0	0	0	0	0	0	0
2016	9	3	33	3	33	3	33
2017	8	8	100	0	0	0	0
2018	9	8	88	1	12	0	0

Figure 3 shows the outcome of patients at 1 month, 1 year and ≥2 years after diagnosis. Out of 30 patients, 4 (13%) children died and 3 (10%) children were lost to follow-up within one month after diagnosis. At 1 year of follow-up, mortality rate increased to 23% as 5 patients died. Only 13 of the total number of patients had records pertaining to their outcome after 2 years. At this time, more patients (62%) had unfavorable outcomes.

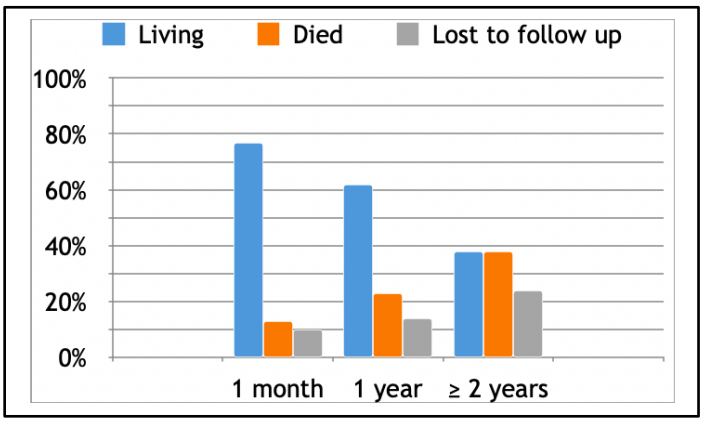

Figure 3: Outcome of pediatric HIV at 1 month, 1 year and ≥2 years after diagnosis

Two out of three children below 12 years of age died 1 month after diagnosis. For older children (12-18 years old), 33% had unfavorable outcomes at 1 month, 29% died or were lost to follow up after 1 year and majority (60)% had an unfavorable outcome after ≥2 years. The results showed that age was not significantly associated with outcome (Table 8).

Table 8: Association of age and outcome of pediatric HIV

Age	Living	Died or Lost to follow up	p- value
1 MONTH AFTER DIAGNOSIS (n=30)			
<12 years old	1	2	0.1190
12-18 years old (adolescent)	22	5	
1 YEAR AFTER DIAGNOSIS (n=21)			
<12 years old	1	2	0.7428
12-18 years old (adolescent)	12	6	
≥2 YEARS AFTER DIAGNOSIS (n=13)			
<12 years old	1	2	0.4895
12-18 years old (adolescent)	4	6	

Our study showed that 17% of children with CD4 count of <200 cells/uL died within 1 month after diagnosis. Similarly, 33% of patients with severe immunodeficiency (CD4 count < 200 cells/uL) died within one year after diagnosis. Only 10 children had baseline CD4 counts and at least 2 years of follow-up. Of this, seven children had a CD4 count below 200 cells/uL and 4 (57%) of them had poor outcome (either died or lost to follow-up). All children with CD4 count of at least 200 cells/uL were still alive after 1 month, 1 year and ≥2 years of diagnosis. Despite these, our study showed that CD4 count is not significantly associated with outcome of pediatric HIV as shown in Table 9.

Table 9: Association of baseline CD4 count and outcome of pediatric HIV

Baseline CD4 Count	Living	Died or Lost to follow up	p- value
1 MONTH AFTER DIAGNOSIS (n=25)			
≥200 cells/uL	7	0	0.3548
<200 cells/uL	15	3	
1 YEAR AFTER DIAGNOSIS (n=18)			
≥200 cells/uL	6	0	0.1618
<200 cells/uL	8	4	
2 YEARS AFTER DIAGNOSIS (n=10)			
≥200 cells/uL	3	0	0.1667
<200 cells/uL	3	4	

DISCUSSION

The Philippines is suffering from an explosive HIV epidemic despite improvements in the overall global data on HIV.[4] Since the first HIV case in 1984, the country has seen changing trends in the socio-demographic characteristics and clinical profile of affected patients.

Based on available data, the period prevalence of pediatric HIV in Western Visayas is 2.2%. This value is lower compared to the national prevalence of 4% reported in the HIV/AIDS and ART Registry of the Philippines (HARP) released last August 2018.[5] Similarly, this is lower than the UNAIDS 2018 data which showed a global prevalence of pediatric HIV at 5%.

From 2016 to 2018, this study noted a disconcerting number of new pediatric HIV infections. From only 3 cases in 2013, the periods 2016 to 2018 showed a 200% increase, with 8-9 new cases diagnosed annually. This confirms the UNAIDS 2018 warning that the Philippines has the fastest-growing HIV rates in the Asia-Pacific region with a 141 percent increase from 2010 to 2016.[6]

These figures are also in parallel with the latest data recorded by HARP which reported an exponential growth of new HIV cases from an average of one case per day in 2008 to an alarming 31 new cases daily. The growing rates may be partly attributed to the Department of Health's (DOH) heightened drive for HIV testing.[7] These numbers should not be taken for granted and other contributing factors should be considered. Results of the 2015 Integrated HIV Behavioral and Serologic Surveillance (IHBSS) reveal that majority of key affected populations such as men who have sex with men (MSM), persons who inject drugs (PWID) and female sex workers (FSW) have limited knowledge on HIV transmission, HIV status and access to HIV support.[8] Another possible explanation for this rising trend is a genotype shift in locally transmitted infections. Recent scientific findings by the National Institutes of Health (NIH) last April 2018 suggest that from the common HIV subtype B, new cases were seen to be caused by a more aggressive and easily transmissible subtype AE.[9]

Review of the socio-demographic profile of pediatric HIV patients in this study revealed that majority (97%) are males consistent with the August 2018 HARP report. Other studies on pediatric HIV conducted in India, Zimbabwe and Nepal also reported a predominance of male patients.[10-15]

Majority of pediatric HIV patients enrolled in WVMC Treatment Hub were adolescents. Indeed, HIV in the Philippines has become a youth epidemic.[16] There were 57 newly diagnosed adolescents aged 10-19 years old in August 2018 alone, accounting for 5% of the total number of new cases. Furthermore, overall HARP data reported that 94% (2,241 out of 2,389) of all pediatric HIV cases were adolescents 10 to 19 years old. Worldwide HIV statistics also documented a similar pattern. In 2017, the UNAIDS emphasized that young people aged 15 to 24 years old have been at the forefront of the HIV/AIDS pandemic, reporting a 170% increase in the number of new HIV infections among this age group.[1]

In this study, majority of infections were acquired through sexual transmission and all of HIV-positive adolescents were men who have sex with men (MSM). This finding is consistent with HARP August 2018 data which revealed that all of 57 newly diagnosed adolescents were infected through sexual transmission. Although male to male sexual contact is also the leading mode of infection in 70% of cases recorded by HARP, other forms of sexual contact (heterosexual and bisexual) were responsible for the remaining infections.[5]

The alarming increase in new HIV cases seen in this study is proportionate to the doubling HIV prevalence particularly among adolescent males/ trans-genders who have sex with males (M/TSM) in the past five years.[17] Furthermore, there are more young people who engage in sex but have limited access to adequate sex education and contraceptive services – a reality faced by countries in the Asia-Pacific, including the Philippines.[18] Findings from the 2015 Integrated HIV Behavioral and Serologic Surveillance (IHBSS) showed that most key affected populations such as MSM start their high-risk behaviors during the adolescent years.[8] A study conducted by the Philippine National AIDS Council (PNAC) in 2017 revealed that a two to three year gap from unprotected coitarche to first contraceptive use has become a major factor to the rising spread of HIV among the youth.[19]

In contrast to studies conducted among pediatric patients in other HIV-plagued countries in Asia and Africa which still showed a predominance of mother-to-child (MTC) transmission, this mode of infection accounted for only 3 (10%) cases in this study.[10-13,15,20,21] All of these children belonged to the younger age group (<12 years old) and were diagnosed at 8 months old, 4 years old and 8 years old, respectively with an average age at diagnosis of 4.2 years old. This data is consistent with other pediatric HIV studies which documented that the average age of diagnosis in most perinatally transmitted infections is before 5 years old.[11-13] According to HARP, a total of 13 children below 15 years old were reported to have been infected through mother-to-child transmission in 2018.[5]

Over-all Philippine data on MTC show a decreasing trend due to effective maternal intervention programs and improved access to ART. Recent data show that MTC accounts for only 6% of all pediatric HIV cases.[3] This study recorded one new pediatric HIV case in 2018, suggesting that vertical transmission remains to be an important contributor to the spread of HIV.

The proximity of the WVMC-HACT Treatment Hub to patients residing in Iloilo City and nearby towns explains the distribution of enrolled patients according to location. However, this study did not include those patients registered in other HIV treatment centers in the region. The WVMC-HACT Treatment Hub is one of five DOH designated HIV Treatment Hubs in Region VI. Despite availability of these HIV care facilities, there is an ongoing challenge to provide young people who are at risk for HIV adequate access to HIV testing and intervention.[17]

Data on clinical symptomatology of pediatric HIV were consistent with findings from similar studies.[13,22-27] More than half of patients (73%) in this study already had signs and symptoms during the first consult similar to results obtained by Ramaswamy et al. and Poudel et al., which reported that 78% and 80% of enrolled children were symptomatic upon diagnosis.[13,22]

Most of the symptoms noted in this study were non-specific, like cough and flu-like symptoms, fever, vomiting and body malaise. Common presenting signs included lymphadenopathy, rashes, weight loss and oral ulcers. Pol et al. reported symptoms that were very similar to the ones obtained in this study such as persistent fever (70.42%), persistent cough (59.15%), loss of appetite (59.15%) and weight loss (56.33%).[25] Fever was noted in separate studies done by Lodha et al. (73.6%), Rajasekaran et al. (36.6%) and Poudel et al.[12,13,24] Lymphadenopathy was a prominent clinical finding in many pediatric HIV studies.[11,22,24] Protein energy malnutrition as a clinical manifestation was not observed in this study due to the lack of nutritional and anthropometric data in the medical records. This is significant to note because several studies showed that failure to thrive, poor nutritional status and malnutrition were common presenting features in most patients.[10,12,22] Other findings seen in previous studies that were not observed in the current study include recurrent diarrhea, anemia and hepatosplenomegaly.

Tuberculosis and pneumonia were the top HIV-related conditions seen among patients in this study and these are comparable to those documented in the literature.[10,13, 22,28,29] Ramaswamy et al. reported tuberculosis (all forms of TB) in 25% of patients, while Shah et al. observed tuberculosis in 35%, candidiasis in 11% and *Pneumocystis carinii* pneumonia in 7%.[10,22] Likewise, an investigation on pediatric HIV in Nepal noted the following co-infections: tuberculosis (16%), bacterial pneumonia (9.3%) and oropharyngeal candidiasis (6.7%).[13]

CD4 count determination is useful in classifying pediatric patients in terms of category of immunodeficiency.[30] In this study, baseline values showed that most (60%) patients had an actual CD4 count of less than 200 cells/uL which classified these children as having severe immunodeficiency. This result is much greater than the findings of Ramaswamy et al. in 2017 which reported only 5% of pediatric patients having severe immunodeficiency (CD4 count range: 11-227 cells/uL). The same study correlated clinical profile with CD4 count, and noted that children with opportunistic infections have lower CD4 values compared to those without opportunistic infections.[22]

The mortality rates of pediatric HIV patients observed in this study were as follows: 13% at 1 month, 23% at 1 year and 38% at 2 years follow up. Majority (80%) of patients who died were observed to have died immediately or within a month after diagnosis, signifying that these cases were picked up at a late stage. In terms of outcome, the 2-year mortality rate is higher at 38% as compared to figures obtained by Shah et al. with only 14% mortality after 22 months.[10] Similarly, these rates are significantly higher when compared to the findings of Poudel et al. which noted zero mortality in a 3.5 year study among HIV-infected children receiving anti-retroviral therapy in Nepal.[13] Lodha et al. reported different survival rates for those

receiving and not receiving antiretroviral therapy.[12] The high mortality noted in this study may be attributed to the following factors: small number of patients, late diagnosis, severe immunodeficiency with low CD4 counts, presence of HIV-related infections and delayed or poor access to ART.

Although raw data would suggest that older patients tend to have poorer outcomes as compared to younger children, this was not statistically significant. Likewise, there was no significant relationship between baseline CD4 count and survival despite numbers suggesting that more patients with CD4 count of <200 cells/uL have died. There may be a limitation in determining the actual relationship between these factors due to unavailability of baseline CD4 count in several patients and the small number of patients involved.

CONCLUSION AND RECOMMENDATIONS

In conclusion, pediatric HIV patients in Western Visayas consist mostly of symptomatic male adolescents who engage in male to male sexual contact. Mother-to-child transmission accounted for a small percentage of cases. Most cases had severe immunodeficiency, and tuberculosis and pneumonia were common HIV-related conditions. Most patients died within a month after diagnosis and the 2-year survival rate was lower as compared to other studies done on pediatric HIV. There was no correlation between age and baseline CD4 count and outcome.

Results from this study may be utilized as baseline information for future studies on pediatric HIV patients, particularly among male adolescents. This subpopulation appears to have the highest risk and significant contributing factors require further investigation. Likewise, there is a need to fully evaluate other parameters such as socio-economic, sexual and behavioral epidemiology of young people living with HIV.

Data obtained from this study may be used to help improve HIV/AIDS prevention, treatment and monitoring in Western Visayas Medical Center. Significant results from this study may be beneficial in establishing local health programs and national government policies to specifically target key affected groups (adolescents, MSM, FSW).

A prospective cohort study on pediatric HIV is recommended. This study may include HIV genotype determination, natural history and presentation, course of disease and complications on follow-up, as well as CD4 count monitoring and clinical response to antiretroviral drugs. This would provide a more accurate and comprehensive correlation between different factors and outcome of patients. Data from other treatment centers in the region should also be included in future studies to provide a more representative profile of pediatric HIV in Western Visayas, Philippines.

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ORIGINAL ARTICLE

ACETIC ACID VERSUS CHLORINE TABLET SOLUTION AS DISINFECTANT OF NON-CRITICAL ENVIRONMENTAL SURFACES

Abegail Sales Basco, RN, MD

Department of Pediatrics
National Children's Hospital

Correspondence:
Dr. Abegail Sales Basco
Email: abegailbasco@gmail.com

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ABSTRACT

Objectives: This study aims to determine the bactericidal activity of 4% acetic acid versus chlorine tablets against gram negative and gram-positive microorganisms based on percentage reduction of microorganisms in hospital surfaces and suggest that it may be an effective alternative disinfectant.

Methodology: This was an experimental study where microbiological sampling of hospital surfaces was used to determine bacterial growth. The study was conducted from November to December 2020 at National Children's Hospital, a 200 bed capacity tertiary government hospital catering to children 0 to less than 19 years old. Non-critical hospital surfaces such as beds, bed rails and bedside tables were swabbed before and after intervention cleaning with chlorine tablets or 4% acetic acid solution.

Result: Pre-swabbing, hospital surfaces showed the presence of Bacillus sp., *Klebsiella pneumoniae* and Coagulase Negative Staphylococcus (CONS). Post-application of 4% acetic acid solution resulted to 100% reduction of Bacillus sp., 70.8% reduction of CONS, and 19.5% reduction of *Klebsiella pneumoniae* while post-application of chlorine tablet solution showed 100% reduction of *Klebsiella pneumoniae* and CONS and 95.2% reduction of Bacillus species.

Conclusion: The use of 4% acetic acid solution significantly reduced more gram-positive than gram-negative organisms and is a highly effective disinfectant against *Bacillus* sp. but is not effective against gram-negative organisms as it does not fulfil the criteria of at least 90 percent reduction in bacterial growth. Chlorine tablet solution is a more effective disinfectant against gram-negative organisms than gram-positive organisms. Acetic acid 4% solution is not an effective alternative disinfectant to chlorine tablet solution, the currently used hospital disinfectant, but maybe used as an adjunct for better reduction of hospital environmental pathogens.

KEYWORDS: *Acetic Acid, Chlorine Tablet, Disinfectant, Healthcare Associated Infection, Bacteria*

INTRODUCTION

Healthcare Associated Infections (HAIs) or nosocomial infections are infections acquired during hospitalization that were not present during the time of admission.[1] It is a serious public health problem with significant consequences both individually and economically. It posts a great risk on the quality of care for patients and is responsible for high morbidity and mortality rates. A report released by the WHO last 2011 showed that HAIs occur in 7 and 10 out of every 100 hospitalized patients in high and low to middle-income countries respectively.[2] As a consequence, HAIs lead to prolonged hospital stay of patients.

The hospital environment is widely and naturally contaminated with microorganisms. These microorganisms dwell on inanimate surfaces surrounding a patient and pose a risk of transmitting these microorganisms to them. In a study done by Weber et al., he found that organisms such as Methicillin-resistant *Staphylococcus aureus (*MRSA), Vancomycin-resistant Enterococcus spp. (VRE), Pseudomonas spp., Acinetobacter spp., and Norovirus can survive for days to weeks on dry inanimate surfaces, while *C. difficile* spores survive for months on environmental surfaces.[3] If the hospital environment is not properly cleaned and disinfected, these organisms pose a risk to newly admitted patients.

Noncritical environmental surfaces are objects which come in contact with intact skin, but not mucous membranes, and it includes bed rails, bedside tables and floors. A study done by Huslage et al. stated that the top five most touched surfaces in hospitals are bed rails, bed surfaces, supply carts, over-bed tables and intravenous pumps based on frequency of contact.[4] Environmental surfaces frequently touched by hand could potentially contribute to secondary transmission by contaminating the hands of healthcare workers, thus disinfection of the patient's environment is critical to reduce the risk of healthcare associated infections.[5]

Healthcare Associated Infection Rate is an important measure to evaluate the quality of service delivery in health care facilities. Through the years, there has been several consensus to improve cleaning and disinfection of environmental surfaces in healthcare facilities to decrease HAI rates.[6] There are many disinfectants that are widely used to clean hospital surfaces such as glutaraldehyde, formaldehyde and chlorine compounds, but a study done by Cortesia et al. showed that vinegar has been used for thousands of years as a common disinfectant.[7] Currently, the hospital uses chlorine tablet compound as the standard cleaning solution. This study aims to determine the bactericidal activity of 4% acetic acid versus chlorine tablets against gram-negative and gram-positive microorganisms based on percentage reduction of microorganisms in hospital surfaces and suggest that it may be an effective alternative disinfectant.

MATERIALS AND METHODS

This is an experimental study where microbiological sampling was used to determine the bacterial growth in selected non-critical hospital surfaces. The study was conducted from November 2020 to December 2020 at a 200 bed Tertiary Government Hospital, which caters to children 0 to less than 19 years old. The study was done in the General ward with the greatest number of healthcare associated infections for the month as validated by the Infection Prevention and Control Committee (IPCC). The study was done with routine terminal cleaning. A staff member from the janitorial service responsible for terminal cleaning was chosen and properly instructed by the trained and qualified supervisor on the correct process of disinfection. Return demonstration was done to ensure that the correct process was followed. Microbiological sampling through swabbing taken before and 1 hour post-application of 4% acetic acid or chlorine tablet solution was done to determine the number of microorganism found in selected non-critical hospital surfaces. The protocol was submitted and approved by the Institutional Review Board of the hospital.

Selection of Environmental Surface

A general ward was selected by the hospital IPCC for routine terminal cleaning. In the designated room, non-critical hospital surfaces which include 3 of each of the bed, bedrail, and bedside table were

randomly selected and underwent cleaning with a soap solution (200g detergent powder in 1 gallon of tap water). Note that in our hospital, monthly water analysis is done and water from the faucet was noted free from coliform bacteria. Cleaning was done by rubbing a damp cloth three times for at least 15 seconds over the surface and allowed to dry. Another clean damp cloth was used to remove the soap solution residue and allowed to dry. The clean cloth that was used was bought individually wrapped, then washed with a detergent powder, and hanged out to dry prior to use. Pre-intervention swab was done within 15 minutes after cleaning. Non-critical hospital surfaces with no bacterial growth in the culture prior to application of any solution were withdrawn from the study.

Application of Solution

Cleaning agents used were commercially available acetic acid (350 ml *Datu Puti*) with 4% acetic acid content, and chlorine tablet solution (*Biospot* effervescent chlorine tablets from the hospital Central Supply Unit) prepared as one 3.25 grams tablet dissolved in 1 liter of tap water. The non-critical hospital surfaces selected were then divided into two parts and assigned randomly for a particular solution. A clean damp cloth was soaked in solution (either 250 ml of 4% acetic acid solution or 250 ml of chlorine tablet solution) and was rubbed three times on the surface for at least 15 seconds and left for 1 hour. After an hour, another clean dry cloth was used to wipe any residue, and microbiological sampling using a swab was done in triplicate within 15 minutes.

Microbiological Sampling

Microbiological sampling and swabbing was done by a single hospital medical technologist who was blinded on the surface under study. Uniform swabbing of each non-critical hospital surface was done using horizontal strokes until all surfaces were covered. Each swab was then cultured in a trypticate soy broth and McConkey agar in the laboratory. Agar plates were incubated overnight and were examined the next day for any bacterial growth.

Statistical Analysis

Levene test for equality of variances was used to calculate if 4% acetic acid is equivalent to chlorine solution and can be an alternative disinfectant. Significance was defined as $p<0.05$.

RESULTS

A representative from each of the non-critical hospital surfaces often touched by patients were included in the study, namely the bed, bed rail and bedside table. Swabbing was done post-cleaning with soap and water. Table 1 shows the microorganisms isolated and the bacterial load measured in colony forming units. Majority of bacteria seen were Coagulase Negative Staphylococcus, Bacillus species and *Klebsiella pneumoniae*. Coagulase Negative Staphylococcus species was seen on all non-critical surfaces in the study. *Klebsiella pneumoniae* had the most number of colonies seen on the bed rail with 9000 cfu after 24 hours of incubation. The bed and bed rail had the greatest number of bacteria seen.

Table 1: Bacterial growth pre-application of acetic acid or chlorine tablet solution

Surface	Bacteria	Colonies
Bed	Bacillus sp.	6000 cfu
	Coagulase Negative Staphylococcus sp.	3000 cfu
	Klebsiella pneumoniae	3000 cfu
Bed Rail	*Klebsiella pneumoniae*	9000 cfu
	Coagulase Negative Staphylococcus sp.	2000 cfu
	Bacillus sp.	1000 cfu
Bedside Table	Coagulase Negative Staphylococcus sp.	3000 cfu

Figure 1 shows the colonies of bacteria as seen on the agar plate after 24 hours of incubation pre-application of solution. Figure 1-A shows the colonies of *Klebsiella pneumoniae*, Coagulase Negative Staphylococcus sp., and Bacillus sp. seen on the bed rail. Figure 1-B shows the colonies of Bacillus sp., Coagulase Negative Staphylococcus sp., and *Klebsiella pneumoniae* seen on the bed. Figure 1-C shows the colonies of Coagulase Negative Staphylococcus sp. seen on the bedside table.

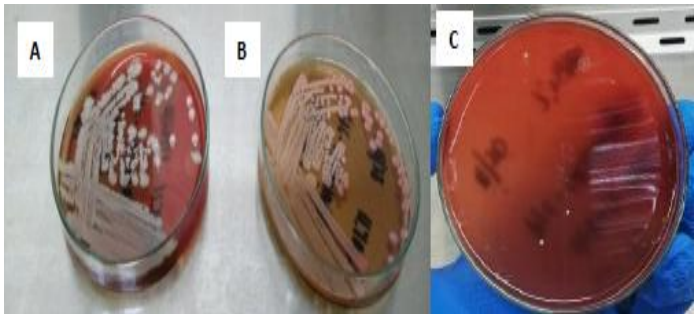

Figure 1: Bacterial colony pre-application of solution. 1-A Colony growth on bed rail. 1-B Colony growth on bed. 1-C Colony growth on bedside table.

The mean colony count on all surfaces contaminated with a specific bacterium and the percent reduction after application of 4% acetic acid solution is shown in Table 2. There was 100% reduction of Bacillus species, 70.8% reduction of Coagulase Negative Staphylococcus species, and 19.5% reduction of *Klebsiella pneumoniae* after application of 4% acetic acid solution.

Table 2: Bacterial reduction post-application of 4% acetic acid solution

Bacteria	Pre-Application (mean colony count) cfu	Post-Application (mean colony count) cfu	Percent Reduction (%)
Bacillus sp.	3500	0	100
Coagulase Negative Staphylococcus sp.	2667	778	70.8
Klebsiella pneumoniae	6000	4833	19.5

Table 3 shows the mean bacterial load measured in colony forming units and the percent reduction after application of chlorine tablet solution.

Table 3: Percent bacterial reduction post-application of chlorine tablet solution

Bacteria	Pre-Application (mean colony count) cfu	Post-Application (mean colony count) cfu	Percent Reduction (%)
Klebsiella pneumoniae	6000	0	100
Coagulase Negative Staphylococcus sp.	2667	0	100
Bacillus sp.	3500	167	95.2

Table 4 shows the percent reduction in bacterial load post-application of the two solutions. Acetic acid 4% solution was better in reducing Bacillus sp. compared to chlorine tablet solution. However, chlorine tablet solution was far more superior in reducing *Klebsiella pneumonia*.

Table 4: Comparison of percent reduction in bacterial load with 4% acetic acid solution vs chlorine tablet solution

Bacteria	Percent Reduction in Bacterial Load with 4% Acetic Acid Solution (%)	Percent Reduction in Bacterial Load with Chlorine Tablet Solution (%)
Bacillus sp.	100	95.2
Coagulase Negative Staphylococcus sp.	70.8	100
Klebsiella pneumoniae	19.5	100

Table 5 shows the Levene Test for Equality of Variances. All p-values obtained were greater than 0.05.

Table 5: Levene Test for Equality of Variances

Bacteria	Solution	Percentage Reduction	p-Value
Bacillus sp.	4% Acetic Acid	100	.363
	Chlorine Tablet	95.2	
Coagulase Negative Staphylococcus sp.	4% Acetic Acid	70.8	.084
	Chlorine Tablet	100	
Klebsiella pneumoniae	4% Acetic Acid	19.5	.064
	Chlorine Tablet	100	

DISCUSSION

Healthcare Associated Infections (HAIs) are one of the major causes of morbidity and mortality among hospitalized patients. In this tertiary Government Hospital where the study was conducted, HAI is one of the main causes of prolonged hospital stay. To address the burden of HAIs, the hospital Infection Control Committee emphasizes strict hand washing, environmental cleaning, disinfection, and patient monitoring & surveillance.

A study done by Boyce on environmental cleaning found that there was lack of time spent in cleaning the hospital environment. This suggests that suboptimal cleaning and disinfection practices cause persistent contamination and increases risk for resistant pathogens.[6]

The Centers for Disease Control and Prevention (CDC) stresses the importance of the environment in transmitting microorganisms and recommends that surfaces near patients be properly disinfected.[8] Many studies showed that hospital acquired pathogens often contaminate non-porous surfaces such as beds, bedside tables, rails and medical equipments.[9] This was consistent with our study where both gram-negative and gram-positive bacteria were isolated on the beds and bed rails. The beds and bed rails are often touched by both the patient and healthcare provider, thus cross-contamination is a possibility especially when hand hygiene practices are not done properly.

The CDC states that there are many factors that can affect the efficacy of disinfection, such as type and level of microbial contamination, concentration of the disinfectant, and exposure time of microorganisms to the disinfectant.[10] In this study, the concentration used was 4% acetic acid since a study done in a hospital in Tuguegarao City, Philippines found that acetic acid concentration of 4-7% is effective as a disinfectant in non-critical hospital surfaces and is commercially available in the market.[11,12] A study from the CDC showed that low chlorine concentrations of <5ppm and 25ppm would have biocidal effect on vegetative bacteria and Mycoplasma sp. respectively in the setting of an absent organic load, and a higher concentration of chlorine at 1000ppm could kill *M. Tuberculosis*.[13] The CDC requires that the concentration of chlorine to be effective for infection control be either 5000-6000ppm or 500-600ppm depending on its intended use.[14] For this study a 1000ppm chlorine concentration was used for general disinfection of non-critical hospital surfaces.

The contact time of microorganism and disinfectant that was used in the study was 1 hour. This gave a longer contact time for the disinfectant and the surface under study. A study done by Abreu et al. showed that for a disinfectant to be effective, a contact exposure of at least 5-10 minutes is needed.[15] A case study done in St. Paul Hospital, Tuguegarao City showed that vinegar inhibits growth of microorganisms after at least 30 minutes to 2 hours of exposure to disinfectant.[11]

In this study, pre-intervention swabbing was done to document the organisms found on hospital surfaces and its microbial load. Three microorganisms were isolated namely Bacillus species and Coagulase Negative Staphylococcus species, both gram-positive organisms, and *Klebsiella pneumoniae*, a gram-negative organism. Findings in our study differ from the study done by Zubair et al. at a hospital in Pakistan which showed that most organisms seen in hospital surfaces pre-disinfection were mainly gram-negative bacteria (56.7%). In our study, the most frequent organism isolated was Coagulase Negative Staphylococcus species comprising 42.9% of isolates. This is consistent with the study done at a hospital in Nigeria and Pakistan where CONS was frequently seen in 28.3% and 22.3% of isolates, respectively. In our study, gram-negative organisms were seen on bed surfaces, consistent with the study of Zubair.[16]

For the year 2019, the top causes of Healthcare Associated Infections in our institution were Coagulase Negative Staphylococcus and *Klebsiella pneumoniae*, similar to the pathogens isolated in our study. *Klebsiella pneumoniae* is one of the most common resistant organisms based on data collected by the Infection Prevention and Control Committee of the hospital for the year 2019. Weber et al. showed that some organisms can survive on surfaces for weeks and spores survive for months,

thus the importance of proper disinfection of patient's surroundings coupled with hand hygiene for all persons in charge of the patient to prevent cross-contamination and infection with multidrug resistant organisms.[3]

Log reduction was used to measure the effectiveness of a disinfectant. Effectiveness of a disinfectant starts with a 1 log reduction which is equivalent to 90 percent reduction of microorganisms. The effectiveness of a disinfectant goes high as the log reduction increases. In our study, chlorine tablet solution was highly effective against gram-negative and gram-positive organisms. This is consistent with a study done by Rweyendala et al. which showed that chlorine tablet solution resulted to a 99.99% reduction in *Pseudomonas aeruginosa*, as well as *Candida albicans, Staphylococcus aureus, Streptococcus mutans* and *Bacillus subtilis* spores.[17] In our study, 4% acetic acid solution was effective against gram-positive organisms consistent with the case study done at St. Paul Hospital, Tuguegarao City.[11] The present study also proved that 4% acetic acid solution is a more effective disinfectant compared to chlorine tablet solution against Bacillus species, which was seen in both the bed and bed rail surfaces. This means that 4% acetic acid solution is clinically significant in reducing gram-positive microorganisms more than gram-negative bacteria.

The efficacy of a disinfectant is affected by the type and level of microbial contamination and its exposure time. However, this study had a limited range of microorganisms that grew on environmental surfaces and only a single exposure time-point was used to test the effectiveness of the solutions.

Levene Test for Equality of Variances was used to identify if there is a significant difference in terms of percentage reduction in bacterial growth between surfaces where the two solutions were applied. All p-values were noted to fall above 0.05, thus there is no statically significant difference between 4% acetic acid and chlorine tablet solution in terms of percentage reduction of microorganisms.

CONCLUSION AND RECOMMENDATIONS

In conclusion, our study showed that there is a significant reduction in the growth of gram-positive bacteria compared to gram-negative bacteria post-application of 4% acetic acid solution, which makes it an effective disinfectant against Bacillus sp. It is not an effective disinfectant for gram-negative bacteria since it does not fulfil the criteria of at least 90 percent reduction in bacterial growth to be qualified as an effective disinfectant. On the other hand, chlorine tablet solution is a more effective disinfectant against gram-negative organisms compared to 4% acetic acid. Acetic acid 4% solution cannot be used as an alternative disinfectant to chlorine tablet solution, the currently used hospital disinfectant, but maybe used as an adjunct for better reduction of hospital environmental pathogens.

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