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Original Article

A Descriptive Study on the Incidence of Stool Colonization and Candidemia Among Premature Infants on Broad Spectrum Antibiotics at the Neonatal Care Unit of the Philippine General Hospital

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ABSTRACT

The recent increase in gram negative organisms resistant to most of the first line antibiotics at our neonatal care unit have compelled the use of Imipenem and Piperacillin/ Tazobactam as alternative first drugs in cases of suspected sepsis. This has raised concerns about the emergence of more opportunistic and pathogenic organisms such as *Candida spp.* Previous studies have documented the ability of these antimicrobials to favor candida colonization of the gut, by altering the micro environment of a possibly compromised gut mucosal defense system. To document the incidence of candida gut colonization and subsequent invasive candida infection, premature infants admitted to the neonatal care unit of the Philippine General Hospital from August 20, 1997 - September 30, 1997 were evaluated. Included were, all premature infants who received (< 12 hrs.) or were to start Penicillin G, Imipenem or Piperacillin/Tazobactam, alone or in combination with other antimicrobials for at least 7 days. Patients who died or whose antibiotics were discontinued before 7 days were excluded. Stool fungal cultures as well as routine and fungal blood cultures were taken at birth, 7, 14, 21 days of antimicrobial therapy, and every 7 days there after until medications were discontinued or systemic antifungals were started. A total of 38 preterm infants were evaluated. Results of our study showed an overall incidence of 76% of stool colonization by candida with 85.7% of patients' stools colonized by 7th day of antibiotics. candida was shed in varying quantities for the duration of the study. No pattern was seen among patients who eventually developed candidemia. *Candida* albicans was the most common stool isolate. Only 32% (12/38) patients subsequently developed candidemia of which 50% were considered significant. *Candida pelliculosa* was the most common isolate in blood. The interval from the time of antibiotic use to developing candidemia was 12.6 days (range 4-21 days) for all cases of blood + candida and 15 days for significant candidemia. The mortality associated with candida was 5.4% (2/37). Factors such as the number of antibiotic days, gavage feeding days, duration of NPO were longer among patients who developed significant candidemia. Foreign literature have documented the gut as a major source of disseminated candida infection among high risk patients whose stools are heavily colonized by *Candida spp*. However, because of the difference of species of candida isolated in the blood and stool in our neonatal care unit, other major sources of nosocomial candida have to be considered such as IV lines. Potential prophylactic antifungals whether oral non absorbable or systemic should take into consideration the most likely port of entry of *Candida spp.*

INTRODUCTION

Recently, changing patterns of resistance and sensitivity of gram negative organisms isolated from neonates with early onset sepsis, have compelled the use Piperacillin/Tazobactam and Imipenem, in combination with other antibiotics, as first line empiric therapy for sepsis at our neonatal care unit. This situation raises concern for a possible increase in neonatal candida infection. At the Philippine General Hospital, the incidence of nosocomial candida infection has increased over the past 3 years: 17.2% (1994), 20% (1995), and 49% (1996); in a study by Go and Genuino at the Neonatal Care Unit, the incidence of candidemia was 3.3% (1993-1994).[18] The increasing incidence in candida infection has been thought to have resulted from antimicrobial use. Studies have shown that Imipenem and

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Vancomycin were independent factors for the development of candidemia.[8] They have been shown to exert their effects by altering the gut microflora. In addition, Piperacillin/Tazobactam has been shown to transiently alter the gut flora.[20]

Candida are usual saprophytes recovered from the human respiratory tract, gastrointestinal tract, vagina and skin. Neonates most frequently acquire *Candida spp.* during delivery as part of the mother's vaginal flora. The infant's gastrointestinal tract then becomes the most predominant site of colonization. Normally, the gastrointestinal tract's protective mechanism is effectively accomplished by an intact mucosa, and a normal bacterial gut flora which competes with colonizing *Candida,* thus preventing overgrowth and subsequent invasive candida infection.[11,12,16] Recently, attention has been focused on the gastrointestinal tract as an endogenous source of *Candida* and in immunocompromised hospitalized patients colonization has been demonstrated to precede systemic infection.[4,8] Premature, and in particular low birth weight infants, however, represents a special population of patients especially at risk for developing nosocomial candidemia.[2,3,4,5] Prior colonization with *Candida* coupled with their immature immune system as well as physical disruption of natural barriers such as the skin and gastrointestinal mucosa or disruption of the normal gastrointestinal flora by antimicrobials greatly increases their risk of developing invasive candida disease.

Although the recovery of *Candida* from the gut may be considered normal, we propose that, in situations where the gastrointestinal tract or general immune system has been compromised, monitoring the load of *Candida* in stools among neonates on broad spectrum antibiotics may be useful in predicting systemic infection despite negative blood cultures or in cases of a single positive blood culture since signs and symptoms of neonatal candidemia are largely non specific.

OBJECTIVES

The objectives of this pilot study are:

1. To determine the incidence of stool colonization and systemic candida infection among premature infants receiving broad spectrum antibiotics.
2. To compare the species of Candida isolated in the stool and in the blood.
3. To determine the time interval between stool colonization and isolation from blood of Candida spp. from the time of birth.
4. To determine changes in colony counts of Candida spp. in relation to duration of broad spectrum antimicrobial therapy.

METHODOLOGY

The study was conducted in the Neonatal Care Unit of the Philippine General Hospital, from August 20, 1997 - October 15, 1997. Included were newly admitted (< 12 hrs.) premature infants admitted and treated for at least 7 days with broad spectrum antibiotics. Patients who died in < 7 days or whose antibiotics were discontinued < 7 days were dropped from the study. On enrollment, a complete history and physical examination was done, the presence of indwelling IV lines, endotracheal tubes, naso gastric tubes, feedings and co-morbidities were noted. Baseline blood and stool fungal cultures were done as soon as possible after birth. Stools were freshly collected and immediately plated on Saboraud's Dextrose agar. Colonies were enumerated and speciated using API Yeast Identification system. Yeasts with (-) germ tube formation were further speciated either at PGH-Bacteriology Dept. or Santo Tomas University Microbiology Department. Colonies of *Candida spp.* were quantitated as follows:

light growth	–	≤20,000
moderate growth	–	>20,000 - 50,000
moderate-heavy	–	>50,000 - 100,000
heavy	–	≥100,000

Patients were followed up daily while cultures were done on days 7, 14, 21 and every 7 days thereafter of antibiotic therapy, until antibiotics were discontinued, or systemic antifungals were started or until the patient's demise. To maximize detection of candidemia, physicians were instructed to include fungal blood work up in addition to routine blood cultures done in between our collection dates. Post mortem fungal cultures were done for patients who died. Data was entered into a standard data collection form and reported as means and percentages.

Outcome of patients were classified as follows:

non-colonizer – stools negative for *Candida spp.* during all collection dates

colonizer – stool positive for *Candida spp.* at any time during collection, this group is further divided into:

1. Stool (+) and significant candidemia – if patients in whom *Candida spp.* isolated in the blood, manifested with signs of candidemia and were treated.

2. Stool (+) and Non-significant candidemia – if patients in whom *Candida spp.* isolated in the blood did not manifest with signs of candidemia and were not treated.

3. Stool colonizer only - if only the stool was + at any time during the collection.

RESULTS

A total of 38 patients were evaluable, of which, 56% were males. Majority (57%) of admitted patients were intubated. The initial antibiotic was Piperacillin/Tazobactam in 76% (29/38), 1 patient was started on IV Penicillin G and an aminoglycoside, while the rest were started on Imipenem and an aminoglycoside. The rest of the patients' characteristics are presented in Table 1. Factors such as age of gestation, birth weight, maternal risk factors, manner of delivery and infant co morbidities failed to show a reliable relationship versus colonization and/or developing systemic infection.

Stool Colonization: Of the 38 evaluable patients, 76% were positive for *Candida* in their stools. Only 1 patient was moderately colonized by day 1. This infant subsequently developed symptomatic candidemia and was successfully treated with systemic antifungals. Majority (85.7%) of infants' stools had been colonized by the 7th post antibiotic day, with another 14.2% of patients only becoming positive by day 14. The quantity of *Candida spp.* varied from each collection date and are

Table 1. Characteristics of patients and their outcome

Characteristics	Non colonized		Stool positive/colonized					
			significant candidemia		*non significant candidemia*		*no candidemia*	
	alive n = 6	*died n = 3*	*alive n = 5*	*died n = 1*	*alive n = 5*	*died n = 1*	*alive n = 15*	*died n = 2*
1. age of gestation								
≤ 28 wks	0	1	0	0	0	1	0	0
29-30 wks	0	1	1	0	1	0	4	0
31-32 wks	1	0	2	0	0	0	3	0
33-34 wks	4	0	1	1	2	0	5	1
35 - ≤ 37 wks	1	1	1	0	2	0	3	1
2. mean birth weight (grams)	1690	1427	1200	1530	1340	700	1618	1950
range of weights (grams)	1043-2290	840-1920	870-1590		1530-1740		1200-2850	
3. maternal risk factors								
URTI	1	0	0	0	1	0	4	1
URTI + fever	0	0	1	0	0	0	2	0
PROM	1	0	0	1	0	0	1	1
CHVD	0	0	0	0	1	0	1	0
pre eclampsia	0	0	0	0	0	0	4	0
pre eclampsia with nephritis	0	1	0	0	0	0	0	0
hypertension	0	0	0	0	0	1	0	0
ASD + eisenmemgerization	0	0	1	0	0	0	0	0
aplastic anemia	0	0	0	0	1	0	0	0
placenta previa	1	1	0	0	1	0	1	0
4. manner of delivery								
vaginal	3	1	4	0	4	1	4	1
cesarian	3	2	1	1	1	0	11	1
5. Initial Antibiotic								
piperacillin/tazobactam	5	1	3	1	5	0	14	2
imipenem	1	2	1	0	0	1	1	0
penicillin G	0	0	1	0	0	0	0	0
6. Intubated	2	3	5	0	2	1	16	1
7. infant co-morbidities								
PDA	0	0	1	0	0	0	0	0
NEC	0	1	1	1	0	0	0	1
NEC + VSD	0	0	0	0	0	0	0	1
pneumothorax	0	1	0	0	0	0	0	0

URTI - upper respiratory tract infection, PROM - premature rupture of membranes, CHVD - chronic hypertensive vascular disease, ASD - atrial septal defect, PDA - patent ductus arteriosus, NEC - necrotizing enterocolitis, VSD - ventricular septal defect

presented in Table 2. *Candida albicans* was the most common stool isolate (see Table 3).

Table 2. Quantity of *candida spp.* in stool during different collection dates

Colonization Status	Quantity of *Candida* in stools		
	day 7 antibiotic treatment (n = 24)	*day 14 antibiotic treatment (n = 14)*	*day 21 antibiotic treatment (n = 7)*
Stool Positive + significant candidemia	light 20% moderate 20% moderate-heavy 66.0%	light 33.3% moderate-heavy 66.6%	moderate 100% (1 patient)
Stool positive + Non significant candidemia	light 20% moderate 40% moderate-heavy 40%	light 40% moderate-heavy 60%	light 33.3% moderate 66.6%
Stool positive only	light 14% moderate 64% moderate-heavy 14% heavy 7.1%	light 16.7% moderate 50% moderate-heavy 33.3%	moderate 100% (1 patient)

*one patient had moderate growth of Candida in stool at birth

Table 3. *Candida* species isolated from the stool

Day	Percent
DAY 7	
C. albicans	73%
C. laurenti	14%
C. famata	4.5%
C. hemicola	9%
DAY 14	
C. albicans	92.3%
C. famata	7.7%
DAY 21	
C. albicans	100%

*2/2 patients
2 patients had stools collected on day 17 - C. albicans

Cases with Disseminated Candidiasis: The incidence of candidemia among preterm infants was 32% (12/38). However, only 50% was regarded as significant and were subsequently treated. All cases who were blood (+) for *Candida,* were stool positive during the 1st 7 days of antibiotic therapy and in one case, was stool positive at birth. *Candida* had been isolated in 2 mortalities: one patient was 28 weeks old who did not receive antifugal therapy, the blood culture result came post mortem, the other patient was received therapy for 5 days. The average time interval from initiation of antibiotics to recovery of *Candida* in the blood whether significant or not, was 12.6 days (range 4-21 days); and 12.6 days (range 4-21 days) in cases of significant candidemia. The average interval from stool colonization to recovery of *Candida* in the blood was 7-8 days (whether candidemia was significant or not) among those with candidemia but not treated. The species of *Candida* found are presented in Tables 4 and 5.

Table 4. *Candida* isolates from blood and stool in patients treated with systemic Antifungals

Candida species	No. of isolates	Percent
1. Blood		
C. pelliculosa	4	66.7
non albicans	2	33.3
2. Stools		
C. albicans	4	66.7
C. tropicalis	1	16.7
C. laurenti	1	16.7

Table 5. *Candida* isolates from blood and stool in patients with (+) blood culture but not treated

Candida species	No. of isolates	Percent
1. Blood		
C. pelliculosa	2	33.3
C. famata	1	16.7
C. albicans	1	16.7
non albicans	2	33.3
2. Stools		
C. albicans	1	16.67
C. famata	1	16.67
C. laurenti	4	66.60

We found the following factors to be relatively prolonged among patients who developed significant candidemia (also see Table 6):

average no. of antibiotic days – **1.7X and 1.5X longer compared to non colonizers and stool colonizers only, respectively.**

average no. of days fed by gavage – **1.8X and 1.6X longer compared to non colonizers and stool colonizers only, respectively.**

average no. of days NPO – **2X and 1.6X longer compared to non colonizers and stool colonizers only, respectively.**

Oral thrush was not found in any of the patients. Diaper dermatitis was found in only 1 patient who eventually developed candidemia and was successfully treated. The most common species of *Candida* isolated from the blood was *Candida pelliculosa.* The species of *Candida* isolated from the stool did not correlate with blood isolates, except in one patient who yielded *C. albicans* in both stool and blood. We also noted a 5-7 day delay in initiation of starting anti-fungals despite previous positive blood cultures.

Table 6. Pertinent clinical characteristics of patients on follow up

Characteristics	Non colonized	Stool positive/colonized		
		significant candidemia	*non significant candidemia*	*no candidemia*
1. mean antibiotic days prior to (+) blood candida	8.9 (range 4-14) days	15.3 (7-21) days	10(6-18) days	13.5 (5-52) days
2. mean no. days NPO	2.9 (< 1-7) days	6.3 (2-19) days	5 (1-10) days	3.8 (< 1-14) days
3. mean no. of days fed by gavage	7 (2-14) days	12.8 (3-16) days	7.6 (2-16) days	8 (3-22) days
4. mean no. days intubated	3.8 (2-7) days	6.7 (3-10) days	11 (7-18) days	7.7 (3-21) days
5. oral thrush	0	0	0	0
6. diaper dermatitis	0	0	1	0
7. IV burns	0	0	0	0

NPO - non per orem, IV - intravenous

DISCUSSION

Recently, the gut as a reservoir for potential pathogens in developing septicemia has received much attention; studies have shown that certain microbial populations were able to translocate from the gut mucosa into the vascular system. *Candida* is one such organism which has demonstrated this ability of translocation.[11] Mucosal colonization, an essential first step in dissemination, is normally inhibited by the presence of anaerobic bacteria through their by-products such as volatile fatty acids, secondary bile acids and undefined complex interactions. Competitive binding of these bacteria to the mucosal surface receptor sites forming dense layers, serves to protect the gut mucosa from *Candida* colonization.[11,15,16] Although normally present in stools of healthy individuals (10^6 to 10^7 col.), in special host situations where local GI mucosal factors are compromised and/or in whom the general immune status has likewise been compromised, heavy stool colonizers have been associated with subsequent invasive candida infection.[4,5,11] In fact, studies have shown that patients whose stool colony count of *Candida* increased by a least 4 log and were treated with vancomycin showed an increased risk of developing candidemia.[5]

During the neonatal period, the environment of the gut changes from that of a sterile organ to one that is populated through successive waves of microorganisms, by a stable population of beneficial bacteria which serves as one of the neonate's first line of defense in the gut against potential pathogens such as *Candida*. Early enteral feedings, especially with breast milk, favors the colonization of more beneficial bacteria.[11,12,19] Broad spectrum antimicrobials such as Imipenem, Vancomycin, Penicillin G, Clindamycin have been shown to alter the gut microflora in favor of *Candida*.[16,19,21] It has been proposed that antimicrobials which include the anaerobes in their spectrum of activity exerts the greatest impact by altering the normal protective GI flora.[6] Breaks in the mucosal barrier as a result of infection or ischemia and a compromised immune defense, further predispose to colonization and subsequent systemic infection.

Although results of our study have established the ability of *Candida* to colonize stools of these infants and that all patients in whom *Candida* was isolated in the blood were all stool colonizers during the first 7 days, the discrepancies of stool and blood isolates of *Candida spp.* have led us to consider another, and perhaps, a major route of entry by candida in cases of disseminated infection. *Candida albicans,* our most common stools isolate, is a common isolate of the GIT, vagina, respiratory tract, skin and may be transferred to inanimate objects such as IV tubings and in dwelling catheters. On the other hand, *Candida pelliculosa,* our most common blood isolates, occurs naturally on inanimate objects and fruits. Its clinical importance has been recognized as a cause of endocarditis secondary to IV drug abuse and catheter related fungemia in neonates.[22]

Results of the possible temporal relation of factors which favored intestinal colonization by *Candida* such as feedings and broad spectrum antimicrobial use and duration, as well as stool colony counts, we previously described could not fully account for the acquisition of systemic candida infection as a result of the discrepancy in *Candida* stool and blood isolates. Unfortunately, we were not able to simultaneously culture other sites, such as IV tubings, parenteral fluids.

CONCLUSIONS AND RECOMMENDATIONS

Among preterm infants who developed significant candidemia were blood culture positive for *Candida* at an average of 15 days (range 4-21 days), after initiation of treatment with piperacillin/tazobactam or Imipenem in combination with other antimicrobials for sepsis. Stool colonization was present as early as birth and majority were colonized by 7 days of antibiotic therapy.

The pathogenesis of systemic candida infection is multifactorial. The inevitable use of broad spectrum

antimicrobials such as Piperacillin/Tazobactam, Imipenem and even Penicillin G in premature infants coupled with abnormal feeding modalities and schedules, invasive therapeutic and diagnostic modalities may increase their risk of developing candidemia as early as the 1st 7 days of intensive care therapy. Although the gut as a major source of *Candida* has been emphasized in foreign literature, different institutions may have different patterns (sites) for acquiring systemic candida infection. Thus vigilance and monitoring of these possible sources may help in developing preventive strategies. In institutions that may have a high rate of acquiring *Candida* through IV lines or other tubings, the use of systemically absorbed prophylactic antifungals may have to be considered.

We suggest that larger population sizes and more accurate methods of stool quantitation of *Candida* colonies be used such as using a pour plate broth dilution method using weighed stools. Using ISOLATOR TUBES rather than conventional blood culture collection methods may further enhance recovery of *Candida*. Further studies may be needed to confirm it systemic candidiasis occurs earlier than 7 days. Finally, we suggest future studies to include culturing of potential ports of entry of *Candida* i.e. IV tubings, in dwelling tubes to determine the most common source of disseminated *Candida* infection.

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