Prevalence and immediate outcome of candida colonized preterm neonates admitted to Special Care Unit of Mulago Hospital, Kampala Uganda

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Abstract

Background: Candida species is the third commonest cause of sepsis among neonates. Colonization by Candida is a predictor for candidemia among preterm neonates.

Objectives: To determine prevalence of early Candida colonization and early outcome among colonized preterm neonates admitted to Mulago hospital Special Care Unit.

Methods: A prospective observational cohort was conducted between December 2008 and April 2009. Preterm neonates aged >72 hours and less than one week were screened for Candida colonization of the groin, oral pharynx and rectum using CHROMagar. Colonized neonates were followed up for 14 days. Blood cultures were done for those with signs of septicaemia.

The Fisher's exact tests and logistic regression were conducted for factors associated with colonization and mortality among colonized neonates. P values of < 0.05 were considered significant and confidence interval of 95% was used.

Results: Candida colonization occurred in 50/213 (23.5%) neonates. Gestational age ≤ 30 weeks was the only factor independently associated with colonization ($p = 0.005$). Of the colonized 14/46 (30.4%) died and 13/46 (28.3%) developed mucocutaneous candidiasis. No candidemia was identified. Multiple site colonization was independently associated with mortality ($p=0.035$).

Conclusion: The consequence of high colonization observed in this study needs to be further elucidated in Uganda.

Key words: Preterm neonates, Candida colonization, Candidemia

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Introduction

The majority of global neonatal deaths take place in low income countries[2] with the leading causes being prematurity (28%) and infections (26%)[2,3]. In Uganda infections and preterm births account for 31% and 25% of all neonatal deaths respectively[4,5]. Although Candida species exist as commensals on the skin, oropharynx and gastrointestinal tract, invasive candidiasis[7-9] may occur among preterm neonates due to immaturity of their immune system[6,7].

Surveys from some parts of the world have revealed an increase in occurrence of invasive candidiasis in neonatal intensive care units (NICUs) especially among preterm neonates[8-10] with outbreaks reported from some NICUs[11]. Studies have found that 7-20% of all Candida colonized preterm neonates develop invasive candidiasis[12-15] and the most important predictor for invasive candidiasis in neonates is colonization by Candida[10,13,16]. Invasive candidiasis is associated with substantial morbidity as well as a high attributable mortality rate (range, 30–75%)[16].

Early diagnosis of invasive candidiasis is challenging hence the shifting of the focus to prevention. Two different Cochrane reviews analyzing antifungal

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prophylaxis using oral/topical nystatin/miconazole and systemic fluconazole among very low birth weight neonates have demonstrated a reduction in invasive fungal disease among infants receiving anti-fungal prophylaxis [17,18].

In this paper, we describe Candida colonization and candidemia in preterm neonates. Since there was no data on the prevalence and outcome of Candida colonization of preterm neonates in our settings, our study findings will provide guidance for care of preterm neonates in Uganda and other low resource settings.

Methods

Study Site: This study was conducted at the Special Care Unit (SCU) in Mulago hospital. Mulago hospital is a national referral hospital for Uganda and a teaching hospital for Makerere University. The SCU is where newborn babies who require specialized care are admitted. The unit admits over 2000 low birth weight babies per year with a 35% mortality among low birth weight babies . The unit has preterm, full term and Kangaroo Mother Care sections.

Most invasive procedures are not carried out in the unit and the available maximum respiratory support is Continuous Positive Airway Pressure (CPAP). The unit is over crowded with most incubators shared by 2 to 3 preterm neonates.

Preterm neonates are discharged from SCU once they are tolerating oral feeds, gaining weight and maintaining stable body temperature while in Kangaroo care irrespective of their weight or gestational age. A Kangaroo clinic runs in the unit twice a week. Discharged neonates are followed up from this clinic till they attain a weight of 2500g.

Study design and sample size:

This was a prospective observational cohort study.

Sample size for prevalence using Kish Leslie method was used since prevalence of Candida colonization was the primary objective. Because no such study had been conducted in Uganda; results from a study in India that found 15.5% preterm neonates colonized by day 3 of life[15] was used in estimating sample size and assuming: Z-Standard normal value corresponding to 95% confidence interval (1.96)

D-Absolute errors between the estimated and true value= 0.05 (5%). P- The prevalence of Candida colonization of (15.5%) after 72 hours of life. n = Z2 P (1-P) / D2n = 1.962 x 0.155(1- 0.155) / 0.052 n (Sample size required) = 201

Assuming 6% loss to follow up: Final sample size = 213

Procedures: Eligible neonates were those < 37weeks of gestational age who were > 72 hours and < 7 days old. Gestational ages were determined using New Ballard Score (NBS) performed within 24 hours of admission.

Mothers of eligible neonates were identified; detailed explanation given and written consent obtained prior to enrolment. Neonates born to mothers who had been on antifungal agents in the last month of pregnancy were excluded. Enrolled neonates were given study numbers and data captured using pre-coded case report forms.

The study staff used sterile swabs to collect specimens from the groin, oral pharynx and rectum by gently rubbing without prior use of antiseptic. Different swabs were used for each site. The swabs were placed in Amies transport medium, labeled with participants study number and site of collection and transported to the laboratory immediately. Specimens were inoculated on CHROMagar (Becton Dickinson, Sparks MD) and incubated at 37^{0}C. Agar plates were examined at the microbiology laboratory (Department of Microbiology Makerere University) for growth at 24, 48 and 72 hours. Candida albicans was identified using CHROMagar while for non albicans fermentation of sugars (Glucose, Sucrose, Lactose, Maltose and Urase) was used.

Colonized neonates were followed-up for two weeks at the SCU and for those discharged earlier as outpatients. Parents to neonates discharged before laboratory results were back were traced in the follow-up clinic on their scheduled visit.

During the follow-up neonates were examined for oral or perianal thrush and for signs suggestive of septicaemia by World Health Organization criteria[1]. Neonates with oral thrush received nystatin suspension and clotrimazole cream for those with diaper rash.

Blood was collected under aseptic technique from neonates with signs of septicaemia, placed in blood culture bottles with Brain heart infusion agar and transported to laboratory for Candida and bacterial isolation.

Data management and analysis: Data from case report forms was entered into EPI DATA package 3.1 then exported into STATA version 10 for analysis. Univariate analysis was conducted for continuous variables (gestation ages, birth weight and mothers' ages) and results summarized as mean, median and standard deviations.

Continuous variables were categorized and then analyzed using Fisher's exact test to determine association with Candida colonization. Factors with p value ≤ 0.2 in the bivariate analysis were included in the logistic regression model to determine factors independently associated with colonization. P values of < 0.05 were considered significant and confidence interval of 95% was used.

Ethical approval: Approval to carry out this study was granted by the Research and Ethics Committee of Makerere University Medical School.

Results

Over a five months period (December 2008 to April 2009), 213 preterm neonates were enrolled in the study. Four study participants were discharged early and lost to follow up. Table 1 shows baseline characteristics of study participants. The mean gestational age and birth weight of the study participants were 31.6 weeks (SD 2.3) and 1469 gms (SD 303) respectively. Forty (18.8%) of the study participants were small for gestational age. All participants received intravenous ampicillin and gentamicin prophylactically; 92% received continuous positive airway pressure and 32% received intravenous aminophylline. Breast milk feeds were initiated in 93% of the study participants prior to enrollment. Two (2) participants received ibuprofen for patent ductus ateriosus and none received surfactant or parenteral lipids. No participant underwent central venous catheterization or intubation and none had commenced kangaroo care prior to enrollment.

Table 1. Baseline characteristics of the 213 study participants at admission.

Variables	Frequency (213)	Percentage (%)
Sex		
Male	101	47.7
Female	112	52.3
Place of birth		
Mulago	138	64.8
Other health facility	52	24.4
Home	23	10.8
Mode of delivery		
Vaginal	174	82.0
Caeserian section	39	18.0
HIV exposure		
Not exposed	175	82.2
Exposed	38	17.8
Gestation Age		
31-36 weeks	150	70.4
≤30 weeks	63	29.6
Birth weights		
≤ 1500 grams	126	59.2
> 1500 grams	87	40.8
Axillary temperature (^{o}C)		
< 36.5	207	97.0
≥36.5	6	3.0
Respiratory rate (breaths/min)		
< 60	167	78.4
≥ 60	46	21.6
Pulse rate (beats/min)		
≥ 120	194	91.1
< 120	19	8.9

Baseline characteristics were taken at admission

Baseline characteristics were taken at admission

The mean age of the mothers involved in the study was 24 years (SD 5.5). Thirty six mothers (18.8 %) were HIV positive, eight were on HAART and 19 (53%) were on cotrimoxazole prophylaxis only. No mother had received antenatal steroids or antifungal treatment in the last month of pregnancy. Seventy eight percent of mothers reported using herbs all of which were taken orally and no mother reported use of herbs per vagina. Table 2 shows Candida species and sites of colonization. Fifty (23.5%) of the 213 enrolled preterm neonates were colonized. The rectum was the most colonized site. Eighteen (36%) neonates had colonization of more than one site and two had all the three sites colonized. Four of the colonized neonates had more than one species of Candida.

Table 2.*Candida* species and sites of colonization

Organisms	n=70 (%)	Oral n = 6	Groin n = 29	Rectal n = 35
Candida albicans	28 (40%)	2	12	14
Candida krusei	21 (30%)	0	10	11
Candida glabrata	13 (18.6)	2	6	5
Candida tropicalis	2 (2.9%)	0	1	1
Unidentified	6 (8.5%)	2	0	4

Four of the colonized preterm neonates had more than one species of Candida, 2 had krusei + albicans, 1 had glabrata + albicans and 1 had glabrata + tropic

Table 3 shows association between baseline characteristics, interventions and early Candida colonization. No association was found between interventions received prior to enrollment and colonization. From multiple regression analysis gestational age ≤ 30 weeks was significantly associated with colonization (p= 0.005). During follow-up, 13/46 (28%) of the colonized neonates developed mucocutaneous candidiasis (oral +/- diaper) occurring between day 6 and 12 of life. Nine (20%) of the colonized neonates developed clinical sepsis, seven had confirmed bacterial septicaemia; Klebsiella pneumonia (5), Eschericia coli (1) and Serratia marcescens (1) while two had no isolated organisms. Candidemia was not found among these neonates. Both neonates who had clinical septicaemia with negative blood cultures had more than one site colonized and one neonate developed mucocutaneous candidiasis.

Table 3 Association between baseline characteristics, interventions and early *Candida* colonization

Variables	*Candida* colonization No (N=163)	Yes (N=50)	OR (95% CI)	p-value
Sex				
Male	78 (47.8%)	23 (46%)	0.93	0.818
Female	85 (52.2%)	27 (54%)	(0.46-1.84)	
Place of birth				
Mulago	108 (66.3%)	30 (60%)	1.00	0.444
Other health facility	36 (22%)	16 (32%)	(0.30-3.30)	
Home	19 (11.7%)	4 (8%)		
Mode of delivery				
Vaginal	131 (80.4%)	43 (86%)	1.50	0.411
Caesarian section	32 (82.0%)	7 (14%)	(0.50-4.30)	
HIV exposure				
Not exposed	130 (80.0%)	45 (90%)	0.43	0.138
Exposed	33 (20.0%)	5 (10%)	(0.13-1.23)	
Gestational age				
31-36 weeks	123 (81.0%)	27 (54%)	2.60	**0.004**
≤ 30 weeks	40 (19.0%)	23 (46%)	(1.20-5.30)	
Birth weight				
≤ 1500grams	90 (55.2%)	36 (72%)	2.08	**0.047**
> 1500grams	73 (44.8%)	14 (28%)	(1.00-4.50)	
Duration of rupture of Membranes				
< 24hours	125(77.0%)	38 (76%)	1.03	0.920
≥ 24 hours	38 (23.0%)	12 (24%)	(0.44-2.28)	
Oral Suction*				
No	148 (91.0%)	48 (96%)	0.41	0.371
Yes	15(9.0%)	2 (4%)	(0.04-1.87)	
CPAP				
No	151(92.7%)	46 (92%)	1.09	1.000
Yes	12(7.3%)	4 (8%)	(0.24-3.84)	
Initiation of breast milk				
Yes	155(95.1%)	45 (90%)	2.15	0.190
No	8(4.9%)	5 (10%)	(0.52-7.86)	
Small for gestational age				
Yes	32 (19.6%)	8 (16.0%)	0.82	0.773
No	131 (80.4%)	42 (84.0%)	(0.42-1.64)	

Variable	COR* (95% CI)	p-value	AOR* (95% CI)	p-value
Gestational age ≤ 30 weeks	2.619 (1.27- 5.33)	0.004	2.60 (1.33-5.10)	**0.005**
HIV exposure	0.437 (0.13-1.23)	0.138	0.45 (0.16-1.22)	0.116
Not Initiated on Breast milk	2.153 (0.52-7.86)	0.190	1.04 (0.3-3.5)	0.994

* Preterm neonates delivered from other health facilities; if suction was not mentioned then it was assumed not done.
COR* Crude Odds ratio, AOR* Adjusted Odds Ratio

Out of 46 colonized neonates 14 (30%) died. Eight died from septicaemia, four from suspected necrotizing enterocolitis (NEC), and two from aspiration of feeds. All neonates who died from suspected NEC had Candida rectal colonization, three had more than one site colonized, and two of them had mucocutaneous candidiasis. All neonates who died from suspected NEC, deteriorated following rapid progression of suspected NEC, no blood for cultures were obtained and parents declined postmortem. Table 4 shows factors associated with mortality among Candida colonized neonates. There was no association between Candida species responsible for colonization with occurrence of mucocutaneous candidiasis, bacterial septicaemia or death. On logistic regression association between multiple sites of Candida colonization and mortality remained statistically significant p=0.035 although the numbers were small.

Table 4. Bivariate and multivariate analysis of Factors associated with mortality among *Candida* colonized preterm neonates.

Variable	N=46 (%)		COR *	p-value
	Died (N=14)	Alive (N=32)	(95% CI)	
Sites colonized†				
> 1site	9(64.3%)	7(22%)	6.4	**0.0084**
1 site	5(35.7%)	25(78%)	(1.34-32.15)	
Mucocutaneous Candidiasis				
Yes	2(14.3%)	11(34.4%)	0.32	0.286
No	12(85.7%)	21(65.6%)	(0.03-1.89)	
Bacterial septicaemia				
Yes	6(43%)	1(3.1%)	23.0	**0.0019**
No	8(57%)	31(96.9%)	(2.14-1102)	
Gestation age				
≤ 30 weeks	12(85.7%)	10(31.3%)	13.2	**0.001**
31-36weeks	2 (14.3%)	22(68.7%)	(2.17-134.5)	
C. Albican* colonization				
Yes	8(61.5%)	13(46.6%)	1.84	0.505
No	5(38.5%)	15(53.6%)	(0.40-8.99)	
C. Krusei* colonization				
Yes	4(31%)	13(46.4%)	0.51	0.498
No	9(69%)	15 (53.6%)	(0.094-2.44)	
C. Non-albican & non- krusei*				
Yes	2(15.4%)	3(10.7%)	1.51	0.645
No	11(84.6%)	25(89.3%)	(0.11-15.1)	

Variable	COR* (95%CI)	p-value	AOR * (95% CI)	p-value
> 1 site colonized	6.242 (1.34-32.1)	0.0084	6.41 (1.143-36.0)	**0.035**
Gestation age ≤ 30weeks	13.2 (2.17-134.5)	0.001	7.07 (1.057-47.3)	**0.044**
Bacterial septicaemia	23	0.0019	9.4	0.08

COR* Denotes Crude Odds Ratio, AOR* denotes Adjusted Odds Ratio

4 preterm neonates were lost to follow up. † 2 preterm neonates who were lost to follow up had >1 site colonized.

* 5 of the colonized preterm neonates had species that were not identified; they were excluded from analysis for association between Candida species colonized with and outcome

Discussion

The prevalence of Candida colonization observed of 23.5% is comparable to 26.2% that was observed by Mandiratta et al in India during the first week of life[15] but higher than those observed in other centers[19-21]. Our prevalence might actually be an under estimation since we did not screen for colonization prior to 72 hours of life yet studies we are comparing with screened study subjects from the first 24 hours of life. Although other studies did not describe the day to day activities of their units, SCU is small, overcrowded with sharing of incubators and mothers are involved in the nursing care. These factors can potentiate person to person transmission of Candida since horizontal transmission has been shown to be an equally important route of Candida acquisition[22,23].

Gestational age ≤ 30 weeks was the only factor independently associated with Candida colonization (p=0.005). This finding is similar to the findings from other studies[15,20,21]. The association of Candida colonization and lower gestational age is partly due to prematurity being associated with compromised immunity [6,7]. The other explanation for this association may lie in the excessive handling that preterm neonates inevitably undergo while in neonatal units that promotes horizontal acquisition[23]. There was no association observed between small for gestational age study participants and Candida colonization (p=0.82), no other studies that we came across looked at small for gestational age and colonization. Our study finding is contrary to knowledge that these neonates are susceptible to infections due to some degree of immunosuppression[24]. One possible explanation for lack of association may be that the small for gestational age neonates tend to be stable hence have less frequent handling and therefore lower risk of horizontal acquisition of Candida.

In our study the odds of being colonized with Candida when a neonate was delivered vaginally was 1.5 fold higher than by caesarian section, however this was not statistically significant (p = 0.411). Findings from other studies demonstrated vaginal delivery as being associated with Candida colonization[15,21,23]. The lack of statistical significance found in our study may be attributed to Prolonged Rupture of Membranes (PROM) ≥24 hours which occurred in 41% of the mothers who delivered by caesarian section and because we did not screen for colonization within first 24 hours, some of the admitted neonates who died at less than 72 hours of age might have been colonized.

After controlling for PROM, the association of colonization and vaginal delivery was not statistically significant (p=0.389). This supports the possibility that Candida colonization identified after 72 hours of life is more likely acquired from the environment[22,23].

PROM for ≥ 24 hours has been associated with ascending infections and neonatal septicaemia. In this study there was no association between PROM and Candida colonization (p= 1.000). This finding correlates with findings from other studies[20,21] but contrary to findings by Mandiratta et al which found a significant association (p=<0.05)(15). Although they didn't specify hours of rupture of membranes, Mandiratta et al screened mothers for Candida vaginal colonization and found 29.8% of mothers were colonized with some isolated species resembling those responsible for neonatal colonization. Maternal HIV has been associated with higher vaginal Candida colonization especially as the mothers' immunological state deteriorates[25]. The association between HIV exposure and Candida colonization was not statistically significant (p = 0.138) even after controlling for mode of delivery (p=0.124). The possible explanation is that most of the HIV infected mothers were receiving HIV care and possibly their immunological status were good hence less carriage of Candida.

Interventions received prior to enrollment were not associated with Candida colonization. The odds of being colonized if breast milk had not been initiated was 2.15(95% CI, 0.525-7.866) although it was not statistically significant (p = 0.190). This finding could be attributed to the presence of lactobacillus in the breast milk that has been demonstrated to reduce gut Candida colonization in preterm neonates[26,27].

Mucocutaneous Candidiasis occurred in 28% of colonized neonates between the 6th and 12th day of life. These findings were similar to those observed in other studies one describing mucocutaneous candidiasis in a third of colonized neonates[20] and another describing the time of occurrence as 2nd to 3rd week of life[28]. Although other studies have reported candidemia in 7-20% of colonized preterm neonates, in our study, we did not find any. This may be due to the fact that our study did not have extremely premature

neonates who are more susceptible and the unit does minimal invasive procedures hence minimizing risk for Candidemia. Although studies have shown that multiple body site colonization with Candida is associated with candidemia[13,29], our study did not find this association. However, mortality was significantly associated with more than one site colonization. The main cause of mortality among our study neonates was bacterial sepsis; the factors predisposing these neonates to bacterial sepsis may be the same factors that predispose them to early Candida colonization[30].

The study limitations included an underestimated prevalence since we did not screen for colonization within 24 hours of life. Our data was skewed with the majority of our study subjects being >30 weeks gestation, since most of the extremely preterm neonates in our unit do not survive beyond 72 hours of life. The study was not powered enough to study associations and our inability to do postmortem on study participants who died especially those with suspected NEC might have compromised identification of invasive candidiasis.

Conclusion: In view of the perceived threat of Candidemia among this population with the high colonization and low levels of candidemia observed in this study, the consequence of early colonization needs to be further elucidated in Uganda

What is already known on this subject

Candida colonization among preterm neonates is a predictor for invasive candidiasis. Antifungal prophylaxis among preterm neonates effectively reduces fungal colonization and invasive disease.

What this study adds

Early Candida colonization of preterm neonates in a low-resource setting is very prevalent. Candidemia is not common.

Conflict of interest

The study funders did not actively participate in the study and none of the authors had any conflict of interest.

Contributorship statement

Abdallah Yaser was the principal investigator; he did the literature search, data collection, entry and the writing up of this work. Philippa Musoke and Jolly Nankunda were involved in study design and methodology; reviewed the write up, data analysis and interpretation of findings. Kaddu Mulindwa guided on laboratory techniques appropriate and reviewed results.

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