Original Article

Neonatal Oral Colonization with Candida in Jos, North-Central Nigeria

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ABSTRACT

Candida tropicalis exists as part of the community of fungi that colonize the oral cavity. The acquisition of the oral colonizers may be altered by factors such as pregnancy events and outcomes, mode of delivery. This pilot study aimed to determine the Candida species that colonize the oral cavity of neonates and some maternal/neonatal factors that affect neonatal oral colonization. This was a cross sectional study in three tertiary health care facility in Jos, North-Central Nigeria. Oral swab samples were collected from the neonates and processed using both phenotypic and molecular techniques. The rate of neonatal oral colonization was 6.9%. The four Candida species isolated and characterized were Candida tropicalis as confirmed by sequencing and phylogenetic analysis. Candida tropicalis is one of the major neonatal oral colonizers. This pilot study emphasizes the need for routine determination of common agents of oral colonization to predict the impact on adult health.

Keywords: Candida tropicalis, Neonate, Candida albicans, Oral cavity.

INTRODUCTION

The human oral cavity is a complex environment for microbial community formation and changes in response to external condition, affecting organisms residing in the cavity.¹ The neonatal oral Candida colonization is believed to be derived from the mother's vaginal mycobiota.² Candida(C)tropicalis exists as part of the community of fungi in the oral cavity.³ The neonatal oral mycobiome involves diverse Candida species including C. tropicalis that have lasting impact on adult health.^{4,5} The first oral colonizers, usually C. albicans followed by other species such as *C. tropicalis*, stimulate the changes that occur in the cavity and thus, favour the growth of subsequent species.⁶⁻⁸ The pioneer oral cavity colonizers then exert selective pressure for some, but not all colonizers of the oral cavity. The acquisition of the pioneer oral cavity colonizers may be altered by several factors which can be maternal and/or neonatal, leading to variation in oral microbial development.^{2,5,9} The factors include, pregnancy events and outcomes, mode of delivery, breast feeding, use of antibiotics, gestational age at delivery and a host of environmental conditions, all have been recognized to affect the neonates fungal gut colonizers.^{2,7,10,11} It is not clear if the factors contribute to shaping the oral colonization and to what extent if they do.

MATERIALS & METHODS

In order to achieve the objectives, a cross-sectional study was designed involving 58 neonates in two tertiary healthcare facilities in Jos, North-Central Nigeria. Oral swab samples were collected from each of the neonates for a culture-dependent molecular study. The swab samples were analysed by conventional phenotypic techniques [Brilliance Candida agar (oxoid) and Saboraud Dextrose agar (oxoid)]. The Candida isolated were store in trypticase soy broth at -20°C.

The DNA extraction was performed with Zymo spin column (Zymo Research Corporation, U.S.A). A PCR assay optimization was performed using specific primer sequences for the internal transcribed spacer -1 and -4 (ITS-1 and ITS-4) regions. The results were validated using sangar sequencing, that employed end-point PCR with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') as f o r w a r d p r i m e r a n d I T S 4 (5' - TCCTCCGCTTATTGATATGC-3') as reverse primer to amplify and sequence the regionsof the 28s ribosomal RNA gene.³

The sequences obtained were analysed with BLAST (Basic Local Alignment Search Tool) sequence comparison algorithm (<u>http://www.ncbi.nlm.nig.gov/BLAST</u>). The phylogenetic analysis was carried out using Bio edit, a sequence alignment editor and MEGA 07, a multiple sequence alignment program, as well as a neighbor joining algorithm. The optimal tree with the sum of branch length = 17.82210188 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method² and are in the units of the number of base substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 478 positions in the final dataset.

Statistical analysis

The data obtained was analysed by means of STATA (version 14IC) statistical software. A 95% confidence interval was applied, considering a p-value lower than alpha error (alpha ≤ 0.05) significance.

RESULTS

The pilot study had 58 neonates with median age of 8 days and oral colonization rate of 6.9%. The fungi isolated were C. tropicalis (Figure 1) from neonates that were more than 3 days old (p=0.48, Table 1).

All four C. tropicalis were isolated from male neonates (p=0.08, Table 1)

The colonization by C. tropicalis in the oral cavity of the neonates based on maternal antenatal clinic attendance was statistically significant (p=0.01, Table 1).

The mode of delivery of the neonates did not significantly correlate with the colonization of the oral cavity of the neonates (p=0.63, Table 1).





Characteristics Total	Candida	+veCandida -ve	chi-square	p-value	
Age (days)			-		
<u><</u> 3	6	0	6	0.49	0.48
>3	52	4	48		
Gender					
Μ	34	4	30	3.03	0.08
F	24	0	24		
Weight (kg)					
<u><</u> 2.5	24	2	22	0.13	0.72
>2.5	34	2	32		
Gestation age at birth	(weeks)				
28-32	4	0	4	0.38	0.83
33-36	11	1	10		
>37	43	3	40		
Antenatal Clinic Atten	dance				
NO	2	1	1	5.9	0.01
YES	56	3	53		
Mode of Delivery					
Ceasarean Section	21	1	20	0.23	0.63
Safe Vaginal	37	3	34		

Table 1. Demographic Characteristics of Neonates with Oral Colonization with Candida in Jos, North-Central Nigeria.

+ve =positive, -ve= negative

DISCUSSION

Currently, Candida is considered a nosocomial pathogen and its ability to colonize the oral cavity contributes to health and disease.^{6,12,13} In the present study, we used culture-dependent techniques to initially characterize the Candida in the oral cavity of neonates, and then molecular techniques using ITS-1 and ITS-4 regions.^{3,7,8,11}

The species of Candida isolated from the oral cavity of the neonates were *C.tropicalis* (100%) with accession numbers: MT490208, MT490209, MT490210, MT490211 in the genebank at NCBI. This finding is not consistent with some studies that reported C.albicans as the most abundant in the oral cavity.^{4,5,10,14} A possible explanation for this variation might be due to differences in the sample size, other maternal factors and geographic location between our study and that of other researchers. A metagenomic study that analysed fungal colonization in neonates across all body sites reported C.tropicalis as the second leading Candida species.^{3,10,12,13,15,16} This further

supports the finding from our study.

Historically, it is known that C.albicansis the most abundant in the oral cavity; several other fungal agents have been identified ranging from Alternaria, Aspergillus, Aureobasidium, Cladosporium, Cryptococcus, Eurotium, and even Fusarium.^{3,6,8,16,17} These were identified using metagenomic analysis of oral samples.

Infant fungal community studies have reported the significant role factors such as birth mode, gestational age at delivery and even antenatal clinic attendance play in neonatal oral fungal colonization.^{2,3,8,18,19} These factors, except the antenatal clinic attendance, did not significantly influence neonatal C.tropicalis colonization in our study. We believe this variation in results may be due to the small sample size and the fact that our methodology was culture-dependent as opposed to metagenomic analysis; hence further studies with larger sample size are required to verify it.

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CONCLUSION

Candida tropicalis colonizes the oral cavity of neonates. Molecular identification of Candida species colonizing neonatal oral cavity is necessary to establish epidemiologic link to infections in adulthood.

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