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Full Length Research Paper

Antimicrobial Susceptibility Pattern of Oral Microbial Isolates among Pregnant Women in Ibadan South-East Local Government Area, Nigeria

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ABSTRACT

Oral pathogenic micro-organisms have been implicated in disease causation elsewhere in the human body especially in pregnancy where they negatively influence birth outcomes. Oral hygiene measures and treatment with appropriate antibiotics prevent this negative effect. Both periodontal diseases and negative pregnancy outcomes are common among pregnant women in Nigeria; however, oral health care is not presently an integral part of antenatal care in our environment. We investigated the types of bacteria present in the mouth of some pregnant women in Ibadan, as well as the sensitivity pattern of these organisms to commonly available antibiotics. Pre-tested questionnaires were used to obtain socio-demographic information as well as oral hygiene and pregnancy history from the participants. This was followed by an intra-oral examination during which an oral swab was taken using a sterile microscopic culturing swab stick and inoculated immediately into Thioglycollate broth. Antimicrobial susceptibility was performed on confirmed pathogens using Kirby-Bauer method. Data was entered into a computer spread sheet. Frequency tables were generated and measures of central tendency calculated. Mean age was 25.28± 4.9 years old. The most prominent oral isolates were *Kliebsiella species* and *Escherichia coli* and these were higher in the second and third trimesters of pregnancy respectively. Anti-microbial susceptibility of isolates was highest for Ceftazidime: 263(99.6%), Rocephin: 247(98.9%), Resistance was highest to Co-trimoxazole 108(44.8%) and Tetracycline: 108(43.7%). The high prevalence of pathogenic, non-commensal isolates in the oral cavity of these women is cause for great concern. General personal as well as oral hygiene measures are proposed for the reduction and elimination of these pathogens.

Key words: Pregnancy, oral microbial isolates, antibiotics, susceptibility

INTRODUCTION

The oral cavity is home to over 600 species of micro-organisms with different families residing in specific locations (Dewhirst *et al.*, 2010). Many of these organisms have been implicated in the aetiology of diseases including caries, periodontitis and systemic conditions such as cardiovascular diseases, strokes and infective endocarditis (Li *et al.*, 2000). The exaggeration of periodontal diseases in pregnancy is well established

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in the literature (Ovadia *et al.*, 2007) so also is the link between periodontal diseases and poor pregnancy outcomes (Lopez *et al.,* 2002; Jeffcoat *et al.,* 2003; Scannapieco *et al.,* 2003; Contreras et al., 2006; Bobetsis *et al.,* 2006; Canakci *et al.,* 2007; Àgueda *et al.,* 2008; Lafzi *et al.,* 2011).

The increased risk for gingivitis and periodontitis in pregnancy is due to increased vascular permeability, presence of the inflammatory modulators PGE_2, Cytokines, GCF, and polymorphonuclear cells in the gingival sulcus. This is also worsened by the presence of periodontal pathogens including *Bacteroides species* (Lieff *et al.*, 2004) and gram-negative anaerobic bacteria *including Prevotella intermedia, Tannerella forsynthesis, Porphyromonas gingivalis, Treponema denticola, Actinobacillus actinomycetemcomitans and Fusobacterium nucleatum* which have been reported to travel through the maternal blood stream to the placenta (Lieff *et al.*, 2004).

Animal studies have raised the possibility that maternal periodontal infections may have long term effects on the infant's development (Bobetsis *et al.*, 2006). While it is true that these perinatal effects are caused by sub-gingival microbes, it is also true that microbial colonization elsewhere in the mouth has influence on periodontal colonization (Wang *et al.*, 2012). It has also been established that maternal oral microbes also have the postnatal effect of easy horizontal transmissibility with attendant infection of the child as has been demonstrated in the case of caries (Van Steenbergen and De Soet, 1998).

It has been reported that obstetric complications linked to periodontal diseases decreased from 10.11% to 1.84% when periodontal therapy was carried out before 28 weeks gestation (Lopez*et al.*, 2002).

This study aimed to determine the pattern of oral microbial isolates and their antibiotic susceptibility pattern in pregnant women in our environment so as to translate this into providing a holistic management of the expectant mother toward achieving favorable pregnancy outcomes.

MATERIALS AND METHODS

Ethical approval was obtained from the Oyo State Ethics Board. The questionnaires were pre-tested on twenty pregnant women at the maternity centre. Three hundred and ninety-five consecutive, pregnant women attending the antenatal clinic of the Idi-Arere Primary Health Care Centre, Ibadan within the study period, were recruited into the study. Following an oral health talk, verbal consent was obtained from the women to participate in the study after duly explaining the steps involved to them.

Pre-tested questionnaires were administered by pre-trained research assistants.

Socio-demographic information as well as oral hygiene and pregnancy history were obtained from the women this was followed by an intra-oral examination. Oral swab was performed by one of the investigators (JUI) using a sterile microscopic culturing swab stick (FL Medical Swab, Plastic stick. w/, Rayon tip; 12 x 150 in Polyproylene test tubes) which was run along the entire buccal and labial sulci of both the upper and lower jaws and then inoculated immediately into Thioglycollate broth (MV010) in a sterile pack.

The broth was then cultured on chocolate, blood and McConkey agars in duplicates. One set was incubated aerobically at 37oC while the other set was incubated anaerobically in an anaerobic gas pack system (Oxoid HP0011A). Isolates were identified by the API 20A and microbacteria identification kits for anaerobes and aerobes respectively. Antimicrobial susceptibility was performed on confirmed pathogens by the Kirby- Bauer disk diffusion method.

Data was entered into a computer spread sheet and analysed using SPSS version 19. Frequency tables were generated and measures of central tendency calculated.

RESULTS

A total of 395 pregnant women participated in the study. The mean age of participants was 25.28± 4.9 years old. Three hundred and eighty-three (97%) of the women were married. Three women (0.8%) were from middle socioeconomic strata while 392 (99.2%) were from low socioeconomic strata.

It was the first pregnancy in 120 (30.4%) participants. Whilst 256 (64.8%) have had between 2 - 4 pregnancies, 19 (4.8%) had more than four pregnancies. Three women (0.8%) were in the first trimester, 100 (25.3%) in the second trimester and 292 (73.9%) in the third trimester of pregnancy. There was evidence of gingivitis in 133 (34.3%) women, with mean gingival index of 1.21±0.34 and mean oral hygiene index of 1.32±0.53. Brushing once daily was the most prevalent oral cleansing practice reported by 256 (65%) of the women.

Klebsiella species was the predominant isolate from 101 (25.6%) of the women. Only 18 (4.6%) of the women had normal oral microbial flora, while 80 (21.05%) yielded no microbial growth. Other organisms isolated are as shown in Table 1.

Table 1:
Pattern of Oral Microbial Isolates

Oral microbial isolates	Frequency (%)
Klebsiella species	101(25.6)
Escherichia coli	81(20.5)
No Microbial growth	80(20.3)
Staphylococcus albus	34(8.6)
Proteus species	28(7.1)
Staphylococcus aureus	25(6.3)
Viridians Streptococcus	7(1.8)
Non Viridians Streptococcus	6(1.5)
Normal Oral Flora	18(4.5)
Pseudomonas species	7(1.8)
Others	8(2.0)
Total	395(100.0)

Table 2:
Distribution of microbial culture by trimester of pregnancy

Microorganisms	Trimester of pregnancy			Total
	First	**Second**	**Third**	
Klebsiella Sp.	0 (0.00)	27 (26.73)	74 (73.27)	101 (100.0)
E. coli	0 (0.00)	23 (28.40)	58 (71.60)	81 (100.0)
No growth	1 (1.25)	15 (18.75)	64 (80.00)	80 (100.0)
Staph. albus	1 (2.94)	7 (20.59)	26 (76.47)	34 (100.0)
Proteus	0 (0.00)	7 (25.00)	21 (75.00)	28 (100.0)
Staph. aureus.	1 (4.00)	6 (24.00)	18 (72.00)	25 (100.0)
Normal flora	0 (0.00)	5 (27.78)	13 (72.22)	18 (100.0)
Strep. viridians	0 (0.00)	3 (42.86)	4 (57.14)	7 (100.0)
Pseudomonas Sp.	0 (0.00)	3 (42.86)	4 (57.14)	7 (100.0)
Non-viridans Streptococcus	0 (0.00)	2 (33.33)	4 (66.67)	6 (100.0)
Others	0 (0.00)	2 (25.00)	6 (75.00)	8 (100.0)
Total	**3 (0.76)**	**100 (25.32)**	**292 (73.92)**	**395 (100.00)**

The pattern of microbial culture whether normal for the oral cavity or not did not vary significantly with parity (p=0.98), trimester of pregnancy (p-0.94) or oral hygiene status (p=0.94) of the women assessed. Distribution of micro-organisms cultured by trimester of pregnancy is as shown in Table 2.

Table 3:
Antibiotic Sensitivity Pattern

Antibiotic	Sensitive Frequency (%)	Resistant Frequency (%)
Augmentin	166 (84.7)	30 (15.3)
Ceftazidine	261 (99.6)	1 (0.4)
Ceftrioxone	252 (97.3)	7 (2.7)
Ciprofloxacin	212 (98.6)	3 (1.4)
Perfloxacin	180 (95.2)	9 (4.8)
Rocephin	244 (98.8)	3 (1.2)
Chloramphenicol	127 (64.8)	69 (35.2)
Tetracyclin	139 (56.7)	106 (43.3)
Co-trimoxazole	133 (55.6)	106 (44.4)
Sparfloxacin	124 (99.2)	2 (0.8)
Ofloxacin	77 (93.9)	5 (6.1)
Ampicilin	40 (54.1)	34 (45.9)
Amoxicilin	27 (92.9)	2 (7.1)

The antibiotic susceptibility pattern among women whose swab yielded organisms that were not the normal oral commensals showed a general trend of susceptibility to parenteral drugs and resistance to drugs administered by the oral route as shown in Table 3.

In our study, the independent sample t-test showed that the type of microbial growth whether normal to the oral cavity or known pathogen did not vary significantly with the oral hygiene or gingival indices (p=0.81 and 0.10 respectively (Table 5).

DISCUSSION

Previous studies assessing the polymicrobial inhabitants of the oral cavity have identified species such as *Streptococcus oralis, constellatus, and mitis* as well as *Selenomonas noxia* as major inhabitants of soft tissue areas such as the buccal sulci (Kolenbrander *et al.*, 2007).

Table 4:
Sensitivity and Resistance to some common antibiotics by microorganisms

ANTIBIOTICS	**ORAL MICROBIAL ISOLATES**														
	BHS	E. Coli	Klebsiella	Microcosertia	No growth	Non heamolytic strep	Proteus	Pseudomonas spp	Serrita m	Staph. albus	Staph. Sp	Strep pneumona e	Strep viridans	Streptous spp	Total
AUGMENTIN															
Sensitive	1 (100)	55 (85.9)	49 (73.1)	1 (100)		2 (100)	14 (93.3)	0 (0.0)	3 (100)	11 (100)	22 (100)		8 (100)	3 (100)	169 (84.9)
Resistant	0 (0.0)	9 (14.1)	18 (26.9)	0 (0.0)		0 (0.0)	1 (6.7)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	30 (15.1)
CEFTAZIDINE															
Sensitive	1 (100)	77 (100)	100 (100)	1 (100)	1 (100)	2 (100)	25 (96.15)	7 (100)	3 (100)	12 (100)	23 (100)	1 (100)	8 (100)	3 (100)	264 (99.6)
Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.85)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
CERFRIOXONE															
Sensitive	1 (100)	74 (96.1)	100 (100)	1 (100)	1 (100)	2 (100)	26 (100)	4 (100)	3 (100)	12 (100)	22 (91.67)	1 (100)	8 (100)	3 (100)	255 (97.3)
Resistant	0 (0.0)	3 (3.90)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.33)	0 (0.0)	0 (0.0)	0 (0.0)	7 (2.7)
CIPROFLAXIN															
Sensitive	1 (100)	61(100)	77(98.7)	1(100)	1(100)	2(100)	21(91.3)	5(100)	3(100)	8(100)	24(100)		8(100)	3(100)	215(98.6)
Resistant	0(0.0)	0(0.0)	1(1.3)	0(0.0)	0(0.0)	0(0.0)	2(8.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)		0(0.0)	0(0.0)	3(1.4)
PEFLOXACIN															
Sensitive	1 (100)	54 (96.4)	63 (96.9)	1 (100)	0 (0.0)	2 (100)	16 (84.2)	3 (100)	3 (100)	8 (100)	21 (95.5)		8 (100)	3 (100)	183 (95.3)
Resistant	0 (0.0)	2 (3.6)	2 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)	1 (100)	0 (0.0)	0 (0.0)	1 (4.6)		0 (0.0)	0 (0.0)	9 (4.7)
ROCEPHIN															
Sensitive	1 (100)	73 (97.3)	90 (98.9)	1 (100)	1 (100)	2 (100)	24 (100)	7 (100)	3 (100)	10 (100)	23 (100)	1 (100)	8 (100)	3 (100)	247 (98.8)
Resistant	0 (0.0)	2 (2.7)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.2)

Table 4 (contd.)

CHLORAMPHENICOL														
Sensitive	1(100)	46(73.0)	23(37.1)	1(100)	0(0.0)	2(100)	10(62.5)	0(0.0)	3(100)	11(91.7)	22(91.7)	0(0.0)	7(87.5)	3(100)	129(65.2)
Resistant	0(0.0)	17(26.1)	39(62.90	0(0.0)	0(0.0)	0(0.0)	6(37.5)	2(100)	0(0.0)	1(8.3)	2(8.3)	1(100)	1(12.5)	0(0.0)	69(34.9)
TETRACYCLIN														
Sensitive	1(100)	46(58.10)	30(33.7)	1(100)	1(100)	2(100)	13(56.5)	0(0.0)	3(100)	11(100)	23(95.8)	0(0.0)	6(75.0)	3(100)	140(56.5)
Resistant	0(0.0)	32(41.0)	59(66.29)	0(0.0)	0(0.0)	0(0.0)	10(43.5)	3(100)	0(0.0)	0(0.0)	1(4.2)	1(100)	2(25.0)	0(0.0)	108(43.5)
SEPTRIN(CO-TRIMOXAZOLE)														
Sensitive	1(100)	42(57.5)	28(31.8)	1(100)	1(100)	2(100)	13(56.5)	0(0.0)	3(100)	10(83.3)	23(100)	0(0.0)	7(87.50)	3(100)	134(55.4)
Resistant	0(0.0)	31(42.50	60(68.2)	0(0.0)	0(0.0)	0(0.0)	10(43.5)	4(100)	0(0.0)	2(16.7)	0(0.0)	0(0.0)	1(12.5)	0(0.0)	108(44.6)
SPARFLOXACIN														
Sensitive	1(100)	44(100)	35(100)	1(100)	1(100)	2(100)	13(86.7)	2(100)	3(100)	8(100)	22(100)	0(0.0)	8(100)	3(100)	143(98.62)
Resistant	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(13.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.38)
OFLOXACIN														
Sensitive	1(100)	26(100)	29(90.6)	1(100)	0(0.0)	1(100)	3(75.0)	4(100)	1(100)	6(100)	1(100)	0(0.0)	4(100)	1(100)	78(94.0)
Resistant	0(0.0)	0(0.0)	3(9.4)	0(0.0)	0(0.0)	0(0.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	5(6.02)
AMPICILIN														
Sensitive		14(51.9)	12(41.4)	1(100)		1(100)	1(50)	1(25)	1(100)	3(100)	1(100)	1(100)	4(100)	1(100)	41(54.7)
Resistant		13(48.2)	17(58.6)	0(0.0)		0(0.0)	1(50)	3(75)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	34(45.3)
AMOXICILIN														
Sensitive		9(100)	6(75)	1(100)		1(100)			1(100)	4(100)	1(100)		3(100)	1(100)	27(93.1)
Resistant		0(0.0)	2(25)	0(0.0)		0(0.0)			0(0.0)	0(0.0)	0(0.0)		0(0.0)	0(0.0)	2(6.9)

On the other hand, *Vellonalla spp*., *Prevotella melaninogenica, Eikenalla corodens , Neisseria mucosa, Actinomyces odontolyticus, Fusobacterium nucleatum* and the sub-species *vicenti* among a wide host of other organisms that colonize the dental area and dorsum of the tongue (Kolenbrander *et al.,* 2007). The microbial culture pattern shown in the present study reveals that organisms that are not normally found in the oral cavity are the most prevalent in this study. These organisms could most readily have been introduced into the mouth by faeco-oral transmission which was done with some frequency.

The most frequent isolate in this study was *Klebsiella pneumonae* an enterobacteriaceae, implicated in meningitis, UTI, neonatal septicemia and brain abscess (Janda and Abbot, 2006) as well as early pregnancy loss and neonatal death (Omwando *et al*., 2006; Torabi *et al.*, 2008). Candy and Leung, 1983, also reported that enterobacteria from the jejunum of infants with protracted diarrhoea showed high oral epithelial adherence.

Escherichia coli, another prevalent microbe observed in this study is linked to preterm delivery, LBW babies, preeclampsia and pregnancy induced hypertension (Khan *et al.,* 2015). It is also reported to show high adherence to oral mucosa in some infants suffering from protracted diarrhoea and malnutrition (Candy and Leung, 1983). Considering that malnutrition and diarrhoea are leading causes of debilitation and death among infants in this environment, our finding is a cause for significant alarm.

Previous studies have also reported epithelial adherence by Streptococcus pyogenes, Streptococcus pneumonea, Staphylococcus aureus and epidermidis (Choo *et. al.*, 1982). These organisms, some of which were found in the mouths of our study participants are known pathogens in humans including neonates and infants causing bacteraemia, sepsis and toxic shock syndrome as well as neonatal death (Davies, 1976).

The fact that one of the common traditional practices in this environment involves the direct transfer of pre-mashed food from the mouth of mothers to their infants is also worrisome as this presents a horizontal post-natal route of disease transmission.

Transient bacteremia as a result of oral infection may facilitate bacterial transmission from the mouth to the uterus usually by haematogenous transmission to the placenta resulting in adverse pregnancy outcome. It has been found that the placenta is not sterile as previously thought. It is reported to harbor a microbiome most similar to that in the oral cavity (Kjersti Aagaard *et al.*, 2014).

Kjersti Aagaard *et al.*, 2014, also observed that women who had full term pregnancies had a different mix of microbes from those with preterm premature deliveries. They proposed that these organisms must have travelled through the mother's blood stream to inhabit the placenta. They then concluded that this could explain why women with periodontal diseases and some other oral infections are at risk of premature delivery.

The sensitivity pattern showed marked increase in resistance to drugs that are primarily oral in their route of administration and are readily available on an over-the-counter (OTC) basis (Mukonzo *et al.,* 2015) as against those that are primarily parenteral prescription drugs. Augumentin, Cephalosporins, and having the lower resistance and higher sensitivity from the isolated strains while Ampicilin, Tetracycline, Septrin and Chloramphenicol have the greatest resistance pattern. This is similar to findings from other studies which assessed uropathogen sensitivity pattern among Ethiopian pregnant women (Ferede *et al*., 2012) and another on oral isolates from healthy and dental unhealthy patients (Gaetti-Jardim and Marqueti , 2010).

In conclusion, oral microbial infections may affect pregnant women and their children through pregnancy to the time of delivery as well as the post-partum period. This study found a high prevalence of non-commensal pathogens in the mouth of the women studied. On account of findings in this study, we propose that proper personal and oral health care be made an integral part of routine ante-natal care

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