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SUSCEPTIBILITY PROFILE OF ANAEROBIC BACTERIA ISOLATES FROM HUMAN ORODENTAL SWABS AT CENTRAL HOSPITAL, BENIN CITY, EDO STATE, NIGERIA

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ABSTRACT

Background*:* Association of oral anaerobes with various life-threatening systemic infections and the increasing resistance of same across the globe poses a great treatment challenge to Physicians; especially in Nigeria where data is limited on their susceptibility. This study evaluates the antimicrobial susceptibility patterns of anaerobic bacteria isolates obtained from human orodental diseases; at Central Hospital, Benin City, Edo State, Nigeria. This is with a view to providing baseline data on local susceptibility patterns; a desirable attribute for improving clinical management of oro-dental infections in the study centre. ***Method*****:** Specimen from sub gingival pockets of extracted teeth were collected with sterile swabs and transported immediately to the laboratory in thioglycollate broth for processing. Standard microbiological methods were used for identification, and antibiotic susceptibility test was carried out on identified isolates using the disk diffusion method as published by Clinical Laboratory Standard Institute for anaerobes. Test antimicrobial agents included; metronidazole, clindamycin, perfloxacin and cotrimoxazole. These were selected in line with the treatment guideline in the study centre. Descriptive analyses were performed on the obtained data. ***Results****: Propionibacterium, Prevotella Streptococcus, Peptostreptococcus, Bacteroides, and Fusobacterium species accounted f*or 37%, 27%, 27% ,5%, 2%, and (2%) of anaerobic isolates. Approximately 70%, 40%, 25% and 15.3% of these isolates were susceptible to fluoroquinolone, clindamycin, metronidazole and cotrimoxazole respectively. ***Conlusion*****:** This study has shown that *Propionibacterium* (37%), *Prevotella* (27%), and *Streptococcus species* (27%) are the most prevalent anaerobic bacteria of dental origin in the study centre. It has also shown that metronidazole routinely used in the prophylaxis and treatment of anaerobic oral diseases at the study centre has reduced efficacy, with only 25% of isolates been susceptible to it. Fluoroquinolones and clindamycins being the most efficacious from our data would be recommended as the drug of choice in the routine management of anaerobic oral diseases in the study centre. This would be in conformity with WHO indicator for rational use of antimicrobial therapy.

KEYWORD: anaerobes, antimicrobial, susceptibility, orodental infections.

INTRODUCTION

Anaerobes are a significant part of oro -dental flora.[1] These bacteria have been documented as major aetiologic agents of serious infections such as *otitis media, sepsis* and *oro dental diseases.*[2] Reports, have associated chronic adult *peridontitis* with high risk *of artherosclerosis, myocardial infarction*, stroke and poorly controlled diabetes.[3] *Porphyromonas gingivalis*, a keystone pathogen in chronic peridontitis has recently been identified in the brain of Alzheimer's disease patients.[4] Although, international monitoring have documented general increase in resistance trends of anaerobes[5]; local surveys in individual hospitals are rarely carried out in Nigeria. Thus this study evaluates the prevalence of oral infections and susceptibility of anaerobic bacteria isolates obtained from human orodental diseases. This is with a view to providing baseline data on local resistance trends; a desirable attribute for improving clinical management of oro-dental infections in the study centre.

MATERIALS AND METHOD

A prospective cross-sectional study of 103 patients who visited the Dental Clinic at the Central hospital Benin city Edo state, Nigeria, was carried out following ethical approval and clearance. Patients for tooth extraction were enrolled in this study, while those for other dental procedures were exempted. Demographic data collected included; sex, age and diagnosis.

Specimen from sub gingival pockets of extracted teeth were collected with sterile swab and transported immediately to the laboratory in thioglycollate broth for processing. Gram staining was performed and each

specimen streaked on *Wilkins Chargarin's agar* supplimented with 10% blood. Internal gas generating system using 4% NaOH solution added to pyrogallor crystals was adopted to obtain anaerobiosis after appropriate validation.[6] Colonial morphology, pigmentation, haemolysis, and fluorescence were noted for observed growth after 72hours incubation. Preliminary identification tests such as Gram staining, spot indole, catalase and carbohydrate fermentation were performed on identical colonies.[7]

Antibiotic susceptibility test was carried out using the disk diffusion standard method for anaerobes as published by Clinical Laboratory Standard Institute.[8] Test antimicrobial agents used were selected based on treatment guideline in the study, they included; metronidazole, clindamycin, perfloxacin and cotrimoxazole. All test media plates were incubated in an inverted position at 37°C for 72hours in an anaerobic chamber. Inhibition zone diameters were measured to the nearest millimetre and interpreted as susceptible, or resistant by comparison with published guidelines for antimicrobial susceptibility testing.[9] All analyses were carried out using SPSS version 22.0 and reported in simple frequency counts as well as percentages for susceptibility of isolates to antimicrobial agents.

RESULTS

A total of 103 (55 males and 48 female) patients were enrolled for the study with mean age of 45years. Eight (8) anaerobic bacteria were identified, with *Propionibacterium species* as the highest frequency of occurrence followed by the *Prevotella species*. *Porphyromonas species* was the lest prevalent (Table 1a and figure 1b).

Totally, 64.6% and 70.6% of isolates were associated with acute apical periodontitis, while 8.3% and 12.7% were associated with dental caries in both female and male patients respectively. Other oral infections accounted for 16.7% (males) and 27.1% (female), (Table 1a and table 1b).

Propionibacterium species (34.3%), *Prevotella species* (27%) and *Streptococcus species* (27%) were the major pathogenic isolate associated with acute apical periodontitis, while *Peptostreptococcus species* (6.0%), *Fusobacterium species* (3.0%) and *Bacteroides species* (3.0%) were least associated with acute apical periodontitis in both sexes (Table 1a and Table 1b).Also *Prevotella species* (29%), *Propionibacterium species* (23%), and *Bacteroides species* (19%) were the major causative organisms associated with dental caries, while *Peptostreptococcus species* (9.5%), *Fusobacterium species* (4.8%) were the least associated with dental caries in both sexes.

Antimicrobial sensitivity tests results showed that 76.5% of the isolates were susceptible to fluoroquinolones since they are rapidly bactericidal and concentration dependent, with predictable clinical outcome. However, reduced susceptibility was observed with clindamycin (40.3%), metronidazole (24.9%), and cotrimoxazole (15.3%) respectively in female patients. While in male patients, 91.8% of the isolates were susceptible to fluoroquinolones, while reduced susceptibility was also observed with clindamycin (34.1%), metronidazole (25.0%), and cotrimoxazole (3.2%) respectively. (Table 2a and Table 2b).

Table 1a: **Association of oral infections with bacterial isolates in female patients.**

ORGANISMS	AAP	DC	DAA	FR	RR	GING	PULP	TOTAL
STREP SPP. (24)	15	1	_	3	2	_	3	**24**
ACTINO. SPP (3)	1	1	_	_	_	1	_	**3**
PROP. SPP (25)	18	_	_	1	3	3	_	**25**
PREV. SPP (23)	18	2	_	_	1	2	_	**23**
BACT. SPP (7)	2	2	_	2	1	_	_	**7**
PEPTO. SPP (8)	5	1	_	_	_	1	1	**8**
PORPHY. SPP (0)	_	_	_	_	_	_	_	_
FUSO (6)	3	1	_	_	1	_	1	**6**
TOTAL	**62**	**8**	_	**6**	**8**	**7**	**5**	**96**

AAP- Acute apical p
eriodontitis, DC-- Dental caries, DAA- Dentoalveolar abscess, FR- Fracture, RR - Retained root, GING-- Gingivitis, PULP - Pulpitis

KEY
Strep spp – streptococcus species
Action spp – actinomyces species
Prop spp – propionibacterium
Prev spp – prevotella species
Bact spp – bacteroides species
Porphy spp – porphyromonas species
Fuso spp – fusobacterium species.

Table 1b: Association of oral infections with bacterial isolates from male patients.

ORGANISMS	AAP	DC	DAA	FR	RR	GING	PULP	TOTAL
STREP SPP. (25)	2	_	_	1	_	_	_	**3**
ACTINO. SPP (2)	18	_	_	1	2	3	_	**24**
PROP. SPP (40)	28	5	1	2	1	2	1	**40**
PREV. SPP (27)	17	4	1	2	_	3	_	**27**
BACT. SPP (7)	2	2	1	1	_	_	1	**7**
PEPTO. SPP (7)	3	2	1	_	_	1	_	**7**
PORPHY. SPP (2)	1	_	1	_	_	_	_	**2**
FUSO (2)	1	_	_	_	1	_	_	**2**
TOTAL	**72**	**13**	**5**	**7**	**4**	**9**	**2**	**102**

KEY
Strep spp - streptococcus species
Actino spp - actinomyces species
Prop spp - propionibacterium species
Prev spp - prevotella species
Bact spp - bacteroides species
Pepto spp - Peptostreptococcus species
Fuso spp – fusobacterium species

Table 2a: Antibiogram of all bacterial isolates from females.

BACTERIAL ISOLATES	Percentage sensitivity of isolates to antimicrobial agents							
	PERFLOXACIN		METRONIDAZOLE		CLOTRIMOXAZOLE		CLINDAMYCIN	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Strep spp (n=24)	12(60%)	8(40%)	4(20%)	16(80%)	1(5%)	19(95%)	4(20%)	16(80%)
Actinospp(n=3)	3(100%)	0(0%)	2(66.7%)	1(33.3%)	2(66.7%)	1(33.3%)	2(66.7%)	1(33.3%)
Propspp.(n=25)	23(92%)	2(8%)	3(12%)	22(88%)	0(0%)	25(100%)	10(40%)	15(60%)
Prevspp.(n=23)	19(82.6%)	4(17.4%)	4(17.4%)	19(82.6%)	5(21.7%)	18(78.3%)	10(44.5%)	13(56.5%)
Bactespp.(n=7)	6(85.70%)	1(14.3%)	2(28.6%)	5(71.4%)	2(28.6%)	5(71.4%)	3(42.9%)	4(57.1%)
peptospp.(n=8)	6(75%)	2(25%)	3(37.5%)	5(62.5%)	0(0%)	8(100%)	2(25%)	6(75%)
Fuso spp.(n=6)	4(66.7%)	2(33.3%)	1(16.7%)	5(83.3%)	0(0%)	6(100%)	2 (33.3%)	4(66.7%)
Porphy spp.(0)	0(0%)	0(0%)	0(0)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Average percentage	76.5%	23.5%	24.9%	75.1%	15.3%	84.7%	40.3%	59.7%

KEY
Strep spp. – Streptococcus species
Actino spp – Actinomyces species
Prop spp – Propionibacterium species
Prev spp – Prevotella species
Bacte spp. – Bacteroides species
Peptp spp. – Peptostreptococcus species
Fuso spp. – Fusobacterium species
Porphy spp – Porphyromonas species

Table 2b: Antibiogram of all bacterial isolates from males.

	Percentage Sensitivity Of Isolates To Antimicrobial Agents							
Bacterial Isolates	**Perfloxacin**		**Metronidazole**		**Clotrimoxazole**		**Clindamycin**	
	Sensitive	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**
Strep spp (n=25)	23(95.8%)	1(4.2%)	6(25%)	18(75%)	1(4.2%)	23(95.8%)	7(29.2%)	17(70.8%)
Actino spp(n=2)	2(100%)	0(0%)	0(0%)	2(100%)	0(0%)	2(100%)	1(50%)	1(50%)
Prop spp.(n=40)	38(95%)	2(5%)	4(10%)	36(90%)	1(2.5%)	39(97.5%)	12(30%)	28(70%)
Prev spp.(n=27)	25(92.6%)	2(7.4%)	5(18.5%)	22(81.5%)	2(7.4%)	25(92.6%)	7(25.9%)	20(74.1%)
Bacte spp.(n=7)	5(71.4%)	2(28.6%)	2(28.6%)	5(71.4%)	1(14.3%)	6(85.7%)	3(42.9%)	4(57.1%)
pepto spp.(n=7)	5(71.4%)	2(28.6%)	3(42.8%)	4(57.1%)	0(0%)	7(100%)	2(28.6%)	5(71.4%)
Fuso spp.(n=2)	2(100%)	0(0%)	1(50%)	1(50%)	0(0%)	2(100%)	1(50%)	1(50%)
Porphy spp.(2)	2(100%)	0(0%)	1(50%)	1(50%)	0(0%)	2(100%)	1(50%)	1(50%)
Average percentage	91.8%	8.2%	25.0%	75.0%	3.2%	96.8%	34.1%	65.9%

KEY
Strep spp. – Streptococcus species
Actino spp – Actinomyces species
Prop spp – Propionibacterium species
Prev spp – Prevotella species
Bacte spp. – Bacteroides species
Peptp spp. – Peptostreptococcus species
Fuso spp. – Fusobacterium species
Porphy spp – Porphyromonas species.

DISCUSSION

High proportion of *Propionibacterium*(34.3%), *Prevotella* (27%) *and Peptostreptococcus* (6.0%) *species* in periodontal diseases may be due to the anaerobic and commensals nature of these bacteria, that enables their survival in periodontal sockets.[10]

The high virulence nature of *Propionibacterium specie*, in peridontal tissues had been attributed to its ability to secrete propionic acid and digestive enzymes that break down peridontal tissues.[11] Thus leading to attachment loss in periodontal diseases.[12] Resultant damage caused by these proteins and the associated inflammation, makes the affected periodontal tissues more susceptible to microbial colonization by opportunistic facultative anaerobic bacteria such as streptococcus.[13]

The presence of *Prevotella species*(23.95% and 26.47% in females and males respectively) in this study corroborate it association with endodontic infections(such as root canal infections, apical periodontitis and periapical lesions); possibly due to it ability to live in biofilm with other microorganisms in the periodontal sockets.[14]

Prevotella growth is stimulated by an increase in gingival crevicular fluid that results from plaque formation usually due poor oral hygiene. The crevicular fluid contains nutrients such as *hemin* and *vitamin K* which are essential for growth of this organisms. *Prevotella* often invade the human buccal epithelial cells, the cellular invasion facillitated by type C fimbrae allows access to a nutritionally rich environment as well as evasion from the host immune defences.[15]

The observed high susceptibility profile of oral anaerobic pathogens to fluoroquinolones in our study, may be due to the broad spectrum of activity and the high tissue penetrating ability of these class of antimicrobials (being active against Gram-negative and Gram positive bacteria). Additionally, the fluoroquinolones destroy bacteria in their dormant, as well as their rapidly dividing state thus giving it a very high kill rate.[16] Low resistance levels to fluoroquinolones observed in our study with about 23.5% of all bacteria isolate may i addition be due to the reported high pharmacokinetic profile with excellent tissue penetration to the target site DNA gyrase and changes elicited in the drug entry/ efflux systems of susceptible bacteria.[17] Clindamycin has been considered the gold standard for treatment of anaerobic infections for decades, but results from our study showed high level resistance (*prevotella species* 56.5%, *Bacteriodes species* 57.1%, and *peptostreptococcus species* 75%); suggesting that this antimicrobial agent may be losing its primacy as the first line drug for management of anaerobic oral infections.[18]

The multi-drugs resistance nature of these bacteria could be due to the presence of plasmid and conjugative transposons.[19,20] The observed low susceptibility of all the isolates to metronidazole (24.9%) may be due to decreased uptake of the drug or altered reduction efficiency.[21] Specific resistance gene (*nim*) conferring resistance to nitro-imidazole have been isolated in different Gram-positive and Gram-negative anaerobic bacteria, including *Bacteriodes species*.[22]

Metronidazole is a 5-nitroimidazole group of drug and is one of the most important agents for the treatment of anaerobic infection. It is a prodrug which must be reduced for the release of the toxic nitroso-residues, that are extremely reactive and able to damage DNA, proteins and membranes of susceptible bacteria.[23] The observed high resistance levels of *Propionibacterium* (88%),

Prevotella (82.6%) and *Peptostreptococcus* (62.8%) species to Metronidazole may be conferred by *nim* genes (*nimA-j)* coding for a nitro-imidazole reductase; this transforms the prodrug to a non-toxic amino-imidazole with no antimicrobial activity. These genes have been reported to share around 70% homology for *Bacteroides and Prevotella species* and it has been associated with moderate to high level metronidazole resistance.[24]

The low susceptibility of the isolates in this study to co-trimoxazole may be due to increase in multi loci induced chromosomal alterations following misuse in most cases, that could lead to decrease in drug permeability.[25]

CONCLUSION

This study has revealed the following prevalence of dental diseases; acute apical *periodontitis* (64%) *dental caries* (12.6%), and *gingivitis(*8.7%) to be the most prevalent oral infections in the study centre. while combined dento-alveolar abscess, retained root, pulpitis and fracture were the least prevalent accounting for 14.7% of oral infections. *Propionibacterium* (37%), *Prevotella* (27%), and *Streptococcus species* (27%) were the most prevalent anaerobes isolated from both sexes. These isolates were most frequently associated with acute apical peridontitis. We have shown that metronidazole routinely used in the prophylaxis and treatment of anaerobic oral diseases at the study centre has reduced efficacy, as over 80% of all anaerobes were resistant to it. Fluoroquinolones and clindamycins being the most efficacious from our data would be recommended as the drugs of choice in the routine management of anaerobic oral diseases in the study centre. For a sustainable drug therapeutic outcome with these oro-dental diseases; the importance of culture, microscopy and susceptibility test of aetiologic agents of infected tooth prior to antimicrobial therapy is highly desirable. This would be in conformity with WHO indicator for rational use of antimicrobial therapy.

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