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SUSCEPTIBILITY PATTERN OF *PREVOTELLA* AND *PORPHYROMONAS* SPECIES FROM PATIENTS WITH ODONTOGENIC INFECTIONS IN LAGOS UNIVERSITY TEACHING HOSPITAL TO AMOXICILLIN

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ABSTRACT

Gram negative anaerobes are associated with periodontal inflammation leading to destruction of the periodontal ligament, supportive bone and subsequent tooth loss. This study was designed to characterize *Prevotella and Porphromonas* species from patients with odontogenic infections and evaluate their susceptibility pattern to amoxicillin a drug empirically administered in Nigerian dental clinics. Isolates were presumptively identified as *Prevotella and Porphyromonas* species by conventional cultural and biochemical analysis. Antimicrobial susceptibility pattern of the isolates to amoxicillin was determined by agar dilution method. *Prevotella* and *Porphyromonas* species showed susceptibility of 80% and 94% to amoxicillin respectively. *Prevotella* and *Porphyromonas sp.* resistant to amoxicillin are involved in oral infections in our population. Correct identification of these species and their corresponding susceptibility pattern will be of public health importance in selecting antibiotics for therapy in order to achieve better treatment outcomes.

KEYWORDS: **Anaerobes, antimicrobial susceptibility pattern, *Prevotella* species, *Porphyromonas* species.**

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INTRODUCTION

Anaerobic gram-negative bacteria species are part of the normal flora of the mouth, upper respiratory tract, intestinal tract and urogenital tract of humans[1]. *Prevotella* and *Porphyromonas* species are prevalent on mucosal surfaces and reach very high concentrations in dental plaque, gingival crevices, tonsillar crypts, the crypts of the tongue, as well as in the colon and the vagina[2, 3].These species are among the most commonly isolated anaerobes in odontogenic infections and the implicated species are *Prevotella melaninogenica, P. intermedia, P. bivia, P. disiens* and *Porphyromonas asaccharolytica, P. endodontalis* and *Porphyromonas gingivalis*[3, 4, 5]. Isolation and identification of anaerobes is usually tedious and costly, particularly in developing countries. This has hindered routine or even periodic testing of clinical anaerobic species[6]. The clinicians rely on empirical guidelines in treating suspected anaerobic infections[7].

Increasing resistance of anaerobes to antibiotics especially beta-lactam drug is well recognized[8]. There is a global emergence of resistant species and variety of genes transferred among genus and species found in the same environment[7]. Majority of the *Prevotella* species and a few species of *Porphyromonas* produce broad spectrum cephalosporinase and penicillinase[8, 9]. It is therefore important that species frequently involved in infections are correctly identified and that susceptible antibiotics are used during therapy. This will reduce treatment failure especially in polymicrobial infections. Therefore, this report details microbiological identification and susceptibility pattern of *Prevotella* and *Porphyromonas species* from oral infections.

MATERIALS AND METHODS

(i) Study Site and Population

Patients attending the Oral Diagnosis Clinic of the Lagos University Teaching Hospital Idi-Araba with a clinical diagnosis of dental caries or periodontal disease were enrolled into this study. Demographic information was obtained from 32 patients attending the Clinic, between April and August, 2011 using a structured questionnaire. Inclusion criteria were patients not on any antibiotics or any form of medication within the last three months and were willing to give their informed consent. The exclusion criteria included patients with malignant tumors, congenital defects and those who opted out of the study. The patients gave their informed consent to be recruited for this study, which was approved by the Research and Ethics Committee of Lagos University Teaching Hospital (LUTH) Idi-Araba Proc. No. ADM/DCST/221/VOL.10 and Institutional Review Board of the Nigerian Institute of Medical Research No: 1RB/11/106.

(ii) Sample Collection

Oral samples were taken from supra-gingival, gingival, sub-gingival sites and carious lesions using sterile paper points which were inserted into periodontal pockets or carious cavities for 1 minute. The paper points were then placed into pre-reduced dental transport media (Anaerobe systems, USA) and transported to the laboratory. Specimens were processed within two hours of collection.

(iii) Cultural Identifications

Specimens were cultured on Fastidious anaerobic agar (FAA) (Lab M) supplemented with 5% defibrinated blood. The plates were incubated at 37^{o}C for 7 days in an anaerobic jar with a sachet of gas generating kit (Oxiod Basingstoke, UK) to generate 90% N_2 and 10% CO_2. An anaerobic indicator (Merck, Germany) was also added to confirm attainment of an anaerobic environment. All brown/black pigmented, slightly raised smooth edged colonies were sub-cultured in FAA to obtain a pure culture. The anaerobic isolates were identified to genus level on the basis of colonial morphology and appearance, Gram stain reaction, pigment production, special antibiotic potency disc and by conventional biochemical reactions[10]. The pure isolates were preserved in

10% skimmed milk and stored at -80°C prior to further analysis.

(iv)Antimicrobial susceptibility testing

The susceptibility of the 18 *Prevotella* and 16 *Porphyromonas* isolates to amoxicillin was performed as recommended by the Clinical Laboratory Standard Institute[11] using the agar dilution method. An inoculum was grown in brain heart infusion broth supplemented with hemin and menadione for 24-48 hours and the turbidity was adjusted to 0.5 McFarland standard. Amoxicillin (Glaxo SmithKline, Philadelphia, PA, USA) was reconstituted according to the manufacturer's instructions and freshly prepared serial two-fold dilutions (ranging from 0.06 – 64 µg/ml) were added to Wilkins-Chalgren agar (Oxiod, Basingstoke, UK) supplemented with hemin (5µg/ml), vitamin K (1µg/ml) and 5% blood. 200µl of the overnight broth was inoculated on the plates and incubated anaerobically for 2 days at 37°C. Control plates without amoxicillin were inoculated with each batch of drug containing plates. Reference strain *Bacteriodes fragilis* ATCC 25285 was included as control. The minimum inhibitory concentration was defined as the lowest concentration of the antibiotic that yielded no bacterial growth.

RESULTS

Thirty-two patients with oral infections attending the Oral Diagnosis Clinic of the Lagos University Teaching Hospital, Idi- Araba were seen from April to August, 2011. Of the 32 patients, 14 (43.8%) had chronic periodontitis, 10 (31.3%) had acute gingivitis, 4 (12.5%) had localized aggressive periodontitis, 3 (9.4%) had dental caries and 1 (3.1%) had dentoalveolar abscess. Seventeen (53.1%) of the 32 patients were males while fifteen (46.9%) were females (Table 1).

Table 1
Distribution of Odontogenic Infections by Gender

Oral infection	Number		Total (%)
	Male	Female	
Chronic periodontitis	7	7	14(43.8%)
Acute gingivitis	6	4	10(31.3%)
Aggressive periodontitis	2	2	4(12.5%)
Dental Caries	2	1	3(9.4%)
Dental Abscess	0	1	1(3.1%)
Total (%)	**17(53.1%)**	**15(46.9%)**	**32(100%)**

Among 32 samples cultured, 29 samples showed growth. A total of 34 pigmented isolates were obtained from the culture. Of these, 18 were identified as *Prevotella species while*16 were *Porphyromonas species.* The rate of antimicrobial susceptibility pattern of *Prevotella* and *Porphyromonas* species to amoxicillin were 80% and 94% respectively (Table 2).

Table 2
Antimicrobial susceptibility of oral isolates of Prevotella and Porphyromonas species to Amoxicillin

Isolates	MIC Range (µg/ml)	MIC obtained (µg/ml)	
		Prevotella spp.	*Porphyromonas* spp.
1	≤ 0.06-32	1.25	-
2	≤ 0.06-32	0.06	-
3	≤ 0.06-32	0.06	-
4	≤ 0.06-16	-	0.25
5	≤ 0.06-16	-	0.06
6	≤ 0.06-32	0.06	-
6B	≤ 0.06-16	-	16
7	≤ 0.06-32	0.06	-
7B	≤ 0.06-16	-	0.06
8	≤ 0.06-32	0.06	-
9	≤ 0.06-16	-	0.25
10	≤ 0.06-16	-	0.06
11	≤ 0.06-32	16	-
12	≤ 0.06-32	0.06	-
12B	≤ 0.06-16	-	1.25
13	≤ 0.06-16	-	0.06
14	≤ 0.06-32	0.06	-
15	≤ 0.06-32	0.06	-
16	≤ 0.06-16	-	0.06
17	≤ 0.06-16	-	0.06
17B	≤ 0.06-32	0.06	-
18	≤ 0.06-16	-	0.25
19	≤ 0.06-16	-	0.06
20	≤ 0.06-16	-	0.25
21	≤ 0.06-16	-	0.06
22	≤ 0.06-16	-	0.06
23	≤ 0.06-16	-	1.25
23B	≤ 0.06-32	0.06	-
24	≤ 0.06-32	32	-
25	≤ 0.06-32	0.06	-
26	≤ 0.06-32	0.06	-
27	≤ 0.06-32	1.25	-
28	≤ 0.06-32	0.06	-
29	≤ 0.06-32	32	-

DISCUSSION

Odontogenic infections particularly chronic periodontitis are frequently seen by dentists in Nigeria and other countries[12,13,14]. The antimicrobial susceptibility pattern of *Prevotella* and *Porphyromonas* species to amoxicillin showed a susceptibility of 80% and 94% respectively. This relatively high susceptibility pattern is in agreement with the work by Kulik *et al.*[3] in which *P. gingivalis* and *P. intermedia* showed 100% susceptibility to amoxicillin. Eighty (80%) of the pigmented *Prevotella* isolates were sensitive to amoxicillin while 20% showed resistance. These decreasing rates of susceptibility of *Prevotella spp.* to antimicrobial agents has also be noted by previous authors[15, 16]. Liu *et al*.[15] reported an increasing trend in the resistance of *Prevotella spp.* to ampicillin over a seven year period. This may largely be due to the production of beta-lactamases by species of *Prevotella*[15, 17 - 20]. Susceptibility of *Porphyromonas* species was rarely reported in surveys and beta-lactamase is rarely produced[19, 20]. However, recent findings have shown varying degrees of resistance to antimicrobial agents by *Porphyromonas spp.* due to beta-lactamase production.

In a study by Japoni *et al*. [17], 12% of *P. gingivalis* isolated from patients with chronic periodontitis were resistant to amoxicillin although 100% were sensitive to amoxicillin/cla-

vulanic acid, indicating that resistance is due to β-lactamase production. Little information exists on the susceptibility of *Prevotella* and *Porphyromonas* spp. in Nigeria[21]. Isolation of *Prevotella* and *Porphyromonas* species shows that they play a role in odontogenic infections thereby supporting previous findings[22, 23]. Our study demonstrates that more male patients had oral infections though the difference between both sexes was not statistically significant ($P \geq 0.05$). This is in agreement with previous findings [13, 23, 24] indicating that both male and female are predisposed to odontogenic infections.

Due to the emergence of resistant anaerobic species, empirical use of antibiotics is discouraged globally. From our study, the majority of the isolates were susceptible to amoxicillin, however, resistance was observed in 20% and 6% of *Prevotella* and *Porphyromnas* species respectively. There is a need to study larger population in order to establish a better understanding of the susceptibility pattern of these species. In cases where resistance to amoxicillin is recorded, a combination therapy of β-lactamase/β-lactamase inhibitor would be of great significance in treating odontogenic infections in a Nigerian population.

CONCLUSION

Amoxicillin is one of the drugs given empirically in several cases of oral infections. Majority of the isolates were susceptibility to amoxicillin. This is an encouraging result because this antibiotic is recommended regularly in dental clinics. However the presence of resistant species should not be overlooked during therapy. It is therefore suggested that antibiotic susceptibility tests be included in the diagnosis of patients with oral infections and surveillance program be put in place to monitor periodically the susceptibility pattern of anaerobes involved in odontogenic infections in our community in order to ensure proper and effective treatment.

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