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EFFECTS OF METRONIDAZOLE AND AMOXICILLIN ON SELECTED ANAEROBES FROM ORAL INFECTIONS

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ABSTRACT: *This study was carried out to determine the "Effect of Metronidazole and Amoxicillin on Prevotella spp and Fusobacterium spp in Patients with Oral Infections Attending Central Hospital Warri. A total of 59 specimens were collected from patients with oral infection. The specimens were studied by culture, biochemical test using analytical profile index 20A kit, polymerase chain reaction test and antibiotic susceptibility test using the agar dilution method. Data was subjected to statistical analysis using ANOVA. Out of 59 specimens collected, 41 patients were infected while 18 patients were non-infected. The highest number of those infected were within the age of 31-40 years and the lowest number was within the age of 1-10 years and 61-70 years. Female patients within the age bracket of 21-40 years and male patients within 41-50 years were the most infected. The most predominant oral infection was caries with 12 (29.3%) and the highest number was seen in mayle with 22 (53.7%) while the lowest was seen in females with 19 (46.2%). Organisms isolated include Prevotella intermedia, Fusobacterium nucleatum, Porphyromonas gingivalis and Peptostreptococcus spp. The most occurring organism was Fusobacterium nucleatum with 15 (36.6%) while the least occurring was Porphyromonas gingivalis with 5 (12.2%). Co-infection of Fusobacterium nucleatum and Prevotella intermedia was seen in 1 (1.7%) patient with periodontitis. The PCR technique identified Fusobacterium nucleatum 4 (9.7%) and Porphyromonas gingivalis 2 (4.9%), the minimum inhibitory concentration of antibiotics on the anaerobes from oral infections. Peptostreptococcus spp was susceptible to metronidazole with the MIC of 0.25µg/ml and all resistant to amoxicillin. Prevotella intermedia was susceptible to amoxicillin with the MIC of 0.125µg/ml and all resistant to metronidazole. Porphyromonas gingivalis was sensitive to both metronidazole and amoxicillin with the MIC of 0.125µg/ml and 0.25µg/ml respectively. Fusobacterium nucleatum was susceptible to both metronidazole and amoxicillin with the MIC of 0.25µg/ml respectively. However, metronidazole was seen to be more effective than amoxicillin in the treatment of oral infection caused by anaerobes. In conclusion, the effectiveness of metronidazole and amoxicillin as the sole therapy in the treatment of oral infections caused by anaerobes may also be of importance in designing approaches to control periodontal infections.*

KEYWORDS: Oral infections, Gram positive and Gram negative anaerobes, analytical profile index 20A, antibiotic susceptibility test, polymerase chain reaction.

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INTRODUCTION

The indigenous microbiota is one of the major defense mechanisms protecting the body against foreign bacteria, and interference with this microbiota can cause harm to the host. Endogenous microorganisms maintain the health of the host by preventing entry of pathogenic bacteria and stimulating the immune response. Members of the indigenous microbiota may however cause local infections if there is disruption in the stability of the habitat (Lim *et al*., 2020).

The migration of commensal oral bacteria to sites not usually accessible to them may lead to infections at non-oral sites. However, some of these species might not be involved in the etiology of the disease but simply be favored by the biological changes caused by true pathogens and host responses to them (Lim *et al*., 2020).

Although much progress has been made in the last 2 decades in the taxonomy of Gram-negative oral anaerobes, the high degree of heterogeneity among commensal bacteria, along with a high similarity with related species, can lead to problematic identification. Furthermore, clonal diversity within pioneering commensal species can be high, and several clones inhabit the oral cavity simultaneously. After initial colonization, commensal bacterial species tend to persist in the mouth for years (Yu *et al*., 2020).

The microbial populations involved in oral infections are known to be highly complex and variable and have not yet been fully identified, although key organisms are generally recognized to be associated with disease progression. Strong positive associations and the concurrent presence of the bacterial species *Campylobacter rectus, Prevotella intermedia, Porphyromonas gingivalis,* and *Tannerella forsythensis* were described in adult periodontitis (Carralo *et al*., 2021). In addition, other bacteria such as *Aggregatibacter actinomycetemcomitans, Eikenella corrodens, Fusobacterium nucleatum*, and *Treponema denticola* are considered putative periodontal-pathogenic microorganisms (Jentsch *et al*., 2020).

The ecological characteristics of the oral cavity make it unique in the body, although it should not be regarded as a uniform environment. The various surfaces of the oral cavity create diverse ecological niches, each with its own particular microbial population. Mucosal surfaces (tongue, cheeks, palate, and lips), teeth, and gingival crevices all form their own particular environment, and have their own specific microbial population based on the physical and nutritional factors that apply to that particular site. A range of habitats that exist at each surface further increases this complexity of the mouth. Moreover, the properties of these environments are constantly changing. Daily changes include food consumption, hot and cold drinks, oral hygiene, and salivary flow. Thus the mouth may be considered a "feast or famine" environment. Not only does the ecology of the oral cavity change during the day, it also changes during the lifetime of the host, affecting the entire oral microbial community. In addition, other occasional events, such as scaling and polishing, dental restorations, and antibiotic therapy influence the residential micro biota (McLeod *et al*., 2021).

Bacterial culturing has been the classic diagnostic method widely used in the study of the composition of dental plaque and is still generally used as the gold or primary standard when determining the utility of a new microbial test in periodontal microbiology (Vasudevan & Stahl, 2020).

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It is generally accepted that anti-infective therapy is the cornerstone of periodontal treatment. The goal of periodontal therapy is to preserve the dentition by arresting, slowing down or reversing periodontal destruction and to prevent the recurrence of the disease. Periodontal treatment includes surgical and sophisticated regenerative techniques as well as mechanical removal of supra and subgingival microbial plagues combined with locally or systemically administered antimicrobial agents in specific groups of patients (Genco & Sanz, 2020).

LITERATURE REVIEW

In healthy human subjects, saliva contains roughly 10^8 bacterial cells/ml and bacterial concentration of the gingival crevice exceeds 10^{11} bacteria/ml (Dorn *et al*., 2003). In accordance with these high numbers of bacteria, between 500 and 1000 species of bacteria can be found in the oral cavity (Haffajee *et al*., 2006). These diverse bacteria can be divided into 2 categories based on their occurrence in the host: normal (resident) and transient (Loesche & Lopatin, 2000). These normal species compose the commensal microbiota and are almost always found in high prevalence within the host. The transient species are less prevalent and in lower numbers. They may consist of transient bacteria, which are temporarily present in the oral cavity but disappear relatively quickly, but the proportion of some of these species may occasionally increase due to environmental circumstances, causing disease in a susceptible host (Finegold & Jousimies-Somer, 1997).

Research works have shown transmission of oral bacteria between individuals. These include infants and young children getting oral microbes from their close contacts, especially from their mothers (Brook & Walker, 2005; Könönen *et al*., 2005) but also from their fathers (Brook & Walker, 2005), and from other individuals with frequent contacts, such as siblings and other children at daycare (Darby & Curtis, 2001). Furthermore, identical clones of some periodontal pathogens and mutans streptococci have been demonstrated to inhabit spouses (Van Steenbergen *et al*., 1993) and the same bacterial clones can be frequently found in all members of a family (Asikainens *et al*., 1996). Variation of bacteria from one site to another in the oral cavity can occur and identical clones of bacteria could be frequently found at different sites in the mouth (Van Steenbergen *et al*., 1993). Also, the changes of bacteria from the mouth to non-oral sites could possibly be mediated through saliva. It is well noted that oral microorganisms can easily be transferred to the pharynx (Bolstad and Jensen, 2008) and the gastrointestinal tract (Dorn *et al.*, 2003) through swallowing.

Oral infections encompass a diverse range of microbial conditions affecting the oral cavity, including the teeth, gums, and surrounding tissues. These infections can result from the proliferation of bacteria, viruses, fungi, or other pathogens, often exacerbated by poor oral hygiene practices. Common oral infections include dental caries (tooth decay), gingivitis (gum inflammation), and periodontitis (advanced gum disease). The oral environment provides an ideal breeding ground for microorganisms, as the warm, moist conditions support their growth. Additionally, factors such as compromised immune function, systemic diseases, and lifestyle choices can contribute to the development and progression of oral infections. Left untreated, oral infections can lead to pain, tooth loss, and even systemic health issues. Bacterial species associated with oral infections include *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Fusobacterium nucleatum*, and *Peptostreptococcus micros*. In addition, Gram negative anaerobic bacilli such

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as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia* have been implicated as important pathogens in oral and non-oral infections, including periodontal disease activity and progression (Bagavad *et al*., 2016). Translocation of periodontal pathogens from one site to another can threaten the outcome of periodontal treatment (Dahlen *et al*., 2004), and the re-emergence of commonly implicated periodontal pathogens after periodontal treatment seems to be mainly from the indigenous microbiota.

MATERIALS AND METHODS

Study Area and Population

The study was primarily hospital-based. It was carried out in Central Hospital Warri, Delta State within the period of three months. All consenting patients from both sexes and from all age groups with oral infections attending the dental clinic of the hospital were eligible to participate in the study.

Ethical Consideration

Ethical clearance for conducting the project was sought from the Hospital's Ethical Committee before commencement of the research.

Specimen Collection

A total of fifty-nine (59) supragingival and subgingival specimens were collected into pre-reduced dental transport media (pre- reduced thioglycolate broth) using a sterile paper point inserted into the periodontal pocket for 1 min and also swab stick was used to collect specimens from patients with abscess, caries and periodontitis. Two paper points were used on each patient; eppendorf tubes containing broth were prepared. One was placed into the sterile pre-reduced transport medium in a transportation vial for culture and the other into a 1.5 ml eppendorf tube containing thioglycollate medium for DNA extraction. The specimen collected was processed within four (4) hours of collection, while the other eppendorf tubes were stored at -86°C and used for PCR (Janson *et al*., 2023).

Specimen Analysis

Swab specimens in dental transport media, thioglycolate broth (Oxoid), was cultured into fastidious anaerobe agar (FAA) (Lab M) plates (20ml agar) supplemented with 5% defibrinated sheep blood by making an inoculum pool with the swab stick and streaking out with a wire loop. The plates were labelled and incubated in an anaerobic jar with a sachet of gas generating kit to generate 90% N_2 and 10% CO_2. Incubation was done at 37^0C for 7 days; the extended period was to allow for pigmentation to appear. FAA was used for subculturing to obtain a pure culture.

Identification of Isolates

The anaerobic isolates were presumptively identified on the basis of colonial morphology; after 7 days of incubation, the anaerobic cultures were first examined for the presence of growth, pattern of haemolysis, pigmentation, pitting of agar and colonial morphology; and appearance, Gram staining reaction and pigment production. Plates were re-incubated anaerobically for an

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additional 7 days for colony characteristics to become more distinct. All isolates obtained from the pure culture were preserved in skimmed milk and stored at -80^{0}C. From the skimmed milk preservation, the stored isolates were subsequently inoculated into freshly prepared FAA plates and used to carry out biochemical tests (Alghamdi, 2022).

Biochemical Tests

(i) Catalase: A 7 day growth on subculture plates was used. A pure colony was touched with a wooden stick and transferred onto the surface of a clean dry slide. A drop of 15% hydrogen peroxide was added to the smear and observed for immediate bubble formation (Cheesebrough, 2006).

(ii) Biochemical test using the API 20A analytical profile index (bioMérieux SA, Marcy-l'Etoile, France): The test was performed according to the manufacturer's instructions. The API 20 A strip consists of 20 microtubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension which reconstitutes the media. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. An ampule of API 20 A medium was opened and 1 ml was dispensed into test tubes. A swab stick was used to harvest all the growth on the FAA plate after 24 hrs incubation. The organism was emulsified in the test tube containing the medium by rotating the swab and rubbing it against the side of the test tube without taking it out of the suspension medium. The suspension was checked for turbidity by comparing it with the Mcfarland standard. Five millilitres of distilled water was distributed into the honeycomb wells of the ampule tray to create a humid atmosphere. An API 20 A strip was placed in the ampule tray and a sterile pipette was used to inoculate the suspension in the test tube into the strip. For the GEL (Gelatin) test, both the tube and cupule were filled with the suspension while for the indole test only the tube was filled and the cupule was filled with mineral oil to prevent the indole from evaporating. The lid was placed in the ampule tray and incubated for 24 hrs under anaerobic conditions. A positive result for indole test was indicated by a red colour while a negative result was indicated by yellow colour. A positive result for the gelatin test was indicated by diffusion of black pigments contained in the test strip while a negative result was indicated by no diffusion of black pigments.

Antibacterial Susceptibility Testing

Preparation of 0.5 Mcfarland turbidity standard: 1% solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water and also 1% solution of barium chloride was prepared by adding 0.5 g of hydrated Barium chloride (BaCl2.2H20) in 50 ml of distilled water. 0.6 ml of the Barium chloride solution was added to 99.4 ml of the sulphuric acid solution (Cheesebrough, 2006).

Antibiotics: The potency of the following antibiotics was used for the Minimum Inhibitory Concentration (MIC), metronidazole, and amoxicillin. The agar dilution procedure recommended by Clinical Laboratory Standard Institute was used (CLSI, 2020). Double dilution of each antibiotic agent (ranging from 0.06-32µg/ml) was incorporated into the sterile molten Fastidious anaerobic agar supplemented with hemin. The agar plates were inoculated and incubated anaerobically for 2 days at 37^{0}C. Control plates without metronidazole and amoxicillin were inoculated and incubated in the same way. The MICs were recorded as the lowest concentration of the antibiotic in the medium that inhibited bacterial growth, gave a

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faint haze of growth or with no more than one discrete bacterial colony. The MICs were interpreted as resistant or sensitive by applying the breakpoints that were proposed by CLSI (2020).

Molecular Analysis

Molecular analysis was performed at The Anaerobe Laboratory, Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Yaba, Lagos.

Bacterial detection by polymerase chain reaction: The DNA extraction was done directly from clinical specimens stored in -80^{0}C, using the boiling method (Haffajea *et al*., 2006). 1.5 ml of the clinical specimen in broth was centrifuged for 5 minutes at 10,000 rpm and the supernatant was discarded. About 500 µl of sterile water was added to the pellet and vortexed to homogenize with the sterile water using a vortex mixer. In an Eppendorf thermomixer comfort (22331 Hamburg, Germany), the specimen was boiled in a heating block (Fisher Scientific) for 10 minutes and vortexed, and then centrifuged at 10,000 rpm for 5 minutes. About 500 µl of the DNA solution was transferred into 1.5 ml Eppendorf tubes. The Nanodrop spectrophotometer (ND-1000, UV/VIS spectrophotometer USA) was used to estimate the concentration and purity of the extracted DNA using a blank solution containing sterile water as the standard, after which the DNA amplification was performed using species-specific primers. The primer set was FN5059S-F (ATTGTGGCTAAAAATTATAGTT) and FN5059S-R for *Fusobacterium* spp, *Porphyromonas* gin-F (AGGCAGCTTGCCATACTGCG) and *Porphyromonas* gin-R (ACTGTTAGCAACTACCGATGT) for *Porphyromonas gingivalis*. Amplification was carried out in a 25 µl reaction mixture containing 12.5 µlGo Taq Green master mix, 0.25µl Upstream primer, 0.25µl Downstream primer, 3µl DNA template and 9µl nuclease-free water to make up the reaction mixture. The amplification reaction was carried out in an Eppendorf Master Cycler gradient. The cycling parameter consisted of an initial denaturation step at 94^{0}C for 3 minutes, followed by 35 consecutive cycles of denaturation at 95^{0}C for 1 minute, the primer-specific annealing temperature at 54^{0}C for 1 minute, and an extension step at 72^{0}C for 2 minutes. This was followed by a final extension step at 72^{0}C for 5 minutes (Lau *et al*., 2004). All PCR products were separated using a 1.5% agarose gel electrophoresis performed in an electrophoresis tank at 100 volts for 2.5 hours (Sigma chemical company), stained with 0.5µg/ml ethidium bromide and photographed under UV transilluminator using a digital camera (Optima UVT 260D.S/N-268002). A DNA ladder digest of 100bp ladder was used as the molecular weight marker.

Statistical Analysis

The results obtained were edited, coded and subjected to different statistical investigations. Mean occurrence was determined for various samples. Analysis of variance (ANOVA) was used to determine the significance at 95% interval. ANOVA was used to investigate which antibiotic is most potent against the bacteria. Percentage susceptibility to the isolated bacteria to different antibiotics was also investigated (Chatzi & Doody, 2023).

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RESULTS

Of the clinical dental conditions, the number of patients with oral infections were: abscess, 5 (12.1%); chronic periodontitis, 9 (21.9%); dental caries, 12 (29.3%); periodontitis, 9 (21.9%); and acute periodontitis, 6(14.6%). Thirty-one (52.5%) of the 59 patients were females and twenty-eight were males (47.5%). The highest number of patients infected was within the age of 31-40 years and the lowest number was within the ages of 1-10 years and 61-70 years. Female patients within the age bracket of 31-40 years and male patients within 41-50 years were the most infected.

Table 1 shows the distribution of oral infections by age group. The highest incidence was seen in the age bracket of 31-40 years while the lowest in the age bracket of 1-10 years and 61-70 years respectively. Statistically, there is no significant difference in the age distribution, $p>0.05$.

Table 1: Age distribution of oral infections among patients attending the Central Hospital, Warri

Age group (years)	Male	Female	Total	Total number infected
1-10	3	5	8	3
11-20	4	3	7	4
21-30	4	7	11	7
31-40	6	8	14	12
41-50	7	5	12	8
51-60	3	1	4	4
61-70	1	2	3	3
Total	28	31	59	41

The distribution of oral infections by sex is as shown in **Table 2**. Caries was observed as the infection with the highest number of occurrence seen in males with 7 (17.1%) and chronic periodontitis in females with 6 (14.6%), while the lowest was Abscess and Acute periodontitis with 2 (4.9%) for males and females with 2 (4.9%).

Table 2: Distribution of oral infections by sex among patients attending Central Hospital, Warri dental clinic

Oral infection	Male (%)	Female (%)	Total (%)
Abscess	2(4.9)	3(7.3)	5(12.1)
Caries	7(17.1)	5(12.1)	12(29.3)
Periodontitis	6(14.6)	3(7.3)	9(21.9)
Chronic periodontitis	3(7.3)	6(14.6)	9(21.9)
Acute periodontitis	4(9.8)	2(4.9)	6(14.6)
Total	22(53.7)	19(46.2)	41(100)

Key

% - Percentage

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The distribution of oral infections by marital status is as indicated in **Table 3**. The oral infections were prevalent among the married with the highest number 26 (44.1%) of those infected. No significant difference was observed statistically, $p>0.05$.

Table 3: Distribution of oral infections by marital status among patients attending Central Hospital, Warri dental clinic

Marital Status	Total number	Number infected (%)	Non- infected (%)
Single	22	15(25.4)	7(11.9)
Married	37	26(44.1)	11(18.6)
Total	59	41(69.5)	18(30.5)

Key

% - Percentage

Table 4 shows the distribution of infections by sex and marital status. Caries was the infection with the highest number of occurrences both by sex and marital status, with 12 (29.3%) while abscess had the lowest occurrence with 5 (12.1%).

Table 4: Distribution of oral infections by sex and marital status among patients at Central Hospital, Warri dental clinic

Oral infection	Number of cases = 59	% Number of cases	Sex		Marital status	
			M	F	S	M
Abscess	5	12.1	2	3	2	3
Caries	12	29.3	7	5	9	3
Periodontitis	9	21.9	6	3	7	2
Chronic periodontitis	9	21.9	3	6	4	5
Acute periodontitis	6	14.6	4	2	0	6
Total	41	100	22	19	22	19

Key

% - Percentage

M - Male F - Female

S - Single M - Married

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Table 5 shows the frequency of occurrence of isolated organisms by culture. The most occurring isolate was *Fusobacterium nucleatum* with 15 (36.6%) while the least occurring was *Porphyromonas gingivalis* with 5 (12.2%).

Table 5: Frequency of occurrence of isolated organisms by culture among patients at Central Hospital, Warri dental clinic

Isolated organism	Number of times	%
Prevotella intermedia	10	24.4
Fusobacterium nucleatum	15	36.6
Peptostreptococcus spp	11	26.9
Porphyromonas gingivalis	5	12.2
Total	41	100

Key

% - Percentage

The co-infection observed among the isolated organisms by culture is indicated in **Table 6**. Co-infection was seen in a patient with periodontitis and the bacterial isolates involved were *Prevotella intermedia* and *Fusobacterium nucleatum*.

Table 6: Co-infection of isolated organisms in patients with oral infection attending Central Hospital, Warri dental clinic

Isolated organism	Number of co-infection	Type of infection	(%)
Prevotella intermedia *Fusobacterium nucleatum*	1	Periodontitis	1.7

Key

% - Percentage

Table 7 shows the minimum inhibitory concentration of antibiotics on the anaerobes from oral infections. *Peptostreptococcus* spp was susceptible to metronidazole with the MIC of 0.25 µg/ml and all resistant to amoxicillin. *Prevotella intermedia* was susceptible to amoxicillin with the MIC of 0.125 µg/ml and all were resistant to metronidazole. *Porphyromonas gingivalis* was sensitive to both metronidazole and amoxicillin with the MIC of 0.125 µg/ml and 0.25 µg/ml respectively. *Fusobacterium nucleatum* was sensitive to both metronidazole and amoxicillin with the MIC of 0.25 µg/ml respectively.

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Table 7: Minimum inhibitory concentration of antibiotics on the anaerobes from oral infections

Bacterial isolates	**Metronidazole** Range tested (µg/ml) Breaking point (µg/ml)	MIC obtained	**Amoxicillin** Range tested (µg/ml) Breaking point (µg/ml)	MIC obtained
Peptostreptococcus spp				
1.	≤ 0.5-32	0.25	≤ 0.5-32	2
2.	≤ 0.5-32	0.5	≤ 0.5-32	0.5
3.	≤ 0.5-32	0.25	≤ 0.5-32	1
4.	≤ 0.5-32	0.25	≤ 0.5-32	8
5.	≤ 0.5-32	0.25	≤ 0.5-32	8
6.	≤ 0.5-32	0.25	≤ 0.5-32	0.5
7.	≤ 0.5-32	0.25	≤ 0.5-32	0.5
8.	≤ 0.5-32	0.25	≤ 0.5-32	0.5
9.	≤ 0.5-32	0.5	≤ 0.5-32	0.5
10.	≤ 0.5-32	0.25	≤ 0.5-32	2
11.	≤ 0.5-32	0.25	≤ 0.5-32	2
Prevotella intermedia				
1.	≤ 0.5-32	2	≤ 0.5-32	0.125
2.	≤ 0.5-32	8	≤ 0.5-32	0.125
3.	≤ 0.5-32	1	≤ 0.5-32	0.125
4.	≤ 0.5-32	1	≤ 0.5-32	0.125
5.	≤ 0.5-32	16	≤ 0.5-32	0.25
6	≤ 0.5-32	0.5	≤ 0.5-32	0.125
7.	≤ 0.5-32	1	≤ 0.5-32	0.125
8.	≤ 0.5-32	2	≤ 0.5-32	0.125
9.	≤ 0.5-32	1	≤ 0.5-32	0.25
10.	≤ 0.5-32	8	≤ 0.5-32	0.125
Porphyromonas gingivalis				
1.	≤ 0.5-32	0.125	≤ 0.5-32	0.25
2.	≤ 0.5-32	0.125	≤ 0.5-32	0.25
3.	≤ 0.5-32	0.125	≤ 0.5-32	0.25
4.	≤ 0.5-32	0.125	≤ 0.5-32	0.25
5.	≤ 0.5-32	0.125	≤ 0.5-32	0.5

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Fusobacterium nucleatum				
1.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
2.	≤ 0.5-32	0.25	≤ 0.5-32	1
3.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
4.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
5.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
6.	≤ 0.5-32	0.5	≤ 0.5-32	0.25
7.	≤ 0.5-32	0.5	≤ 0.5-32	0.25
8.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
9.	≤ 0.5-32	0.25	≤ 0.5-32	0.125
10.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
11.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
12.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
13.	≤ 0.5-32	0.25	≤ 0.5-32	0.25
14.	≤ 0.5-32	0.5	≤ 0.5-32	0.25
15.	≤ 0.5-32	0.25	≤ 0.5-32	0.25

The identification of bacterial isolates using PCR is as shown in **Table 8**. Organisms subjected were *Fusobacterium nucleatum* with 15 (36.6%) and *Porphyromonas gingivalis* with 5 (12.2%), out of which 4 (9.7%) and 2 (4.9%) were positive for both *Fusobacterium nucleatum* and *Porphyromonas gingivalis* respectively. The total positive for the PCR was 6 (14.6%) and 14 (34.1%) was negative.

Table 8: Identification of isolated organisms from patients at Central Hospital, Warri using PCR

Isolated organism	Number of positive	% Positive	Number of Negative	% Negative
Fusobacterium nucleatum	4	9.7	11	26.8
Porphyromonas gingivalis	2	4.9	3	7.3
Total	6	14.6	14	34.1

Key

% - percentage

Table 9 shows the comparison between culture and PCR in identification of bacterial isolates in patients with oral infections. Percentage of isolated organisms by culture is 20 (48.8%) while the percentage of isolated organisms by PCR is 6 (14.6%).

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Table 9: Comparison between culture and PCR in identification of anaerobes in patients with oral infections at Central Hospital, Warri dental clinic

Isolated organism	Culture	%	PCR	%
Fusobacterium nucleatum	15	36.6	4	9.7
Porphyromonas gingivalis	5	12.2	2	4.9
Total	20	48.8	6	14.6

Key

% - percentage

Figures 1 and **2** show the amplification bands *Fusobacterium nucleatum* and *Porphyromonas gingivalis* at 360bp and 404bp respectively using gel electrophoresis of specie specific primer.

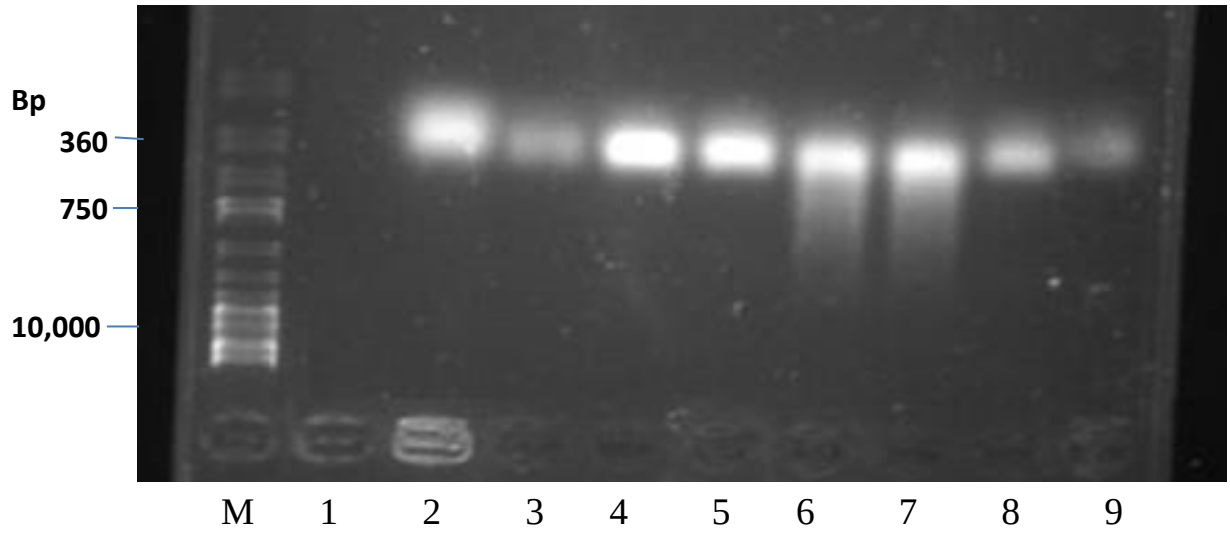

Figure 1: Lane M, 1Kb DNA ladder, Lane 1 negative control, Lane 2 positive control, Lanes 3,7,9 no amplification bands, Lanes 4-7 shows amplification bands of *Fusobacterium nucleatum* at 360Bp, in gel electrophoresis using specie specific primer.

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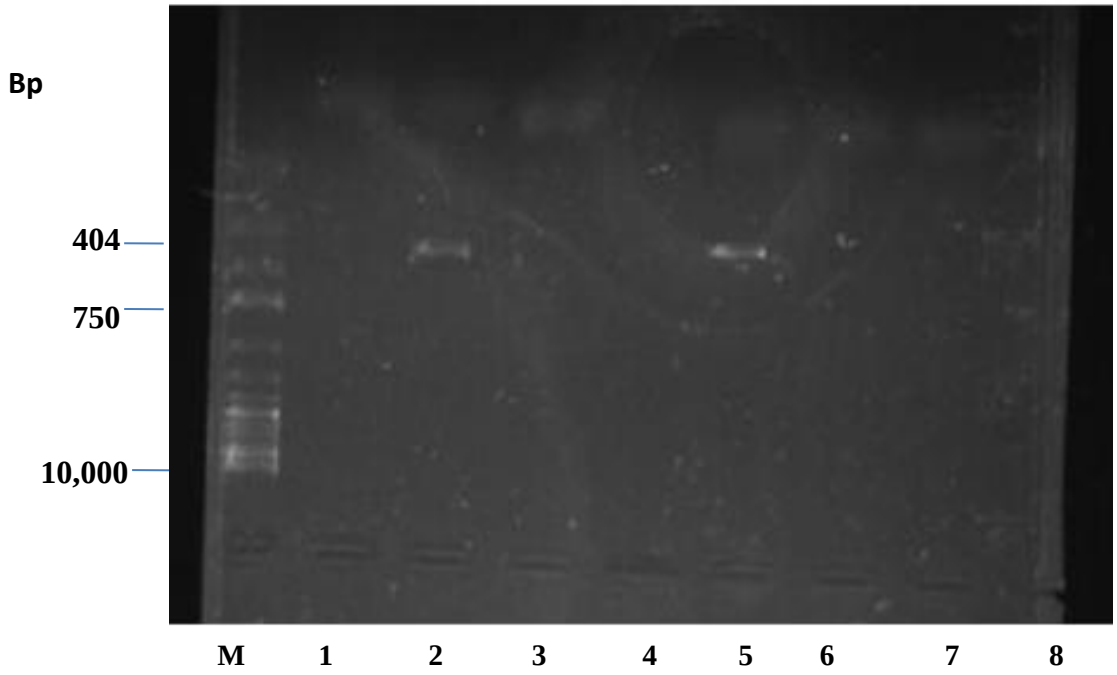

Figure 2: Lane M, I Kb DNA band, Lane 1 negative control, Lane 3-4 and 6-8 shows no amplification bands, Lane 2 and 5 shows amplification bands of *Porphyromonas gingivalis* at 404bp in gel electrophoresis using specie specific primer.

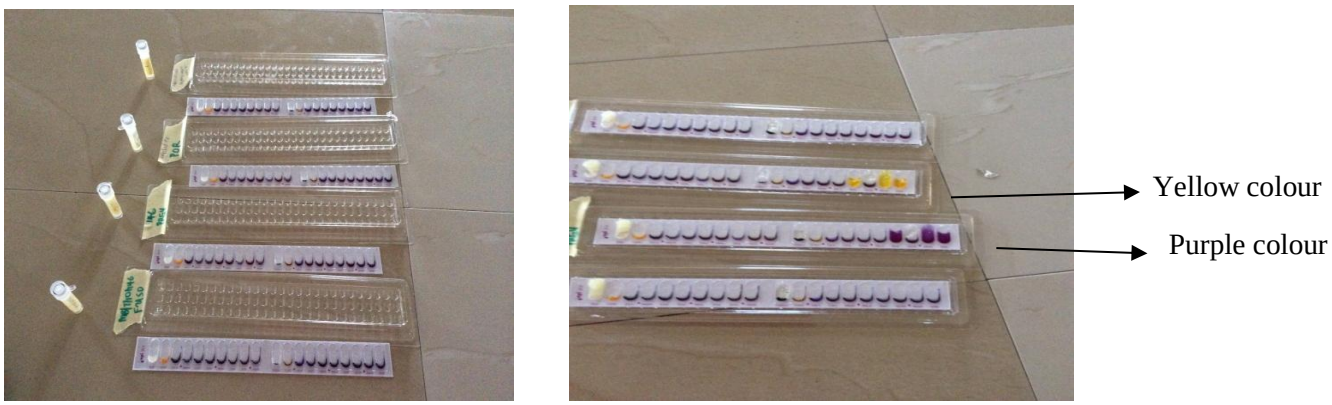

Figure 3: API 20A Biochemical test kit for Anaerobes

the organism to the test reagent (yellow showing positive and purple indicating negative).

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DISCUSSION

The effectiveness of the antimicrobials in the treatment of mixed anaerobic infections of the oral cavity is undermined by empirical selection of drugs and the problem of self-medication. The strict anaerobes play a major role in the pathogenesis of chronic or aggressive periodontitis and the systemic use of antimicrobial agents may improve the treatment of such infections when associated with local procedure and modification of oral hygiene standards (Ahuja *et al*., 2012). This study was carried out to determine the effects of metronidazole and amoxicillin on selected anaerobes in patients with oral infections. The synergistic effects of metronidazole and amoxicillin when used together in the treatment of subgingival microbiota appear to be more effective and more lasting in reducing periodontal pathogens than either amoxicillin or metronidazole alone used in treatment.

A study aiming to determine the changes in subgingival microbial profiles in chronic periodontitis patients receiving either systematically administered amoxicillin or metronidazole as an adjunct of scaling and root polishing found that both antibiotics were useful in rapidly lowering counts of periodontal pathogens. It was found that some species of *Fusobacterium* were markedly decreased during metronidazole or amoxicillin administration but ended to increase once the antibiotics were withdrawn (Dabija-Wolter *et al*., 2018). In this study, a total of 59 specimens were collected and 41 were positive. The organisms isolated were *Peptostreptococcus* spp, *Prevotella intermedia*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*; this is in agreement with the study carried out by Dabija-Wolter *et al*. (2018) on patients with periodontitis. The predisposing factors to oral infection have been linked to diabetes mellitus, obesity, excess intake of carbohydrates, surgery, cigarette smoking and also excess intake of sweets and confectionaries (Kammaj *et al*., 2007).

The present study showed that *Fusobacterium nucleatum* had the highest occurrence of 15 (36.6%) among the isolated organisms; this is in agreement with the work done by Bolstad *et al. (*2008) which emphasizes that *Fusobacterium* spp. is the most common in supragingival and subgingival plaque in both healthy individuals and patients with oral infections. Occurrence of *Prevotella intermedia* with 10 (24.4%) was linked to the work done by Haffajee *et al.* (2006) which described an association between *Prevotella intermedia* and periodontal diseases at an occurrence of 31(52.5%). Co-infection was also observed with periodontitis and the bacterial isolates involved were *Prevotella intermedia* and *Fusobacterium nucleatum* with 1 (1.7%). This may suggest the continuous changes in oral flora due to connection with the external environment (Saad, 2022) or strong ecological relationship between these microbial species (Anyiam *et al*., 2014). The highest incidence of oral infection was seen in the age bracket of 31-40 years while the lowest in the age bracket was 1-10 years and 61-70 years respectively. The prevalence of oral infection was seen in caries with 12 (29.3%), and higher in males with 22 (53.7%) than in females with 19 (46.2%). Our findings can be compared with the findings of Ankita and Pratibha (2020) which indicated the age group of 31-40 years as having highest oral infection, and also higher incidence in males. The higher incidence of oral infection in males could be because men are more likely than women to develop destructive periodontal disease, which is reflected in the sexual dimorphism of vulnerability to periodontitis (Shiau, 2018). This may also be attributed to less visit by men to the dentist and negligence when it concerns oral hygiene. Furthermore, men tend to ignore their oral health, practise poorer oral hygiene habits, and experience higher rates of periodontal disease, oral cancer and dental trauma (Lipsky *et al*., 2021).

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Antibiotics susceptibility revealed that *Peptostreptococcus* spp was susceptible to metronidazole with the MIC of 0.25µg/ml and all were resistant to amoxicillin. *Prevotella intermedia* was susceptible to amoxicillin with the MIC of 0.125µg/ml and all were resistant to metronidazole. This is in accordance with the work done by Castillo *et al*. (2022), which emphasizes that *Prevotella intermedia* was sensitive to amoxicillin. *Porphyromonas gingivalis* was sensitive to both metronidazole and amoxicillin with MIC values of 0.125µg/ml and 0.25 µg/ml respectively. *Fusobacterium nucleatum* was susceptible to both metronidazole and amoxicillin with the MIC of 0.25 µg/ml, which is in agreement with the work of Paolantonio *et al*. (2004), where penicillin and metronidazole were used to treat patients with periodontitis. The results of this present investigation agree with those of previous studies by Lopez *et al*. (2011) that showed metronidazole and amoxicillin as the sole therapy in progressive untreated adult periodontitis reduced the proportion of *P. intermedia* and *P. gingivalis* and allowed a significant improvement in clinical condition. However, this showed metronidazole to be more effective than amoxicillin in the treatment of oral infection caused by these isolated anaerobes, as also agreed with previous studies conducted by Sonja *et al*. (2010) and Anyiam *et al*. (2014).

This study highlighted the organisms subjected to PCR to be *Fusobacterium nucleatum* with 15 (36.6%) and *Porphyromonas gingivalis* with 5 (12.2%). Our findings with PCR showed *Fusobacterium nucleatum* to be 2 (4.9%) while *Porphyromonas gingivalis* was 1 (2.4%.) In comparison between bacterial culture and PCR analysis, we obtained 20 (48.8%) of the bacterial isolates by culture and 3 (7.3%) by PCR. This is in line with the work done by Lau *et al*. (2004) and Verner *et al*. (2006) where PCR analysis was compared with bacterial culturing in periodontal pathogen identification. Importantly, anaerobic pathogens linked to oral infections identified using the PCR technique employed in this work have demonstrated its efficacy.

LIMITATION

We acknowledge limitations in study. The study's constraint was that *Prevotella intermedia* and *Peptostreptococcus* spp was not subjected to polymerase chain reaction as at the time when this research was carried out.

CONCLUSION

The effectiveness of metronidazole and amoxicillin as the sole therapy in the treatment of oral infections caused by anaerobes may also be of importance in designing approaches to control periodontal infections in undesired populations with limited access to dental care, as metronidazole and amoxicillin are comparatively inexpensive agents. However, it is essential to be cautious in extending this therapy to patients who have received prior periodontal therapy. A better treatment response would be expected using most therapies in subjects whose subgingival microbiota has been minimally altered by periodontal therapy. It is advisable to maintain good oral hygiene and restrain from excess intake of low fiber carbohydrate diets and sugary foods which can contribute to oral infections. In addition, the frequency of the scaling and polishing when sought for cosmetic reasons should be minimized.

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Additional studies in a large number of subjects are needed to determine, in the long term, whether the therapeutic modality used in this current study can produce bacterial resistance, and whether non removal of sub gingival calculus can reduce the risk for recurrence of oral infections.

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