Original Research Article

PHENOTYPIC CHARACTERIZATION AND ANTIBIOGRAM OF NON-ORAL BACTERIA ISOLATES FROM PATIENT ATTENDING DENTAL CLINIC AT FEDERAL COLLEGE OF DENTAL TECHNOLOGY AND THERAPY MEDICAL CENTER ENUGU

ABSTRACT

Background and Objectives: Antibiotic-resistance among microbiota found within the oral cavity is a growing concern due to extensive use of antibiotics in dental practice both for therapeutic and prophylactic reasons, but has so far received little attention in recent time. The aim of this study was to determine the antibiogram of non-oral bacteria isolates from patient attending dental clinic at Federal College of Dental Technology and Therapy Medical Center Enugu.

Methodology: A total of two hundred (200) oral swab samples were collected from patients with dental disease, placed in sterilized Brain Heart Infusion broth and immediately transported to the Microbiology Laboratory Unit of Federal College of Dental Technology and Therapy Enugu (FEDCODTTEN), for bacteriological analysis using standard microbiological methods for isolation and characterization. Antibiogram studies of non-oral bacteria was performed using the Kirby–Bauer disk diffusion method and the results were interpreted using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. Multiple antibiotic resistance index (MARI) was determined for MDR non-oral bacteria.

Result: Phenotypic characterization of non-oral bacteria revealed an occurrence rate of *S. aureus* 35(17.5%) followed by *E. coli* 18(9.0%), *Salmonella typhi* 16(8.0 %) and *K. oxytoca* 4(2.0%) as the least predominant bacteria species. Among the oral site, Lower Right Quadrat showed increase isolation rate of 30(15.0%) bacteria followed by Lower Left Quadrat 23(11.5%) while Upper Right Quadrat accounted 15(7.5 %) with the least isolation rate. There was no statistically significant difference in the prevalence of non-oral bacteria in Right quadrant and Left quadrant samples from dental disease patient ($p < 0.05$). Non-oral bacteria isolate exhibited 57.1-100% resistant to Ertapenem, colisitn, amoxillicin, azetronam, colistin, ampicillin and clindamycin with MARI ranged from 0.4-0.7, indicating high level of MDR but were susceptible to ciprofloxacin 77.8%, gentamicin 100% and imipenem 100%.

Conclusion: The high antibiotic resistant and increase MDR outcome reported among non-oral bacteria in this study calls for strengthened efforts in antibiotic stewardship and infection prevention and control measures in dental practices with the need to implement regular awareness programs at time interval to control and manage MDR resistance bacteria through judicious use of antibiotic to re-establish dominance over MDR non-oral bacteria implicated in dental diseases.

***Keywords**: Non-oral bacteria, oral cavity, antibiotic-resistant, dental disease patient*

1. INTRODUCTION

Non-oral bacteria are transient or non-resident pathogenic bacteria that are not generally considered a common part of the oral microbiota [1]. The oral microbiota have been reported to contain more than one hundred thousand (1000) species of bacteria [2,3,4] belonging to the genera *Streptococcus, Enterococcus, Staphylococcus, Actinomyces, Peptostreptococcus, Eubacterium, Treponema, Corynebacterium, Bacteroides, Lactobacillus, Fusobacterium, Leptotrichia, Campylobacter, Prophyromonas,* etc., [5,6] with only a few proportions of these bacteria are associated with dental disease such as periodontitis, gingivitis, dental caries, etc.,[7,8]. However, the invasion and colonization of the oral cavity by non-oral bacteria such as *Staphylococci*, enterococci and Gram-negative enteric rods (GNRs) depict an imbalance of oral flora in the oral cavity [6,9,10]. The presence and proliferation of non-oral bacteria in the oral cavity have been associated with several oral diseases such as caries, periodontitis, gingivitis, and more systemic diseases such as rheumatoid arthritis, endocarditis and cystic fibrosis [1,6].

Antibiotics are widely used in dental-related issues, both for therapeutic and prophylactic reasons [11]. The lack of proper identification of non-oral bacteria especially in dental disease patients with severe infections increases the use of broad-spectrum antibiotics. Dental surgeons frequently prescribe antibiotics with apprehension that the oral cavity contains a huge number of microorganisms as normal flora which can cause infections in their patients [12]. As a result of this antibiotics overuse, bacteria found within the oral cavity exhibit resistance to commonly available antimicrobial agents with limited therapeutic option [13, 14]. Within the oral niche, the spread of resistant oral/non-oral bacteria and antimicrobial selection pressure within the oral cavity has been the main drivers of antibiotic resistance [15,16] amongst dental disease patients. In recent times, non-oral bacteria are also the current most serious Multidrug-resistant organisms (MDROs) [17,18].

Most of these non-oral bacteria pathogens found in the oral cavity of a patient with dental disease that was easily treatable have shifted away toward more resistant bacteria. This dilemma has raised significant concern for community-acquired and nosocomial infection prevention and control, as these bacteria become a reservoir of resistant determinants that are easily transferred to other oral microbiota through Horizontal Gene Transfer (HGT).

It is important to note that the acquisition of Antibiotic Resistance Genes (ARGS) through their HGT is facilitated through biofilm formation composed of oral and non-oral bacteria. Within the oral cavity, one of the most common groups of bacteria that are of medical importance in healthcare today is Gram-negative bacteria, which together with other highly important MDR Gram-positive pathogens of the non-oral cavity. In dental disease patients, the eradication of this non-oral bacteria from the dental plaque or biofilm seems to be more challenging due to their high MDR profile to antimicrobial agents has raised the probability of treatment failure and reinfection [6, 19].

Generally, data about the prevalence of antibiotic resistance on non-oral bacteria are difficult to find, particularly in countries where antibiotics are easily obtainable Over The Counter (OTC). Despite the untenable rate of antibiotic-resistant bacterial infections reported in dentistry in most published studies in Nigerian [4,6,20,21], there is a substantial gap in the surveillance of these non-oral bacteria in several Nigerian cities especially in Southeastern Nigeria where limited studies have been done on the prevalence of resistant oral and non-oral bacteria [4,6]. Hence, it worthwhile investigating the antibiotic resistance of non-oral bacteria that inhabit or colonizes the oral cavity among patients attending FCDTTEN to optimize treatment and decrease mortality rates.

2. MATERIALS AND METHODS

2.1 Patient Recruitment and Sample Collection

The study was carried out at Federal College of Dental Technology and Therapy, Trans-Ekulu, located at latitude 6°29'07.1"N and longitude 7°29'42.5"E in Enugu, Nigeria. Patient undergoing dental restoration and antibiotic treatment were excluded from the study while patient diagnosed with active dental disease were included. Glycol-thymoline solution (Kress and Owen Company, Middletown, New Jersey, U. S. A) was administered to patients to disinfect the oral cavity before examination and sample collection. A total of two hundred (200) oral swab sample were collected from right and left quadrat of dental disease patient. A sterile swab moistened with a sterile Physiological Buffer Saline (PBS) solution, was aseptically swabbed or wiped gently on the portion of the affected tooth cavity of patients with dental disease attending dental clinics at Federal College of Dental Technology and Therapy Medical Center Enugu. The collected oral swab samples were transported immediately to Microbiology laboratory unit of FCDTTEN, Nigeria for bacteriological analysis.

2.2 Processing of Clinical Specimens

The collected swab specimens were suspended in a sterilized Brain-heart infusion broth (Merck Co., Germany) and incubated at 37^{o}C for 18-24 hrs. A loopful of the turbid bacterial growth were plated onto Cetrimide agar, Mannitol salt agar, *Salmonella/Shigella* agar, MacConkey agar (Merck Co., Germany), and incubated at 37^{o}C for 18-24 hrs. Bacterial colonies showing typical characteristics on selective and differential media were aseptically purified by sub-culturing onto Brain-heart infusion agar (Merck Co., Germany) and incubated at 37^{o}C for 24 hrs. Pure cultures of the bacterial isolates were carefully examined macroscopically and microscopically for their cultural morphology and cellular characteristics respectively. Isolates were characterized based on their colonial morphology (color, consistency, texture), microscopic techniques (Gram staining and motility test) and biochemical characteristics, including oxidase, indole, citrate utilization, triple sugar iron test, methyl red, Voges-Proskaeur test, coagulase test, catalase and carbohydrate fermentation tests such as mannitol, sucrose, glucose and lactose as described by Iroha *et al.* [22]. Further bacterial strain confirmation was performed using VITEK 2 System (bioMerieux, France) [23].

2.3 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method as outlined in the current Clinical and Laboratory Standards Institute (CLSI) guidelines [24] guidelines. In brief, overnight culture of the test bacterial suspension ($1x10^{6}$ colony forming unit per milliliter (cfu/ml) were adjusted to 0.5 MacFarland turbidity standard and were spread over the entire surface of solidified Mueller-Hinton agar using a sterile cotton-tipped swab stick. This was allowed to stand for 15 mins to enable the inoculated organisms to pre-diffuse. The following antibiotics: ampicillin (30 µg), amoxicillin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), Colistin (10 µg) Gentamicin (5µg), Clindamycin (15µg), ciprofloxacin (5 µg), imipenem (10 µg), Ertapenem (10 µg), aztreonam (30 µg) were aseptically placed onto the surfaces seeded solidified Mueller-Hinton plates with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37°C for 18-24 hrs and zones of inhibition after 24 hrs of incubation was taken. The inhibition zone diameters (IZD) around each antibiotic disk were measured using a calibrated transparent ruler and recorded in millimeters. A standardized table was used to determine if each bacterium was 'resistant', 'intermediate,' or 'sensitive.' For analysis, isolates with intermediate or resistant results was merged as resistant [23, 24].

2.4 Multiple Antibiotic Resistance Index (MARI)

Non-sensitivity to one or more agents in at least three categories of antimicrobials was determined i.e., number of antibiotics to which test isolate displayed resistance (x) and (y) the total number of antibiotics to which the test organism has been evaluated for sensitivity [23].

2.5 Data Analysis

Basic descriptive statistics such as frequency distribution was calculated. Statistical analysis was performed using the statistical package for social sciences (SPSS) computer software (Version 25), IBM software, USA. Comparison between categorical variables was calculated using Independent Samples T-test. Results were considered statistically significant if the p value was less than 0.05 ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Distribution of non-oral bacteria from oral cavity of dental disease Patient

Distribution of non-oral bacteria isolated from oral cavity of patients with dental disease are shown in Table 1 below. Oral cavity of infected patients with dental disease harbored overall occurrence rate of 89(44.5%) non-oral bacteria comprising of high prevalence of *S. aureus* 35(17.5%) followed by *E. coli* 18(9.0%), *Salmonella typhi* 16(8.0 %) and *K. oxytoca* 4(2.0%) as the least predominant bacteria species. Amongst the oral site, Lower Right Quadrat showed increase isolation rate of 30(15.0%) bacteria followed by Lower Left Quadrat 23(11.5%) and Upper Right Quadrat 15(7.5) with the least isolation rate. There was no statistically significant difference in the prevalence of non-oral bacteria in Right quadrant samples and Left quadrant from patient ($P < 0.05$).

3.2 Antibiogram of non-oral bacteria isolated from oral cavity of dental disease Patient

Amongst the non-oral bacteria isolate, *P. aeruginosa* demonstrated resistant to Azetronam 77.8 %, Ceftazidime 77.8 %, Ceftriaxone 88.9%, Ertapenem 88.9%, Colistin 100 % but were sensitive to Gentamicin 66.7 %, and 100 % for both Ciprofloxacin and imipenem (Table 2). The proportion of *E. coli* resistant to Colistin and Ceftazidime accounted 100% while low resistant proportion of 5.6 % and 22.2 % was exhibited against Gentamicin and Ciprofloxacin but were 11.1 %, 22.2%, 27.8 % susceptible to Azetronam, Ceftriaxone and Ertapenem respectively. *Salmonella tyhi* resistant to Colistin, Ceftriaxone, Ertapenem, Ceftazidime accounted 100 %, 68.7 % , 62.5 % and 50.0 % respectively while 75.0 %, 87.5 %, 100% of the isolate were susceptible to ciprofloxacin, Gentamicin and imipenem respectively. *K. pneumoniae* were more sensitive to imipenem 100 %, Ciprofloxacin 100 %, Gentamicin 85.7 % but revealed high resistant proportion to Ceftazidime 100 %, Ertapenem 100 % colistin 100 % and 71.4 % for both Azetronam and Ceftriaxone. Resistant to Gentamicin, Colistin and Azetronam was 50.0 %, 100% and 75.0 % for *K. oxytoca*. *S. aureus* were extremely resistant to Ampicillin, Amoxicillin and Clindamycin recording 100% while resistant to Ceftazidime, Ceftriaxone and Ertapenem accounted 85.7 %, 62.9 % and 57.1 % respectively. Both Gram-positive and Gram-negative non-oral bacteria were 100 % susceptible to Imipenem as shown in Table 2. The result showed that all the non-oral bacteria were resistant to two or more antibiotic inferring multidrug resistant with MARI ranging from 0.4-0.7 (Table 3)

Table 1: Distribution of non-oral bacteria isolated from oral cavity of dental disease Patient

ORAL SITE									
Right Quadrat	**No. of Sample**	***P. aeruginosa* (%)**	***E. coli* (%)**	***Salmonella typhi* (%)**	***K. pneumoniae* (%)**	***S. aureus* (%)**	***K. oxytoca* (%)**	**Occurrence (%)**	***P-value***
Lower	**49**	4(2.0)	7(3.5)	5(2.5)	0(0.0)	14(7.0)	0(0.0)	**30(15.0)**	**.4781**
Upper	**37**	0(0.0)	2(1.0)	6(3.0)	0(0.0)	5(2.5)	2(1.0)	**15(7.5)**	
Left Quadrat									
Lower	**53**	2(1.0)	9(4.5)	1(0.5)	2(1.0)	7(3.5)	2(1.0)	**23(11.5)**	
Upper	**61**	3(1.5)	0(0.0)	4(2.0)	**5**(2.5)	9(4.5)	0(0.0)	**21(10.5)**	
Total	**200**	**9(4.5)**	**18(9.0)**	**16(8.0)**	**7(3.5)**	**35(17.5)**	**4(2.0)**	**89(44.5)**	

Table 2: Antibiogram of non-oral bacteria isolated from oral cavity of dental disease Patient

Antibiotic (µg)	***P. aeruginosa* (n= 9)**		***E. coli* (n=18)**		***Salmonella tyhi* (n=16)**		***K. pneumoniae* (n=7)**		***K. oxytoca* (n=4)**		***S. aureus* (n=35)**	
	R (%)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Ampicillin (30)	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	35(100)	0(0.0)
Amoxicillin (30)	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	35(100)	0(0.0)
Azetronam (30)	7(77.8)	2(22.2)	16(88.9)	2(11.1)	7 (43.8)	9(56.3)	5(71.4)	2(28.6)	3(75.0)	1(25.0)	*NA*	*NA*
Ceftazidime (30)	7(77.8)	2(22.2)	18(100)	0(0.0)	8 (50.0)	8 (50.0)	7(100)	0(0.0)	3(75.0)	1(25.0)	30(85.7)	5(14.3)
Ceftriaxone (30)	8(88.9)	1(11.1)	14(77.8)	4(22.2)	11(68.7)	6(31.3)	5(71.4)	2(28.6)	3(75.0)	1(25.0)	22(62.9)	13(37.1)
Ertapenem (30)	8(88.9)	1(11.1)	13(72.2)	5(27.8)	10(62.5)	6(37.5)	7(100)	0(0.0)	4(100)	0(0.0)	20(57.1)	15(42.9)
Imipenem (30)	0(0.0)	9(100)	0(0.0)	18(100)	0(0.0)	16(100)	0(0.0)	7(100)	0(0.0)	4(100)	0(0.0)	35(100)
Colistin (10)	9(100)	0(0.0)	18(100)	0(0.0)	16(100)	0(0.0)	7(100)	0(0.0)	4(100)	0(0.0)	*NA*	*NA*
Clindamycin (15)	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	35(100)	0(0.0)
Ciprofloxacin (5)	0(0.0)	9(100)	4(22.2)	14(77.8)	4(25.0)	12(75.0)	0(0.0)	7(100)	0(0.0)	4(100)	13(37.1)	22(62.9)
Gentamicin (15)	3(33.3)	6(66.7)	1(5.6)	17(94.4)	2(12.5)	14(87.5)	1(14.3)	6(85.7)	2(50.0)	2(50.0)	10(28.6)	25(71.4)

***Key: R**=Resistance, **S**=Susceptibility, **n**=number of isolate, **%**-Percentage, **NA**=Not Applicable*

Table 3: Multiple Antibiotic Resistant Index (MARI) of Non-oral bacteria isolated from oral cavity of patients with dental disease

Non-oral bacteria	Resistant Antibiotics	Mean Average MARI
P. aeruginosa	*ATM, CRO, CAZ, ETP, CT, CIP, G*	0.6
E. coli	*ATM, CRO, CAZ, ETP, CT, CIP, G*	0.6
Salmonella species	*ATM, CRO, CAZ, ETP, CT, CIP, G*	0.4
K. pneumoniae	*ATM, CRO, CAZ, ETP, CT, G*	0.5
S. aureus	*AMP, AMX, CRO, CAZ, DA, ETP, CIP, G*	0.7
K. oxytoca	*ATM, CRO, CAZ, ETP, CT, G*	0.5

Key: AMP=Ampicillin, AMX= Amoxicillin, ATM=Azetronam, CRO=Ceftriaxone, CAZ= Ceftazidime, ETP=Ertapenem, IMP=Imipenem, CT=Colistin, DA=Clindamycin, CIP=Ciprofloxacin, G= Gentamycin, MARI-Multiple Antibiotic Resistant Index

3.4 Discussion

The present study identified one bacterial isolate of Gram-positive origin (*S. aureus*) and five Gram-negative bacteria (*Salmonella typhi, P. aeruginosa, Escherichia coli, K. pneumoniae,* and *K. oxytoca*) in oral swab samples from dental disease patients. The prevalence of these non-oral bacteria reflect on the highly diverse microbiota of the oral cavity. There was a higher carriage rate of non-oral bacterial isolates (89.0%) in the oral swab culture. The increased carriage could be accrued to the inability to maintain or adhere to proper oral hygiene due to poor oral health. These results were similar to the high prevalence documented data in Germany 72.2% and India 77% [25,26] but varied from the low carriage rate reported in Chile 17.6%, Brazil 31.2%, Latin-American 34.4% [27,28,29] and in two studies 26.6% and 34.5% in Jos and Ogun state Nigeria [20,21. The reasons for these observed variations can be accrued to the differences in the socio-economic status of the studied population, geographical regions, sample size, and method employed for bacteria characterization.

Nevertheless, there is a controversy about whether non-oral bacteria are merely transient or unique to this niche but in recent times substantial evidence in different studies has highlighted the role of these bacteria in dental disease either with coordinated co-operative behaviors in the presence of normal oral microbiota [1,6,25,30,31,32]. Additionally, some of these non-oral bacteria isolates from the oral cavity of patients with dental disease in this study, have been reported to be genetically different from strains from other parts of the human body [33], which could potentially lead to another understanding of the ecosystem of the oral cavity.

The most frequent bacteria found in this study were *Staphylococcus aureus* 35(17.5%). But in contrast with other studies, this bacteria appear as the second most frequently found Gram-positive cocci after Streptococci, especially species from the viridans group in Ogun State, Nigeria [21] and other Countries [25,34,35, 36,37] but in line with the studies from two studies in Nigeria were 53.4%,14.2% [20,38] and a study in Poland 91.8% [30] *Staphylococcus aureus* predominant the Oral cavity. Earlier findings have also revealed that *S. aureus* was found at higher levels in the oral cavity and with greater prevalence, in periodontitis than in non-periodontitis subjects [19,39] while Fritschi *et al*.[40] found higher levels of *S. aureus* in aggressive than chronic periodontitis subjects. Consequently, *S. aureus* was pointed out as a contributor to the microbial profiles that could differentiate between aggressive and chronic forms of the disease. Presumably, this discrepancy observed may be associated with the type of sample collected from the oral cavity, as the *Staphylococci* analyzed in previous studies were isolated from plaque, saliva from the oral cavity

Earlier, the *Staphylococcus* species were not considered a member of the oral flora. Until Smith *et al*. [41] noted that the *Staphylococcus* species are more frequent colonizers of the oral cavity than previously thought. As initial colonizers of the tooth surface, they play a major role in the establishment of the early biofilm community. *Staphylococcus aureus* and other anaerobes use the enzyme glucansucrase to convert sucrose into a sticky, extracellular, dextran-based polysaccharide that allows the bacteria to cohere, forming plaque. (Sucrose is the only sugar that bacteria can use to form this sticky polysaccharide [42]. These microorganisms all occur naturally in the oral cavity and are normally harmless. However, failure to remove plaque by regular tooth-brushing allows them to proliferate, unchecked, and thereby build up in a thick layer, which can by their ordinary metabolism cause various dental diseases to the host [42]. The ability of *S. aureus* to proliferate in the oral cavity is due to its arsenal of virulence factors that are coordinately expressed during different stages of infection, such as superantigens, toxins such as β-toxin, matrix-binding surface adhesins, biofilm formation, and tissue-degrading enzymes such as proteases, lipases, nucleases, and collagenases [1,31,43].

The second most predominant non-oral bacteria identified in this study were *E. coli* 18(9.0%). The non-oral bacterial frequency in this study slightly agrees with those of three studies in Nigeria. For instance, Anejo-Okopi *et al.* [20] reported *Escherichia coli* (7.1%) and Enitan *et al.* [21] reported *Escherichia coli* (3.3%) and 44 *E. coli* isolated from the dental disease reported in Enugu [6]. *Escherichia coli*, a Gram-negative motile organism, is naturally found in the intestinal tract, but has been isolated from urine, pus, cerebrospinal fluid, and blood in addition to the fecal specimen. Strains of *E. coli* have been recognized to cause diarrhoeal diseases some of which include the enterotoxigenic *E. coli*, the enteropathogenic *E. coli*, the enteroinvasive *E. coli* and most recently the enterohaemorrhagic *E. coli*. The organism is the most pathogenic organism found in the urinary tract of humans and is one of the major organisms implicated in wound infection and meningitis and bacteremia in neonates [6, 21]. And recently, *E. coli* has been incriminated in active caries lesions, gingivitis, and periodontitis [6, 21, 44]. The most studied virulence factors of this strain include lipoteichoic acid, gelatinase, biofilms, surface adhesins, aggregation substance, hyaluronidase, cytolysin toxin, sex pheromones and extracellular superoxide. Each of these factors might be associated with many phases of periapical inflammation, systemic diseases and endodontic infections [45, 46].

Salmonella typhi from this study accounted 16(8.0%). However, a recent study in the same setting was the first to report its prevalence in chronic periodontitis and gingivitis patient [6]. So far, no study has elucidated it pathogenicity and potential role in the enhancement of virulence in mixed periodontitis disease or other related dental diseases. But it is important to note that enteriobacteriaceace family of which *Salmonella* species is a member are mostly implicated in numerous dental diseases and they are characterized by high pathogenic potential as they elaborate various enzymes which can degrade basement membrane laminin [26, 47] inactivate complement components [26], produce extracellular leukotoxins [26,48] and suppress lymphocyte proliferation [26]. In addition, they are also highly tissue invasive [26, 48]. They have also been shown to persist after periodontal debridement [26] and have been also implicated as a key pathogen in cases of refractory periodontitis [26, 47, 49]. All these findings favor the hypothesis that enteriobacteriaceace might be involved in the pathogenesis of periodontal disease and other dental diseases. The presence of *Salmonella typhi* s in the study population supports the idea that the oral cavity may act as a reservoir and a source of dissemination of these microorganisms to other areas of the body.

The occurrence of non-oral bacteria isolates among the study patients revealed that 9(4.5%) of the *P. aeruginosa* were recovered from disease dental patients. Nevertheless, its role as a transient member of the oral microbiome or a possible pathogen has fully been explored. However, studies using molecular biology methods have revealed that its presence in the oral cavity is underestimated and it is much higher in complex biofilms [50, 51]. Moreover, these species have many virulence properties such as the ability to adhere to and form biofilms on tissues and abiotic surfaces [49], along with their ability to produce and secrete extracellular enzymes and toxins 47, 49] as well as the expression of multiple antimicrobial resistance elements [52]. *P. aeruginosa* has also been identified in the periodontal pockets of immunocompromised subjects [53] and might be an important pathogen in periodontitis and gingivitis [39, 54]. Lately, oral *P. aeruginosa* has been associated with oral squamous cell carcinoma [55] and chronic kidney disease [56]. Additionally, focal necrotizing lesions have been found in the oral mucosa of HIV-positive patients, which are different from periodontal disease patterns and are related to the presence of oral *P. aeruginosa* [51].

Likewise, *K. pneumoniae* 7(3.5%) and *K. oxytoca* 4(2.0%) which was isolated in this study has been reported by other researchers [6, 25]. This genera is usually present in the respiratory tracts and feces of about 5% of normal individuals (Enitan *et al.,* 2020). It causes chest infections and occasionally severe bronchopneumonia with lung abscesses. They can produce extensive hemorrhagic necrotizing consolidation of the lungs. It can also cause urinary tract infections and bacteremia with focal lesion in debilitated patients [21, 57]. It is ranked among the top ten bacterial pathogens responsible for hospital-acquired infections and is second only to *E. coli* as a urinary tract pathogen [57]. *K. pneumoniae* and *K. oxytoca* has been implicated in oral infection because of their ability to degrade proteinaceous substances in the mouth resulting in bad breath [6, 21, 25, 58].

Regarding the occurrence of this non-oral bacteria among the studied population, the following could be the possible risk factor: an infrequent visit to the dental clinic, poor oral hygiene and dental care, continuous use of toothbrush even when it is long overdue for a change, recent dental surgery, the practice of oral sex among some folks; pathogens from the vaginal of an infected female partner can be inoculated into the oral cavity of the male partner during oral sex; thus, pre-disposing the latter to oral infections, as well as poor hand/toilet hygiene.

Antibiotics are widely used in dental caries and other dental-related issues, both for prophylactic and therapeutic reasons to patients before massive dental procedures. Multiple studies reported that dental surgeons frequently prescribed inappropriate antibiotics which ultimately promote antimicrobial resistance [59, 60, 61].

The outcome of this study showed that *S. aureus* was extremely resistant to Amoxillicin, ampicillin and clindamycin ranging from 92.9-100%. Of clinical importance, clindamycin is one of the most frequently prescribed antibiotics by dental practitioners [25, 62]. Notably, the German guidelines on odontogenic infections recommend amoxicillin and other penicillin/derivative (ampicillin) for empiric antibiotic therapy, while clindamycin is only recommended in cases of penicillin allergy [35]. In line with these findings, Meinen *et al.* [25], Heim *et al*. [36] and Poeschl *et al.* [63] reported similar clindamycin resistance rates for *S. aureus* in Poland, Germany and Austria.

Due to *S. aureus* high clindamycin, ampicillin and amoxillicin, resistance proportions (>50%), treatment options may be very limited, which is a concern since these results indicate that *S. aureus* is frequently found in oral infections. However, the evolution of *S. aureus* strain has been traced to the acquisition of the exogenous gene (*mec*A) which is part of the Staphylococcal cassette chromosome *mec* (SCCmec) (types I–VII) [23, 64]. The *mecA* gene codes for an additional penicillin-binding protein (PBP2a), a peptidoglycan transpeptidase, which can confer resistance to all *β*-lactam antibiotics including penicillin derivatives, cephalosporins, and other antibiotics class in this study.

A substantial resistance was observed to colistin in the present study as all Gram-negative bacteria showed 100% resistance to colistin. Although this strain's colistin-resistant profile is scarce in dentistry but few studies in other areas have reported the spread of colistin-resistant *K. pneumonia, E. coli* and *Pseudomonas aeruginosa* [65, 66, 67, 68, 69]. Gram-negative bacteria resistant to colistin were commonly observed in this study and may depict the persistence of colistin-resistant in the area study. Such trend could be linked to exposure to sublethal doses of colistin as a last-line antibiotic in the treatment of recurrent or complicated enterobacteria infections.

Regarding the antibiotic-resistant pattern of non-oral bacteria isolates recovered from dental disease patients, the majority of the Gram-negative bacteria displayed 50-100% resistant proportion to Azetronam, colistin, ceftazidime, ceftriaoxone and Ertapenem. This observation substantiates the findings from other studies on dental disease [25, 70] and reports from non-oral human clinical samples [57, 71, 72, 73]. Bacterial resistance to most of these antibiotics may primarily be due to the production of extended-spectrum β- lactamase enzyme which confers resistance to a wide spectrum of the antibiotics rendering them inactive though not screened in this study.

Moreover, although resistances against cephalosporins and carbapenem in this oral bacteria were relatively high, it is worrying that resistances against these antibiotic classes increased over time, which underlines the importance of continuous efforts in antibiotic stewardship. In the studied setting, it could be envisaged that about 10% of all antibiotics are prescribed by dentists. It could be estimated that approximately one-third of all outpatient antibiotic prescriptions are unnecessary and thereby contribute to the development of antibiotic resistance. The potential overuse of antibiotics (e.g., in antibiotic prophylaxis) is rarely addressed in dentistry, but a recent study by Löffler and Böhmer. [74] showed that a combination of audit and feedback and education on antibiotics could help as an intervention in hospital dental care and outpatient dental settings.

Nevertheless, the non-oral bacteria having MAR index of 0.4 and above is worrisome. This finding correlates with the known fact which states that MAR index values > 0.2 indicates the existence of isolate from high–risk contaminated source with frequent use of antibiotics, while values ≤ 0.2 show bacteria from source with fewer antibiotics usage [21, 23]. The high frequency of multiple antibiotic resistance might be a reflection of inappropriate use of antimicrobials, lack of laboratory diagnostic tests, and unavailability of guidelines for the selection of antibiotics. Regular and frequent use of antibiotics in dental infection may often cause long-term public health troubles by leading to the development of resistant microbes including multidrug-resistant pathogens.

This study further showed that antibiotics such as imipenem, ciprofloxacin and Gentamicin were very potent and can be used for the effective treatment of oral and dental infections because of their *in vitro* effect on the isolates considering the high level of sensitivity observed. The effectiveness of these drugs substantiates existing studies [75, 76, 77]. Therefore empirical treatment must be well guided by laboratory investigations through accurate antibiotic susceptibility testing.

4 Conclusion

This study shows that the oral cavity of the dental disease patient is associated with a wide range of different non-oral bacteria. *S. aureus* 35(17.5%) was the most frequently identified bacteria followed by *E. coli* 18(9.0%) and *K. oxytoca* 4(2.0%) as the least predominant bacteria species. These non-oral bacteria are the most frequently identified pathogens in hospitals and dental practices. The high antibiotic resistance and increase MDR of 0.4-0.7 outcome reported in this study calls for strengthened efforts in antibiotic stewardship and infection prevention and control measures in dental practices. Importantly, it's ideal for one to see his/her Dentist once at time interval of six months for a clean-up and dental check-up to forestall the possibility of developing dental disease and other oral diseases. Furthermore, molecular studies (a); are needed to better understand the genetic diversity of some of these bacteria strains colonizing the oral cavity of patients with dental disease and isolates from other parts of the human body, (b) empirical treatment must be well guided by accurate antibiotic susceptibility testing, (c) as antibiotics are frequently used in the treatment of oral infections it is important to identify the extent of resistance to these drugs, by using primers for commonly encountered antibiotic resistant gene and their mobile Genetic Element.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

All authors declare that written informed consent was obtained from the patient or care-giver of the patient before collection of sample.

ETHICAL APPROVAL

The approval and consideration for this study was gotten from the research and ethical committee of Federal College of Dental Technology and Therapy, Enugu with ethical clearance number FCDTT/DEC/VOL21/2021/707.

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