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Phenotypic Characterization and Antibiogram of Non-Oral Bacteria Isolates from Patients Attending Dental Clinic at Federal College of Dental Technology and Therapy Medical Center Enugu

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 patients attending dental clinic at Federal College of Dental Technology and Therapy Medical Center Enugu (FEDCODTTEN)
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, 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 Multidrug Resistant (MDR) non-oral bacteria.
Results: 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 quadrant showed increase isolation rate of 30(15.0%) bacteria followed by lower left quadrant 23(11.5%) while upper right quadrant 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 patients ($P < 0.05$). Non-oral bacteria isolate exhibited 57.1-100% resistant to Ertapenem, colisitn, amoxillicin, azetronam, colistin, ampicillin and clindamycin with Multiple Antibiotic Resistant Index (MARI) ranged from 0.4-0.7, indicating high level of multi-drug resistance but were susceptible to ciprofloxacin 77.8%, gentamicin 100% and imipenem 100%.
Conclusion: The high antibiotic resistant and increase multi-drug resistance 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 multi-drug resistance bacteria through judicious use of antibiotic to re-establish dominance over multi-drug resistance non-oral bacteria implicated in dental diseases.

Keywords: Non-oral bacteria; oral cavity; 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 has 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. Patients undergoing dental restoration and antibiotic treatment were excluded from the study while patients 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 quadrant 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 FEDCODTTEN, 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 24 hours. After overnight incubation, a loopful of the turbid bacterial growth were plated onto a sterilized solidified Cetrimide agar, Mannitol salt agar, *Salmonella/Shigella* agar, MacConkey agar (Merck Co., Germany), and incubated at 37^{o}C for 24 hours. 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 hours. 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]. 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 minutes 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 24 hours and zones of inhibition after 24 hours of incubation were 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 were 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., the 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 Isolate from Oral Cavity of Dental Disease Patients

Distribution of non-oral bacteria isolated from oral cavity of dental disease patients are shown in Table 1. 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 non-oral bacteria species. Amongst the oral site, lower right quadrant showed an increase isolation rate of 30(15.0%) bacteria followed by lower left quadrant 23(11.5%) and upper right quadrant 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 patients ($P < 0.05$).

3.2 Antibiogram of Non-oral Bacteria Isolates from Oral Cavity of Dental Disease Patients

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 % were 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 a 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 isolates from oral cavity of dental disease Patients

Oral site									
	No. of sample	***P. aeruginosa* (%)**	***E. coli* (%)**	***Salmonella typhi* (%)**	***K. pneumoniae* (%)**	***S. aureus* (%)**	***K. oxytoca* (%)**	**Occurrence (%)**	***P-value***
Right quadrant									
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 quadrant									
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 isolates from oral cavity of dental disease patients

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 dental disease patients

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

3.3 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 [21]. 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* [65-69]. 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 sub-lethal 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 [57,71-73]. 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 indicates that the oral cavity of the dental disease patients harbors numerous non-oral bacteria and these bacteria are the most frequently identified pathogens in hospitals and dental practices. The high antibiotic resistance and increased 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.

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.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zaatout N. Presence of non-oral bacteria in the oral cavity. Arch Microbiol. 2021; 203(4)2:747–2760.
2. Mahasneh SA, Mahasneh AM. Probiotics: A promising role in dental health. J Dent. 2017;5(4):26.
3. Gao L, Xu T, Huang G, Jiang S, GuChen YF. Oral Microbiomes: More and more importance in oral cavity and whole body. Protein Cell. 2018;9(5):488–500.
4. Ngwu JN, Uzoeto HO, Emaimo J, Okorie C, Mohammed ID, Edemekong CI, Peter IU, Ezeh C, Chukwu E, Adimora EE, Ani SE, Oke B, Moses I. B, Nwakaeze EA, Otu JO, Chukwunwejim C R, Egbuna RN,

Ikusika BA, Adagiri P, Iroha IR. Antibiogram of biofilm forming oral streptococci species isolated from dental caries patients visiting federal college of dental technology and therapy, Enugu Nigeria. Int J Res Rep Dent. 2022;5(1):12-25.

5. Donkor ES, Kotey FCN. Methicillin-resistant *Staphylococcus aureus* in the oral cavity: implications for antibiotic prophylaxis and surveillance. Infect Dis Res Treat. 2020;13:1–8.
6. Iroha IR, Mohammed ID, Moses IB, Ngwu NJ, Uzoeto HO, Oladimeji, AS, Ikemesit, UP, Onuora LA, Ewa NJ, Edemekong CI. Molecular characterization of enterobacteriacea isolated from gingivitis and periodontitis patients and the antimicrobial activity of mouth wash agents. Sci. Afr. 2022;15:1-106.
7. Kreth J, Merritt J, Qi F. Bacterial and host interactions of oral streptococci. DNA Cell Biol. 2009;28(8):397–403.
8. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner ACR, Wade WG. The human oral microbiome. J. Bacteriol. 2010;192(19): 5002–5017.
9. Al-Ahmad A, Muller N, Muller N, Wiedmann-Al-Ahmad M, Hellwig E. Endodontic and salivary isolates of *Enterococcus faecalis* integrate into biofilm from Human salivary bacteria cultivated in vitro. J Endo. 2009;35(7):986–991.
10. Van Winkelhoff AJ, Rurenga P, Wekema-Mulder GJ, Singadji ZM, Rams TE. Non-oral gram-negative facultative rods in chronic periodontitis microbiota. Microb Pathogen. 2016;94:117–122.
11. Roda RP, Bagán JV, Bielsa JMS, Pastor EC. Antibiotic use in dental practice. A review. Med Oral Pathol Oral Circle Bucal. 2007;12:186-92.
12. Peedikayil FC. Antibiotics: Use and misuse in pediatric dentistry. J Indian Soc Periodont Prev Dent. 2011;29:282–287.
13. Zhi-Wen Y, Yan-Li Z, Man Y, Wei-Jun F. Clinical treatment of pan-drug resistant bacterial infection consulted by clinical pharmacist. Saudi Pharm J. 2015;23(4):377–80.
14. Shebl RI, Mosaad YO. Frequency and antimicrobial resistance pattern among bacterial clinical isolates recovered from different specimens in Egypt. Cent Afri J Pub Health. 2019;5(1):36–45.
15. Duthey B. Priority medicines for Europe and the World: A public health approach to innovation. WHO Background Paper. 2013;6-7.
16. World Health Organization (WHO). Antimicrobial Resistance Fact sheet N°194"; 2014.
17. Schiavetti B, Wynendaele E, De Spiegeleer B. CIOMS guide to vaccine safety communication. WHO Drug Inf. 2018;32(1):23-45.
18. Fahim NAE. Prevalence and antimicrobial susceptibility profile of multidrug-resistant bacteria among intensive care units patients at Ain Shams University Hospitals in Egypt—A Retrospective Study. J Egypt Pub Health Assoc. 2021;96:7-34.
19. Souto R, de Andrade AFB, Uzeda M, Colombo APV. Prevalence of non-oral pathogenic bacteria in subgingival biofilm of subjects with chronic periodontitis. Braz J Microbiol. 2006;37(3):208–215.
20. Anejo-Okopi JA, Okwori AEJ, Michael G, Okojokwu OJ, Audu O. Bacterial profile associated with dental caries in Jos, Nigeria. Adv Res. 2015;4(6):371-377
21. Enitan SS, Oluremi AS, Ochei JO, Akele RY, Usiobeigbe SO, Emmanuel I, Enitan CB, Tajudeen RO. Assessment of oral bacterial profile and antibiogram of patients attending dental clinic of a private tertiary hospital in Ogun State, Nigeria. Saudi J Oral and Dent Res, 2020;5(1): 11-23.
22. Iroha IR, Orji JO, Onwa NC, Nwuzo AC, Okonkwo EC, Ibiam EO, Nwachi AC, Afuikwa FN, Agah VM, Ejikeugwu EPC, Agumah NB, Moses IB, Ugbo E, Ukpai EG, Nwakaeze EA, Oke B, Ogbu L, Nwunna E. Microbiology practical handbook. (Editor;Ogbu. O), 1st Edition. Charlieteximage Africa (CiAfrica Press). 2019;Pp:344.
23. Peter IU, Ngwu JN, Edemekong CI, Ugwueke IV, Uzoeto HO, Joseph OV, Mohammed ID., Mbong EO, Nomeh OL, Ikusika BA, Ubom IJ, Inyogu JC, Ntekpe ME, Obodoechi IF, NseAbasi PL, Ogbonna IP, Didiugwu CM, Akpu PO, Alagba EE, Ogba RC, Iroha IR. First Report Prevalence of Livestock Acquired Methicillin Resistant Staphylococcus aureus (LA-MRSA) Strain in South Eastern, Nigeria. IOSR J Nurs Health Sci. 2022;11(1)-50-56.
24. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing;twenty-eighth edition (M100). Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

25. Meinen A, Reuss A, Willrich N, Feig M, Noll I, Eckmanns T, Al-Nawas B, Markwart R. Antimicrobial resistance and the spectrum of pathogens in dental and oral-maxillofacial infections in hospitals and dental practices in Germany. Front Microbiol. 2021;12:676-108.
26. Ranganathan AT, Sarathy S, Chandran CR, Iyan K. Subgingival prevalence rate of enteric rods in subjects with periodontal health and disease. J Indian Soc Periodontol. 2017;21(3): 224–228.
27. Herrera D, Contreras A, Gamonal J, Oteo A, Jaramillo A, Silva N, Sanz M, Botero JE, Leon R. Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. J Clin Periodontol. 2008;35:106-113.
28. Colombo AP, Teles, RP, Torres MC, Rosalem W, Mendes MC, Souto RM, Uzeda M. Effects of non-surgical mechanical therapy on the *Subgingival microbiota* of Brazilians with untreated chronic periodontitis: 9-month results. J Periodontol. 2005;76:778-784.
29. Gamboa F, García DA, Acosta A, Mizrahi D, Paz A, Martínez D. Presence and antimicrobial profile of gram-negative facultative anaerobe rods in patients with chronic periodontitis and gingivitis. Acta Odontology Latinoam. 2013;26:24–30.
30. Garbacz K, Kwapisz E, Piechowicz L, Wierzbowska M. *Staphylococcus aureus* isolated from the oral cavity: phage susceptibility in relation to antibiotic resistance. Antibiotics. 2021;10:13-29.
31. Lima BP, Hu LI, Vreeman GW, Weibel DB, Lux R. The oral bacterium *Fusobacterium nucleatum* binds *Staphylococcus aureus* and alters expression of the staphylococcal accessory regulator sarA. Microb Ecol. 2019;78(2):336–347.
32. Scoffield JA, Wu H. Oral Streptococci and nitrite-mediated interference of *Pseudomonas aeruginos*a. Infect Immun. 2015;83(1):101– 107.
33. Vidana R, Sullivan A, Billstrom H, Ahlquist M, Lund B. *Enterococcus faecalis* Infection in Root Canals—host-derived or Exogenous Source? Lett Appl Microbiol. 2011;52(2):109–115.
34. Farmahan S, Tuopar D, Ameerally PJ. The clinical relevance of microbiology specimens in head and neck space infections of odontogenic origin. Britain Journal of Oral Maxillofacial Surgery. 2014;52:629–631.
35. Al-Nawas B, Karbach J. S3 Leitlinie: Odontogene Infektionen Online verfügbar unter. Available:https://wwwawmforg/uploads/tx_szleitlinien/007-006l_S3_Odontogene_Infektionen_2017
36. Heim N, Faron A, Wiedemeyer V, Reich R, Martini M. Microbiology and antibiotic sensitivity of head and neck space infections of odontogenic origin. Differences in Inpatient and Outpatient Management. J Cranio-Maxillofac Surg. 2017;45:1731–1735.
37. Haque M, Sartelli M, Haque SZ. Dental infection and resistance: Global health consequences. Dent J. 2019;**7**:22- 10.
38. Daniyan S, Abalaka M. Prevalence and susceptibility pattern of bacteria isolates of dental caries in a secondary health care institution, Nigeria. Shiraz E-Med J. 2011;12(3):135-139.
39. Persson GR, Hitti J, Paul K, Hirschi R, Weibel M, Rothen M, Persson RE. *Tannerella forsythia* and *Pseudomonas aeruginosa* in subgingival bacterial samples from Parous women. J Periodontol. 2008;79(3):508–516.
40. Fritschi BZ, Albert-Kiszely A, Persson GR. *Staphylococcus aureus* and other bacteria in untreated periodontitis. J Dent Res. 2008;87(6):589–593.
41. Smith AJ, Jackson MS, Bagg J. The ecology of staphylococcus species in the oral cavity. J Med Microbiol. 2001; 50(11):940–946.
42. Verkaik MJ, Busscher HJ, Jager D, Slomp AM, Abbas F, van der Mei HC. Efficacy of natural antimicrobials in toothpaste formulations against oral biofilms in vitro. J Dent. 2011;39(3):218–24.
43. Merghni A, Ben Nejma M, Helali I, Hentati H, Bongiovanni A, Mastouri M. Assessment of Adhesion, invasion and cytotoxicity potential of oral *Staphylococcus aureus* strains. Microb Pathog. 2015;86:1–9.
44. Oztan MD, Kiyan M, Gerceker D. Antimicrobial effect, in-vitro, of gutta-percha points containing root canal medications against yeasts and *Enterococcus faecalis*. Oral Surg Oral Med Oral Pathol Oral Radiol Endo. 2006;102(3): 410-416.
45. Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: Relationship to

endodontic disease. Crit Rev Oral Biol Med. 2004;15:308–320.

46. Komiyama EY, Lepesqueur LSS, Yassuda CG, Samaranayake LP, Parahitiyawa NB, Balducci I Koga-Ito CY. Enterococcus species in the oral cavity: Prevalence, virulence factors and antimicrobial susceptibility. PLOS One. 2016;11(9):16-3001.
47. Pihl M, Chavez de Paz LE, Schmidtchen A, Svensater G, Davies JR. Effects of clinical isolates of pseudomonas aeruginosa on staphylococcus epidermidis biofilm formation. Fed Euro Med Soc Immunol Med Microbiol. 2010;59(3):504–512.
48. Gonçalves MO, Coutinho-Filho WP, Pimenta FP, Pereira GA, Pereira JA, Mattos-Guaraldi AL. Periodontal disease as reservoir for multi-resistant and hydrolytic enterobacterial species. Newlett Appl Microbiol. 2007;44:488–94.
49. Smith RS, Iglewski BH. *P. aeruginosa* Quorum-sensing systems and virulence. Curr Opin Microbiol. 2003;6:56-60.
50. Wade WG. The oral microbiome in health and disease. Pharmacol Res. 2013; 69(1):137–143.
51. Souza L C D, Lopes FF, Bastos EG, Alves MC. Oral Infection by *Pseudomonas aeruginosa* in patient with chronic kidney disease -a case report. J Braz Nephrol. 2018;40(1):82–85.
52. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare?. Clin Infect Dis. 2002;34(5):634–640.
53. Nakou M, Kamma J, Gargalianos P, Laskaris G, Mitsis F. Periodontal microflora of HIV infected patients with periodontitis. Anaerobe. 1997;3(23):97–102.
54. Vieira Colombo AP, Magalhaes CB, Hartenbach FARR, Martins do Souto Maciel da Silva-Boghossian RC. Periodontal-disease-associated biofilm: A reservoir for pathogens of medical importance. Microb Pathog. 2016;94:27–34.
55. Al-Hebshi NN, Nasher AT, Maryoud MY, Homeida HE, Chen T, Idris AM, Johnson NW. Inflammatory bacteriome featuring *Fusobacterium nucleatum* and *Pseudomonas aeruginosa* identified in association with oral squamous cell carcinoma. Sci Report. 2017;7(1):1834.
56. Simoes-Silva L, Ferreira S, Santos-Araujo C. Oral Colonization of staphylococcus species in a peritoneal dialysis population: a possible reservoir for PD-related infections? Can J Infect Dis Med Microbiol. 2018;57:89-94.
57. Iroha IR, Okeh EN, Moses IB, Nwakaeze EA, Ugbo EN, Kalu AC, Onuora AL, Ude IU. Prevalence and antibiotic susceptibility patterns of extended spectrum beta-Lactamase-producing *Klebsiella oxytoca* isolated from urine samples of patients visiting private laboratories in Abakaliki Metropolis Afri J Microbiol Res. 2019a;13(28):538-543.
58. Goldberg S, Cardash H, Browning H. Isolation of *Enterobacteriaceae* from the mouth and potential association with malodor. J Dent Res. 1997;76:1770-1775.
59. Marra F, George D, Chong M, Sutherland S, Patrick DM. Antibiotic prescribing by dentists has increased: Why?. J Am Dent Assoc. 2016;147:320–327.
60. Maslamani M, Sedeqi F. Antibiotic and analgesic prescription patterns among dentists or management of dental pain and infection during endodontic treatment. Med Princ Pract. 2017;27:66–72.
61. Teoh L, Stewart K, Marino RJ, McCullough MJ. Part 1. Current prescribing trends of antibiotics by dentists in Australia from 2013 to 2016. Austrian Dent J. 2018;63:329–337.
62. Halling F, Neff A, Heymann P, Ziebart T. Trends in Antibiotic Prescribing by Dental Practitioners in Germany. J Cranio-Maxillofac Surg. 2017;45:1854–1859.
63. Poeschl PW, Spusta L, Russmueller G, Seemann R, Hirschl A, Poeschl E. Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endo. 2010;110:151–156.
64. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Robel RK, Kiflay GR, Tesfu T. Methicillin-resistant *Staphylococcus aureus* (MRSA): Prevalence and antimicrobial sensitivity pattern among patients—a multicenter study in Asmara, Eritrea. Can J Infect Dis Med Microbiol. 2018;9: 832-1834.
65. Marjani MFA, Mohammed NR, Abd SY, Mansour RF. Efflux pumps in colistin resistant *Pseudomonas aeruginosa* Isolates in Baghdad. Int J Infect and Drug Resist. 2015;3(11):680–685.

66. Abd El-Baky RM, Masoud SM, Mohamed DS, Waly NGFM, Shafik EA, Mohareb DA, Elkady A, Elbadr MM, Hetta HF. Prevalence and some possible mechanisms of colistin resistance among multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa*. J Infect Drug Resist. 2020;13:323-333.
67. Ammar A, Hafida H, Sana D, Leila S, Mouna M, Messaoud B, Nadia G, Boubaker ER. Emergence of plasmid mediated colistin resistance gene mcr-1 in carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates from Algeria: A new successful resistance combination toward a therapeutic impasse? Int J Sci Res. 2020;76:4-1.
68. Wang Y, Liu F, Hu Y, Zhang G, Zhu B, Gao GF. Detection of mobile colistin resistance gene mcr-9 in carbapenem resistant *Klebsiella pneumoniae* Strains of human origin in Europe. J Infect. 2020;80(5):578-606.
69. Ngbede E O, Adekanmbi F, Poudel A, Kalalah A, Kelly P, Yang Y, Adamu AM, Daniel ST, Adikwu AA, Akwuobu CA, Abba P O, Mamfe LM, Maurice NA, Adah MI, Lockyear O, Butaye P, Wang C. Concurrent resistance to carbapenem and colistin among enterobacteriaceae recovered from human and animal sources in Nigeria is associated with multiple genetic mechanisms. Front Microbiol. 2021;12:740-348.
70. Yadav K, Prakash S, Yadav NP, Sah RS. Multi-drug resistance of bacterial isolates among dental caries patients. Janaki Med Coll J Med Sc. 2015;3:37–44.
71. Ugbo EN, Anyamene CO, Moses IB, Ariom TO, Agumah N B, Chukwunwejim CR, Egbule CU, Emioye AA, Okata-Nwali OD, Aneke CJ, Ugadu IO, Osu BO. Isolation and molecular characteristics of extended spectrum beta-lactamase-producing uropathogenic *Escherichia coli* isolated from hospital attendees in Ebonyi State, Abakaliki. Afr J Biotechnol. 2020; 19(11):829-835.
72. Ibrahim ME. High antimicrobial resistant rates among gram-negative pathogens in intensive care units: a retrospective study at a tertiary care hospital in Southwest Saudi Arabia. Saudi Med J. 2018;39:10-35.
73. Azab KSM, Abdel-Rahman MA, El-Sheikh HH, Azab E, Gobouri AA, Farag MMS. Distribution of extended-spectrum β-Lactamase (ESBL)-encoding genes among multidrug-resistant gram-negative pathogens collected from three different countries. Antibiotics. 2021;10:24-7.
74. Löffler C, Böhmer F. The effect of interventions aiming to optimize the prescription of antibiotics in dental care—a systematic review. PLOS One. 2017;12:188-61.
75. ECDC. Antimicrobial resistance in the EU/EEA (EARS-Net) – Annual Epidemiological Report 2019. Stockholm: European Centre for Disease Prevention and Control; 2020.
76. Yadav K, Prakash S. Antibiogram profiles against polymicrobial pathogens among dental caries patients at Janaki Medical College Teaching Hospital, Nepal. Int J Appl Dent Sci. 2015;1:156–162.
77. Gaetti-Jardim EC, Marqueti AC, Faverani LP, Gaetti-Jardim Júnior E. Antimicrobial resistance of aerobes and facultative anaerobes isolated from the oral cavity. J Appl Oral Sci. 2010;18:551–559.

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