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Molecular characterization of Enterobacteriacea isolated from gingivitis and periodontitis patients and the antimicrobial activity of mouth wash agents

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ABSTRACT

Enterobacteriaceae communities in gingival crevice grow as biofilms (plaque) on teeth and gum surfaces in periodontal pockets; and are implicated in chronic periodontitis due to periodontal therapy failure. This study was designed to determine the frequency of antibiotic resistant genes in Enterobacteriaceae isolated from gingivitis and chronic periodontitis patients visiting dental clinics (FCDT & T) in Enugu, and the antibacterial activity of some commercially-produced mouth washing agents. A total of 178 clinical enterobacteria species previously isolated and characterized from gingivitis and periodontitis patients comprising *K. pneumoniae* (n=65), *E. coli* (n=44), *Salmonella* species (n=35) and *K. oxytoca* (n=34) were collected. Isolated bacteria were re-characterized using standard microbiology procedures which include culturing, Gram staining, biochemical test, and sugar fermentation test. Antibacterial activity of selected brandsof mouth wash agents namely, Oral B, Pearl drop smoker, Dentyl active, and Listerine, were assessed by agar well diffusion assay. Screening for the presence of blaCTX-M, blaSHV, and blaOXA resistance genes was done by polymerase chain reaction (PCR). The mouth wash agents used in this study had antibacterial activity against isolated enterobacteria species at various concentrations with the highest inhibitory zone diameter (IZD) of 23 mm recorded for *Salmonella* species. PCR analysis revealed the presence of blaCTX-M (100 %), blaOXA (100 %), and blaSHV (92.4 %) genes respectively in the isolated enterobacteria species. Enterobacteriaceae that harbor different antibiotic-resistant genes were present in periodontitis and gingivitis patients visiting FCDT & T, Enugu. This might possibly contribute to the destruction of gingival and periodontal tissues, and the spreading of multidrug-resistant bacterial strains in patients, thus making treatment difficult.

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Introduction

Periodontitis, a chronic inflammation induced by pathogenic bacteria, is a major cause of tooth loss, especially among adults by irreversibly destroying the underlying supporting teeth bone [1]. Gingivitis is a reversible localized inflammation of the gingiva. It is also an inflammation and a peri-implant mucositis which manifests around dental implants [2]. Periodontal disease is initiated by specific invasive oral pathogens that colonize dental plaque biofilms on tooth surface. Bacteria are the primary etiological agent in periodontal disease. Moreover, periodontal diseases have been known to cause localized or systemic medical conditions, such as respiratory problems, rheumatoid arthritis, cardiovascular disease, chronic kidney disease, impairment of cognitive function, metabolic disorders, and various cancer risks [3, 4]. There are seven typical types of periodontitis that affect children, adults, and the elderly people [2]**.** Due to this, there has been a continued increasing interest in monitoring periodontitis.

Gram-negative rods (GNR) in the Enterobacteriaceae family have stood out in clinical settings due to the severity of infections they cause, with significant antibiotic resistance traits. An aggravating factor of antimicrobial resistance by GNR has been the continued emergence of beta-lactamase-producing strains [5]. The occurrence and possible colonization by Enterobacteriaceae may not be an issue of health concern since the oral cavities are not their usual habitat. However, oral cavity colonization by other enteric rods (Pseudomonadaceae e.g. *Acinetobacter baumanii* and *Pseudomonas aeruginosa*) has gained relevance in recent times, it is needful to consider their presence and possible role in oral cavity region [6]. Recently, enteric rods, among other Enterobacteriaceae, gained relevance because of their ability to produce an array of virulence factors which results in life-threatening infections such as septicaemia, urinary tract and lower respiratory tract infections, and nosocomial infections [7, 6]. The difficult aspect of the involvement of Enterobacteriaceae in periodontal diseases is their role since the exact roles of these organisms in the pathogenesis of periodontitis have not been fully established in many other research. Some studies have identified them as transient colonizers while others maintained that they are elements of the subgingival flora in a harmonious relationship with microbes in the periodontal region [8]. However, their specific role is unclear since these organisms are foreign to subgingival flora. They have also been identified in the tongue, tonsils, and other oral cavity ecological niches [9]. Some of the factors that affect their distribution in the subgingival dental plaque are hygienic practices, geographic area, and diet with varying impact from one population to another [10]. Mouth wash agents are usually used in reducing the oral cavity's microbial load, or in controlling or reducing bad breath, depending on the type of ingredients in the oral rinse. The antimicrobial activity of these mouth wash agents make it very useful in controlling gingivitis and supragingival plaque. It is mostly utilized before oral and periodontal surgery is done, including implant placements and tooth extraction procedures. The combination of mechanical and chemotherapeutic (antimicrobial mouth rinses) approaches have been used effectively to control plaque, prevent gingivitis and periodontal diseases [11, 12]. This has also been observed among enteric rods isolated from the oral cavity; hence, this present study investigated the antimicrobial activity of different mouth wash agents and the frequency of antibiotic resistant genes (ARGs) in Enterobacteriaceae isolated from gingivitis and chronic periodontitis patients visiting Federal College of Dental Technology and Therapy (FCDT & T) clinics, Enugu State, Eastern Nigeria.

Methods

Bacterial isolates collection

A total of 178 clinical enterobacteria species isolates comprising *K. pneumoniae* (n=65), *E. coli* (n=44), *Salmonella* species (n=35), and *K. oxytoca* (n=34) previously isolated and characterized from gingivitis and periodontitis patients were collected from the Federal College of Dental Technology and Therapy (FCDT & T) clinics, Trans-Ekulu, Enugu State, Nigeria. The enterobacteria species were re-characterized so as to re-confirm their initial identities using standard microbiology techniques which includes culturing, Gram staining, biochemical tests, and sugar fermentation test [13].

Antibacterial activity of mouth wash agents

Determination of the antibacterial activity of 4 commercially-produced mouth wash agents; Oral B, Pearl drop smoker, Dentyl active, and Listerine was done using the agar well diffusion assay. Mouth wash agents were selected based on consumers' popularity in the study area. Briefly, isolated Enterobacteriaceae were streaked on the surface of Mueller-Hinton agar (MHA) plates. An 8 mm cork borer was used to make wells on the MHA plate. Different dilutions (100 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL) of the mouth wash agents were used to fill each hole and the inoculated MHA plates were incubated for 24 h at 37 °C. After the incubation period, inhibition zone diameter (IZD) or area of clearance around the well was measured to the nearest millimeter and interpreted [14].

Polymerase chain reaction (PCR) screening for antibiotic resistant genes (ARGs)

Bacterial isolates were screened for antibiotic resistant genes (ARGs) such as blaOXA, blaSHV, and blaCTX-M as previously described (Olivier *et al.*, [15, 16]) using specific primers (Table. 1). Deoxyribonucleic acid (DNA) extraction was carried out as described by Munday *et al.* [17]. The PCR mastermix consisted of 5 μl Green buffer 5X, 1μl of each primer (forward and

Table 1
Oligonucleotide primers used for PCR.

S/No	Primers	Primer Sequence Direction	Annealing Temp. (°C)	Amplicon Size (bp)
1	blaCTX-M-forward	CGCTTTGCGATGTGCAG	50	550
	blaCTX-M-reverse	ACCGCGATATCGTTGGT		
2	BlaSHV-forward	ATGCGTTATATTCGCCTGTG	60	400
	BlaSHV-reverse	TGCTTTGTTATTCGGGCCAA		
3	blaOXA-forward	GCGCGATCTGGTTCACTCG	56	500
	Bla OXA-reverse	AGTCGACAGTTGCGCCGGC		

Table 2
The Inhibition Zone Diameter (IZD) of Enterobactericeae recovered from patients with periodontitis and gingivitis against Oral B mouth wash agent.

Enterobacteriacea	Periodontal/ gingivae sites	Mouth-wash Agent Concentration (mg/mL)				
		100	50	25	12.5	6.25
E. coli	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	12 mm	11 mm	Nil	Nil	Nil
	ULJ	17 mm	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
Salmonella species	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	LLJ	16 mm	14 mm	14 mm	13 mm	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
K. pneumoniae	LRJ	16 mm	14 mm	13 mm	Nil	Nil
	LRJ	15 mm	13 mm	Nil	Nil	12 mm
	LLJ	Nil	Nil	Nil	Nil	Nil
	LLJ	13 mm	11 mm	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
K. oxytoca	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	12 mm	10 mm	Nil	Nil	Nil
	URJ	13 mm	Nil	Nil	Nil	Nil
	URJ	15 mm	12 mm	Nil	Nil	Nil

Key: LRJ-Lower Right Jaw, LLJ-Lower Left Jaw, URJ-Upper Right Jaw, ULJ-Upper Left Jaw, Nil-No Inhibition

reverse)10 μM, 0.65 μl of dNTPs 10 mM, 0.12 μl of Gotaq 0.5 U/μl, 2 μl of DNA template, and 15.25 μl of purified water to make up a total reaction volume of 25 μl. PCR was done under the following conditions: initial denaturation step at 96 °C for 5 min, followed by 35 cycles consisting of denaturation at 96 °C for 1 min, annealing temperature at 50 °C, 56 °C, and 60 °C for blaCTX-M, blaOXA, blaSHV respectively [18, 19] at 1 min, primer extension at 72 °C for 1 min and final extension for 10 min. Electrophoresis of the PCR products was carried out for 30 min at 80 V on 1 % agarose gel.

Statistical analysis

Statistical analysis for the comparative evaluation of categorical variables was performed using SPSS 17.0 version statistical software package using the ANOVA and Tukey post-hoc multiple comparison test tools. Results were only considered to be statistically significant if the p value was less than 0.05 ($p < 0.05$).

Results

Oral B mouth wash showed high inhibitory zone diameter (IZD) of 17 mm at 100 mg/mL concentration against E. coli isolated from lower left jaw of patients with periodontitis and gingivitis (Table. 2). The least concentration of 6.25 mg/mL revealed IZD of 12 mm against *Klebsiella pneumoniae* recovered from lower right jaw of patients with periodontitis and gingivitis (Table 2).

Pearl Drop Smoker mouth wash agent exhibited no antibacterial activity on *Klebsiella pneumoniae* isolates at all concentrations tested. The mouth wash agent revealed IZD of 13 mm at 25 mg/ml concentration against *K. oxytoca* isolated from lower right jaw (Table. 3).

Dentyl Active mouth wash demonstrated high IZD of 19 mm at 100 mg/mL against *K. pneumoniae* while no IZD was recorded at 12.5 mg/mL and 6.25 mg/mL concentrations against *E. coli*, *Salmonella* spp, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* recovered from patients with periodontitis and gingivitis.

Table 3
The Inhibition Zone Diameter (IZD) of Enterobactericeae recovered from patients with periodontitis and gingivitis against Pearl Drop Smoker mouth wash agent.

Enterobacteriaceae	Periodontal/ gingivae sites	Mouth-wash Agent Concentration (mg/mL)				
		100	50	25	12.5	6.25
E. coli	LRJ	11 mm	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	ULJ	20 mm	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
Salmonella species	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	LLJ	15 mm	14 mm	13 mm	12 mm	9 mm
	ULJ	15 mm	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
K. pneumoniae	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
K. oxytoca	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	13 mm	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil

Key: LRJ-Lower Right Jaw, LLJ-Lower Left Jaw, URJ-Upper Right Jaw, ULJ-Upper Left Jaw, Nil-No Inhibition

Table 4
The Inhibition Zone Diameter (IZD) of Enterobactericeae recovered from patients with periodontitis and gingivitis against Dentyl Active mouth wash agent.

Enterobacteriaceae	Periodontal/ gingivae sites	Mouth-wash Agent Concentration (mg/mL)				
		100	50	25	12.5	6.25
E. coli	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	16 mm	11 mm	10 mm	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
	ULJ	12 mm	10 mm	Nil	Nil	Nil
Salmonella species	LRJ	23 mm	18 mm	14 mm	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	ULJ	13 mm	Nil	Nil	Nil	Nil
	ULJ	Nil	17 mm	16 mm	Nil	Nil
K. pneumoniae	LRJ	19 mm	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	14 mm	12 mm	9 mm	Nil	Nil
	LLJ	16 mm	11 mm	10 mm	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
K. oxytoca	LRJ	12 mm	14 mm	10 mm	Nil	Nil
	LRJ	11 mm	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil

Key: LRJ-Lower Right Jaw, LLJ-Lower Left Jaw, URJ-Upper Right Jaw, ULJ-Upper Left Jaw, Nil-No Inhibition

Listerine mouth wash had no antibacterial activity against *E. coli* and *Klebsiella oxytoca*. However, IZD of 10 mm and 14 mm were recorded against *Salmonella* spp while IZD of 16 mm and 17 mm at 100 mg/ml concentration were recorded against *Klebsiella pneumoniae* (Table. 5).

The prevalence of the bla_{CTX-M}, bla_{OXA}, and bla_{SHV} resistance genes in enterobacteria species isolated from gingivitis and periodontitis patients were 100 %, 100 %, and 92.4 % respectively.

Bla_{CTX-M} resistance gene was harboured by *Klebsiella pneumoniae* 48 (45.2 %), *Escherichia coli* 15 (14.2 %), *Salmonella* spp 23 (21.7 %), and *Klebsiella oxytoca* 20 (18.9 %) while bla_{SHV} resistance gene was harboured by *Klebsiella pneumoniae* 48 (45.2 %), E. coli 12 (11.3 %), Salmonella spp 18 (17.0 %), and *Klebsiella oxytoca* 20 (18.9 %) (Table. 6). Bla_{OXA} resistance gene was harboured by *Klebsiella pneumoniae* 48 (45.2 %), *E. coli* 15 (14.2 %), *Salmonella* spp 23 (21.7 %), and *Klebsiella oxytoca* 20 (18.9 %). Co-existence of bla CTX+SHV+OXA in enterobacteria species was observed in *Klebsiella pneumoniae* 48 (45.2 %), *E.*

Table 5
The Inhibition Zone Diameter (IZD) of Enterobactericeae recovered from patients with periodontitis and gingivitis against Listerine mouth wash agent.

Enterobacteriaceae	Periodontal/ gingivae sites	Mouth wash agent concentration (mg/mL)				
		100	50	25	12.5	6.25
E. coli	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
Salmonella species	LRJ	10 mm	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	LLJ	Nil	Nil	Nil	Nil	Nil
	ULJ	14 mm	Nil	Nil	Nil	Nil
	ULJ	Nil	Nil	Nil	Nil	Nil
K. pneumoniae	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	LLJ	17 mm	Nil	Nil	Nil	Nil
	LLJ	16 mm	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
K. oxytoca	LRJ	Nil	Nil	Nil	Nil	Nil
	LRJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil
	URJ	Nil	Nil	Nil	Nil	Nil

Key: LRJ-Lower Right Jaw, LLJ-Lower Left Jaw, URJ-Upper Right Jaw, ULJ-Upper Left Jaw, Nil-No Inhibition

Table 6
Distribution of antibiotic resistant genes (ARGs) in Enterobacteriaceae recovered from periodontal and gingivitis patients.

Enterobacteriaceae	Single (%)			Co-existence (%)			
	CTX-M	SHV	OXA	CTX-M +SHV+OXA	CTX-M + SHV	CTX-M + OXA	SHV + OXA
K. pneumoniae	48(45.2)	48(45.2)	48(45.2)	48(45.2)	48(45.2)	48(45.2)	48(45.2)
E. coli	15(14.2)	12(11.3)	15(14.2)	12(11.3)	12(11.3)	15(14.2)	12(11.3)
Salmonella sp.	23(21.7)	18(17.0)	23(21.7)	18(17.0)	18(17.0)	23(21.7)	18(17.0)
K. oxytoca	20(18.9)	20(18.9)	20(18.9)	20(18.9)	20(18.9)	20(18.9)	20(18.9)
Total	106(100)	98(92.4)	106(100)	98(92.4)	98(92.4)	106(100)	98(92.4)

coli 12 (11.3 %), *Salmonella* spp 18 (17.0 %), and *Klebsiella oxytoca* 20 (18.9 %). Co-existence of bla CTX-M+SHV in isolated enterobacteria species was observed in *Klebsiella pneumoniae* 48 (45.2 %), E. coli 12 (11.3 %), *Salmonella* species 18 (17.0 %), and *Klebsiella oxytoca* 20 (18.9 %) while the bla CTX-M+SHV co-existence was observed among *Klebsiella pneumoniae* 48 (45.2 %), E. coli 12 (11.3 %), *Salmonella* spp 18 (17.0 %), and *Klebsiella oxytoca* 20 (18.9 %). Co-existence of bla CTX-M+OXA was observed in *Klebsiella pneumoniae* 48 (45.2 %), *E. coli* 15 (14.2 %), *Salmonella* spp 23 (21.7 %), and *Klebsiella oxytoca* 20 (18.9 %) (Table. 6). Figures SM1, SM2, and SM3 show PCR amplification gel pictures of blaSHV, blaCTX-M, and blaOXA resistance genes harboured by enteric bacteria from periodontal and gingivitis patients.

Discussion

This study investigated the frequency of antibiotic-resistant genes in Enterobacteriaceae isolated from gingivitis and chronic periodontitis patients visiting Federal College of Dental Technology and Therapy (FCDT & T) clinics, Enugu and the antibacterial activity of some commercially-produced mouth washing agents. Among the commercial mouth wash agents screened, Dentyl active (containing cetylpyridinium chloride and triclosan) showed a high inhibitory activity with an inhibition zone diameter (IZD) of 23 mm at 100 mg/mL against *Salmonella* species. Triclosan, an antimicrobial agent which affects fatty acid biosynthesis in bacterial cells inhibits enoyl reductase enzyme [20], while cetylpyridinium chloride (CPC), an ammonium quaternary which affects microbial lipids and proteins is effective in the prevention of dental plaque, and in reducing the incidence of gingivitis [21, 22]. Their synergistic effect may have resulted in an increased inhibitory effect against our test isolate. The in vitro activity of each of these individual compounds in mouth wash products against oral *Streptococcus* species has been documented [23]. This may have contributed to the FDA consideration of mouth wash agents containing 0.1 % CPC as safe for short term use [24]. On the other hand, Pearl Drop Smoker contains antimicrobial compounds such as sodium lauryl sulfate, sodium fluoride, CPC, and pegylated hydrogenated castor oils (PEG 40), but were unable to cause potential inhibitory effect on periodontal pathogens in this study. This has also been reported by other components, especially detergents used in the cosmetics industry, such as pegylated hydrogenated castor oils have been reported to have no antibacterial activity [25]. The antagonistic interaction between these components may in effect can-

cel out the antimicrobial activity of Cetylpyridinium chloride (CPC) which may in turn account for the poor antimicrobial activity observed with the pearl drop smoker mouth wash agent in our study.

Listerine is known to have antimicrobial property due to the presence of thymol, sodium benzoate and benzoic acid and alcohol being one of its constituents which results in microbial enzymatic inhibition, cell wall destruction, and extraction of lipopolysaccharides [11]. In this present study, the antimicrobial activity of Listerine was observed only at 100 mg/mL concentration with inhibition zone range of 10–17 mm. This is in concord with other studies [26, 11] which reported 43.8 % - 60 % reduction in recoverable plaque bacteria after rinsing with Listerine mouth wash agents. Pan et al. [27] also observed 78.7 % bactericidal effect by Listerine against *Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Fusobacterium nucleatum*.

The inhibitory effect of Oral B mouth wash with IZD of 12 mm at 6.25 mg/ml against *K. pneumoniae* is in line with the Oral B mint mouth wash bactericidal activity at 11–12 mm against oral streptococcal species [23]. It is worth-noting that Oral B, Listerine, and Dentyl Active mouth wash agents produced bacteriostatic effect mostly at 100 mg/ml concentration. There was no significant difference in the antimicrobial activity exhibited by the different mouth wash agents against the tested periodontal pathogens ($p = 0.426$). However, there was a significant difference in the antimicrobial activity of Peal Drop Smoker (S.D. = 5.50) and Dentyl Active mouth wash agents (S.D. = 4.65), $p = 0.03$.

It was very clear that peculiarities in mouth rinse effectiveness depends on different concentrations of the mouth rinse. In addition, the combination of different active ingredients may contribute to their synergic effect [28]. These variations sometimes make it difficult to determine the clinical effectiveness of mouth rinses. It is also sometimes difficult to obtain the activity of mouth wash agents because the exact concentrations of ingredients were not stated by the manufacturers which may have contributed to the observations recorded. It is worth mentioning that these results may not correspond to the actual behaviours of tinctures in vivo because they are not exposed to the same conditions found in periodontal sites. Nevertheless, this study has revealed the action of these agents and further clinical investigations. Although, there are pockets of studies which reported the isolation of pathogenic bacteria in periodontal and gingival diseases, but reports on the molecular characterization of enterobacteria associated with oral health for antibiotic resistance genes (ARGs) are scarce. Our study was able to fill this gap as we employed molecular techniques in characterizing multidrug-resistant ESBL-producing enterobacteria implicated in periodontitis and gingivitis. The frequency of $bla_{CTX-M,}$ bla_{SHV} and bla_{OXA} resistance genes in the isolated Enterobacteriaceae in our study was 100 %, 100 % and 92.4 % respectively. In agreement with our study, Ouedraogo et al. [19] reported that 94 % of E. coli that were identified as ESBL producers harboured $bla_{CTX-M-15}$ gene. The frequency of occurrence of bla_{CTX-M} among ESBL-producing E. coli and K. pnuemoniae in this study was 14.2 % and 45.2 % respectively. This was in accordance with high occurrence frequency of bla_{CTX-M} resistance gene among ESBL-producers as reported by Rossolini *et al*. [29] and Canton & Coque [30] for *E. coli* (30–90 %) and *Klebsiella pneumoniae* (10–60 %). The prevalence of bla_{SHV} resistance gene among *K. pnuemoniae* in this study was 45.2 %. This was close to the 32 % reported in Nigeria by Olowe et al. [31] and less than the 100 % reported in Iraq by Salman & Ghaima [32]. Beta-lactamase genes occur frequently by combination ($bla_{OXA,}$ $bla_{CTX-M,}$ and bla_{SHV} genes) than singularly; this has changed their epidemiology considerably because they combine the expansion of specific clonal dissemination with mobile genetic elements, where these strains also carry plasmids mediating antibiotic resistance genes to other drug classes, such as exhibiting high frequency of resistance to aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, and tetracycline [32]. The bla_{OXA} and bla_{SHV} genes co-existed in 11.3 % of *E. coli* and is in contrast with findings of Handal et al. [18] in which β-Lactamase genes (OXA and SHV) were not detected by PCR in 53 subgingival bacteria from refractory periodontitis patients while Dureja et al. [33] reported that bla_{OXA-10} and bla_{SHV}-like resistance genes were not detected in any of the ESBL-producing *E. coli* isolates from healthy humans. Additional investigations showed that four ESBL-producing *E. coli* isolates harbouring bla_{CTX-M} and bla_{SHV} genes also harboured bla_{OXA} gene while one *E. coli* isolate positive for both $bla_{CTX-M-15}$ and bla_{OXA-1} genes has been reported [19]. Expression of combination genes (bla_{CTX-M} and bla_{SHV}) was observed in 17.0 % of *Salmonella* spp while other studies reported *Salmonella* isolates to be negative for the bla_{SHV} gene and bla_{CTX-M} gene [34]. The prevalence of bla_{CTX-M} and bla_{SHV} genes among *K. oxytoca* in this study was 18.9 %. This was similar to the prevalence of 20 % and 22.5 % reported for the bla_{CTX-M} and bla_{SHV} genes respectively in Nigeria [31].

According to literature, our work is the first study in Nigeria that simultaneously reported a high frequency of ESBL resistance genes (blaCTX-M, blaSHV, and blaOXA) in enterobacteria implicated in cases of periodontitis and gingivitis and the in vitro activities of some currently used commercial mouth wash agents against these ESBL-producing pathogens. This will go a long way to fully understand the current antimicrobial resistance trends associated with oral pathogens and the effectivity of some commonly used mouth wash antimicrobial agents used in reducing the oral cavity's microbial load, controlling bad breath or plague, and in turn preventing gingival and periodontal diseases. According to literature, our work is the first study in Nigeria that simultaneously reported a high frequency of ESBL resistance genes (blaCTX-M, blaSHV, and blaOXA) in enterobacteria implicated in cases of periodontitis and gingivitis and the *in vitro* activities of some currently used commercial mouth wash agents against these ESBL-producing pathogens. This will go a long way to fully understand the current antimicrobial resistance trends associated with oral pathogens and the effectivity of some commonly used mouth wash antimicrobial agents used in reducing the oral cavity's microbial load, controlling bad breath or plague, and in turn preventing gingival and periodontal diseases. Presence of beta-lactamase gene in pathogenic bacteria implicated in periodontal disease showed that oral cavities are reservoirs of bacterial resistance determinants [18]. The external environment is in constant interaction with the oral microbial flora, and invading bacteria descending to the gut usually pass through this region. Thus, a two-way resistance determinant exchange mechanism between food and the oral microbial flora is likely to occur [18,

35]. Our study has been able to show that enterobacteria implicated in periondontitis and gingivitis harbor ESBL resistance genes which are critical elements/factors of their pathogenesis. We were also able to assess the antimicrobial activities of some currently used commercial mouth wash agents which are used in oral health. Information from our study will greatly help to fully understand the current antimicrobial resistance trends associated with oral health and the effectivity of commonly used mouth wash agents employed in decolonization/killing of oral pathogens to prevent oral diseases. Our research will drive more studies and be a template for further research directed as investigating the growing antimicrobial resistance trends of enterobacteria implicated in oral health.

Enough attention has not really been paid to oral health in Africa. Even though oral cavities are not the normal habitats of enterobacteria, their oral cavity colonization has continued to gain strong relevance increasingly. Our study has been able to show that multidrug-resistant bacteria are gradually becoming a menace to oral health and an emerging challenge to oral hygiene. The high frequency of enterobacteria harbouring antibiotic-resistant resistance genes in periodontitis and gingivitis cases in our study area could possibly contribute to the destruction of gingival and periodontal tissues, thus making treatment difficult. In fact, these pathogens have been implicated as a major cause of tooth loss, especially among adults by causing irreversible destruction of the underlying supporting teeth bone. If this menace is not curtailed, the identified pathogenic abilities of the enterobacteria implicated in periodontitis and gingivitis in our study could further exacerbate and become a very difficult public health problem which could affect the overall well-being of people. It is therefore imperative to carry out continued antimicrobial resistance surveillance of oral pathogens implicated in periodontitis and gingivitis so as to fully understand their pathogenesis and roles in the oral cavity region. This will greatly help in managing and preventing oral diseases such as bad breath, periodontitis, and gingivitis.

Conclusion

Our study is the first to show the presence of $bla_{CTX-M,}$ bla_{SHV}, and bla_{OXA} resistance genes from Enterobacteriaceae recovered from periodontal patients in South-Eastern Nigeria. The frequency of $bla_{OXA,}$ $bla_{CTX-M,}$ and bla_{SHV} genes among Enterobacteriaceae isolated from periodontitis and gingivitis patients was high with the co-existence of different beta-lactamase genes in isolated bacteria. The mouth wash agents (Oral B, Pearl drop smoker, Dentyl active, and Listerine) used in this study had antibacterial activity against periodontal pathogens from patients visiting dental clinics (FCDT & T) in Enugu, Eastern Nigeria. However, several studies are required to identify and fully understand the different genetic determinants of beta-lactamase production by Enterobacteriaceae isolated from patients with gingival and periodontal diseases.

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Declaration of Competing Interest

Authors declare no conflict of interest.

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