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Genotypic Detection of Dominant Bacteria in Dental Caries in Uyo, Nigeria

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Abstract

Dental caries remains one of the most prevalent and persistent oral health challenges globally, with nearly universal incidence across populations. The disease is increasingly complicated by the emergence of antibiotic-resistant bacteria, a phenomenon largely driven by biofilm formation and the acquisition of resistance genes. This study aimed to identify the predominant bacterial species implicated in the etiology of dental caries in Uyo, Nigeria, and to characterize their associated antibiotic resistance genes. A total of 120 clinical samples were analyzed using the *VITEK 2 Compact System* (*bioMérieux*) for bacterial identification and antibiotic susceptibility testing. Molecular detection of three extended-spectrum β-lactamase (ESBL) genes—*CTX-M*, *TEM*, and *OXA*—was performed via PCR using standard thermal cycling conditions on an *ABI 9700 Applied Biosystems* platform. Among the 27 isolates recovered, Gram-negative bacteria constituted 66.7%, with *Burkholderia cepacia* complex

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being the most prevalent (25.9%). *Burkholderia cepacia* exhibited high sensitivity to Amikacin and Tobramycin but showed marked resistance to Ceftazidime. Of the *B. cepacia* isolates, 6 (85.7%) underwent *16S rRNA* sequencing, confirming their identity as *Burkholderia cepacia* (n=4) and *Burkholderia cenocepacia* (n=2). *CTX-M* genes were detected in all sequenced isolates (100%), while *TEM* genes were present in one isolate (16.7%) and *OXA* genes were absent. These findings underscore the potential public health threat posed by ESBL-producing *B. cepacia* complex strains in dental caries, highlighting the urgent need for targeted antimicrobial stewardship and enhanced surveillance in oral healthcare settings.

Keywords: *Burkholderia cepacia* complex; Dental caries; ESBL genes; *CTX-M*; Antibiotic resistance; *16S rRNA* sequencing.

INTRODUCTION

Dental caries is known to be a transmissible, infectious oral disease that leads to demineralization of the tooth and the formation of cavities. It is one of the most chronic oral diseases globally. According to Gewargis (2019), Ritter *et al.* (2015), Lamont and Egland (2015), Udoh *et al.*, (2025) and Onwuezobe *et al.*, (2025), the aetiology of caries is multifactorial and involves various complex interaction of host factors such as salivary components; dietary factors such as availability of fermentable carbohydrates on the tooth surface and microbial factors (presence of cariogenic oral flora, biofilm) over time. Worthy of note, is the fact that dental caries emergence and progress are complex, as not all persons with teeth, biofilm, and are consuming carbohydrates will have caries over time. Ritter *et al.* (2015), documented that various modifying risk and protective factors leads to the dental caries onset. At the tooth level, caries emergence is identified by localized demineralization and loss of tooth structure. According to Onwuezobe *et al.*, (2025), this often leads to tooth pain and other forms of discomfort. In the biofilm cariogenic bacteria metabolize fermentable carbohydrates for energy, and organic acid by-products are produced. The organic acids, when present in the biofilm for a long time, may lower the pH in the biofilm to below its critical level of 5.5 for enamel, and 6.2 for dentin. As the pH lowers, calcium and phosphate are removed from the tooth to the biofilm in order to get to an equilibrium, thereby leading to loss of minerals in the tooth (demineralization). If the pH in the biofilm becomes neutral after a while, then the concentration of soluble calcium and phosphate will be supersaturated relative to that in the tooth. At such instance,

minerals are channelled to partially demineralized enamel (remineralization). In situations whereby demineralization process persists more than remineralization process, cavity formation sets in, and there is gradual dissolution and destruction of calcified tissues of the teeth. At this point several symptoms of caries becomes more noticeable. Such as; tooth discomfort especially when cold or hot food hits the tooth, tooth sensitivity when eating sweet or sour foods, tongue feels the roughness of the enamel. This gradually leads to the loss of affected tooth, unless treatment with antibiotic is received on time to stop further progress. However, several research findings have shown that many bacteria aetiological agents of caries are resistant to commonly used antibiotics. In a study by Anejo-Okopi *et al.* (2015), antimicrobial sensitivity test of dental caries revealed that out of nine bacteria isolates, three were resistant to Vancomycin, one to Chloramphenicol, and four to Erythromycin. Of the observed isolates, only *Enterobacter* species was resistant to all the three selected antibiotics while *Bacillus subtilis* and *Staphylococcus aureus* were resistant to Erythromycin and Vancomycin. For Chandrabhan *et al.* (2012), antimicrobial sensitivity of seven isolates from dental caries showed that four were resistant to Vancomycin, Chloramphenicol, and Erythromycin. Maripandi *et al.* (2011), similarly reported that dental caries bacteria were resistant against Vancomycin, Chloramphenicol, Penicillin, Bacitracin and Streptomycin. This study therefore was necessitated by the facts that in Uyo, the study centre, there were little or no information about the predominant bacterial causes of dental caries, the pattern of antibiotic resistance and the genes involved in such.

MATERIALS AND METHODS

Study design and bacterial isolates. This was a descriptive cross-sectional hospital-based study. A hundred and twenty (120) swab samples from patients with dental caries attending the dental clinics of University of Uyo Teaching Hospital (UUTH) and Saint Luke's General Hospital Anua, were processed in the Medical Microbiology and Parasitology Laboratory of University of Uyo Teaching Hospital. Ethical approval for the study was gotten from both centres before commencing the study. Bacterial species were identified using standard laboratory methods, including Gram staining, biochemical test using Vitek 2 System (bioMe´rieux). culture was initially done on Brain Heart Infusion (BHI) broth incubated for 24 hours at 37^0C, those with evidence of growth were sub-cultured on Nutrient Agar (NA), Blood agar (BA), MacConkey agar (MAC) and Cystine Lysine

Electrolyte Deficient (CLED) and incubated at 37 ^{0}C over night for further processing (Cheesbrough, 2006).

Antimicrobial susceptibility tests. Antibiotic susceptibility test was done for all isolates using the Vitek 2 System (bioMe´rieux). The Vitek 2 System (bioMe´rieux) Gram-negative reagent cards (test kits) for antibiotic susceptibility tests consist of; Ampicillin, Ampicillin/Sulbactam, Piperacillin, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Trimethoprim/Sulfamethoxazole.

Interpretation of antibiotic susceptibility testing. Antibiotic susceptibility reactions of isolates were obtained within 12-18 hours of insertion of the antibiotic susceptibility test kits containing the isolates and interpretation based on turbidity was categorized as; 'Resistant' 'Intermediate' or 'Susceptible'. The isolates that were non-susceptible to at least one agent in three or more antimicrobial categories were regarded as multiple-drug-resistance (MDR) (Magiorakos *et al*., 2012). Control strains that were used to test the performance of the methods used in this research were *Staphylococcus aureus* ATCC 25923 for Gram-positive isolates, and *Escherichia coli* ATCC 25922 for Gram-negative isolates (Cheesbrough, 2006).

Molecular detection of antibiotic resistance genes of dominant isolates (B. cepacia) - Extended Spectrum Beta- Lactamases (ESBLs) detection. Gel electrophoresis was used for the detection of DNA by ultra-violet (UV) transilluminator. The polymerase chain reaction (PCR) assay was performed to detect the antibiotic resistance gene for *B. cepacia*. The primer used was designed by Alpha DNA company, Canada. Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 1µl of 10mg/ml ethidium bromide (Sigma, USA) and run at 80V for 1.5hr. A single band was observed at the desired position on UV transilluminator (Cleaver, UK), and bands photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products (Saladin *et al*., 2002, Ensor *et al.* 2009 and Dallenne *et al.,* 2010).

Amplification of resistance genes.

***Amplification of CTX-M genes*:** The CTX-M genes from the isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR: 5'-ACCGCGATATCGTTGGT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microliters for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 ^{0}C for 5 minutes; denaturation, 95 ^{0}C for 30 seconds; annealing, 52 ^{0}C for 30 seconds; extension, 72 ^{0}C for 30 seconds for 35 cycles and final extension, 72 ^{0}C for 5 minutes. The product was resolved on a 1 % agarose gel at 120 V for 25 minutes and visualized on a UV transilluminator for a 550 bp product size (Saladin *et al.*, 2002 and Roschanski *et al.,*2014).

Amplification of TEM genes: The TEM genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TTACCAATGCTTAATCAGTGAG-3' primers (Ensor *et al.* 2009 and Roschanski *et al.,*2014), on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microliters for 35 cycles. The PCR mix included: X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 ^{0}C for 5 minutes; denaturation, 95 ^{0}C for 30 seconds; annealing, 55 ^{0}C for 30 seconds; extension, 72 ^{0}C for 30 seconds for 35 cycles and final extension 72 ^{0}C for 5 minutes. The product was resolved on a 1% agarose gel at 120 V for 25 minutes and visualized on a UV transilluminator for a 400 bp product size.

Amplification of OXA genes: The TEM genes from the isolates were amplified using the OXA-1F: 5'- AGCCGTTAAAATTAAGCCC-3' and OXA-1R: 5'-CTTGATTGAAGGGTTGGGCG-3' primers (Dallenne *et al.,* 2010), on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microliters for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95

^{0}C for 5 minutes; denaturation, 95 ^{0}C for 30 seconds; annealing, 47 ^{0}C for 30 seconds; extension, 72 ^{0}C for 40 seconds for 35 cycles and final extension, 72 ^{0}C for 5 minutes. The product was resolved on a 1% agarose gel at 120 V for 25 minutes and visualized on a UV transilluminator for a 911 bp product size.

Statistical analysis. Statistical analysis was conducted using SPSS, version 22.0 (Chicago, IL, USA). Associations between variables were considered statistically significant at p-values less than or equal to 0.05 ($p \leq 0.05$).

RESULTS

Of the 120 plaque samples, 18 (15.0%) yielded growth of Gram-negative bacterial isolates, 9 (7.5%) yielded growth of Gram-positive bacterial isolates, while 93 (77.5%) yielded no significant growth on culture (Figure 1) (Udoh *et al*., 2025 and Onwuezobe *et al*., 2025). *Burkholderia cepacia complex* 7 (5.8%) were the dominant bacterial isolates (Udoh *et al*., 2025). *Klebsiella pnuemoniae* 1 (0.8%), *Acinetobacter spp* 1 (0.8%), *Enterobacter cloacae ssp dissolvens* 1 (0.8%), *Enterococcus faecalis* 1 (0.8%), *Enterococcus spp* (non *E. faecalis*) 1 (0.8%), *Pediococcus pentosaceus* 1 (0.8%), *Kocuria kristinae* 1 (0.8%) were the least dominant bacterial isolates. For antibiotic susceptibility pattern of the Gram-negative isolates, Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole were the most sensitive, while Ceftazidime was the most resistant. *B. cepacia complex* isolates were most sensitive to Amikacin and Tobramycin, and were most resistant to Ceftazidime (Table 1). Agarose gel electrophoresis of 16s gene of six *B. cepacia* isolates was carried out (Udoh *et al*., (2025). Lanes 1- 6 represent a 16s band (1500 bp), Lane M represents the 100 bp molecular ladder. The 16S rRNA of the isolates showed percentage similarity to other species at 100 %. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Burkholderia* sp (*Burkholderia cepacia* complex - Bcc), which revealed a closely relatedness to *Burkholderia cepacia* (4 isolates) and *Burkholderia cenocepacia* (2 isolates), as illustrated in Figure 2. Agarose gel electrophoresis showing the amplified resistance genes for CTX-M gene and TEM gene of six *B. cepacia* isolates. Lanes 1-6 represent a gene band; 550 bp for CTX-M genes (Figure 3) and 400 bp (Figure 4) for TEM genes. Lane M represents the 100 bp molecular ladder. The CTX-M

genes (6: 100%) was the dominant, TEM genes were only found in one B. cepacia isolate (1: 16.7%), while OXA genes was not present in all the six *B. cepacia* isolates (0: 0%).

Table 1: **Antibiotic susceptibility of dominant *B. cepacia* isolates**

Antibiotic	**Resistant (%)**	**Intermediate (%)**	**Sensitive (%)**
Ampicillin	1 (14.3)	5 (71.4)	1 (14.3)
Ampicillin/Sulbactam	1 (14.3)	4 (57.1)	2 (28.6)
Piperacillin	2 (28.6)	4 (57.1)	1 (14.3)
Cefazolin	4 (57.1)	2 (28.6)	1 (14.3)
Cefoxitin	1 (14.3)	4 (57.1)	2 (28.6)
Ceftazidime	5 (71.4)	1 (14.3)	1 (14.3)
Ceftriaxone	0 (0.0)	3 (42.9)	4 (57.1)
Cefepime	4 (57.1)	3 (42.9)	0 (0.0)
Ertapenem	1 (14.3)	5 (71.4)	1 (14.3)
Meropenem	3 (42.9)	3 (42.9)	1 (14.3)
Amikacin	0 (0.0)	2 (28.6)	5 (71.4)
Gentamicin	0 (0.0)	3 (42.9)	4 (57.1)
Tobramycin	0 (0.0)	2 (28.6)	5 (71.4)
Ciprofloxacin	0 (0.0)	3 (42.9)	4 (57.1)
Levofloxacin	1 (14.3)	4 (57.1)	2 (28.6)
Nitrofurantoin	0 (0.0)	4 (57.1)	3 (42.9)
Trimethoprim/Sulfamethoxazole	1 (14.3)	2 (28.6)	4 (57.1)

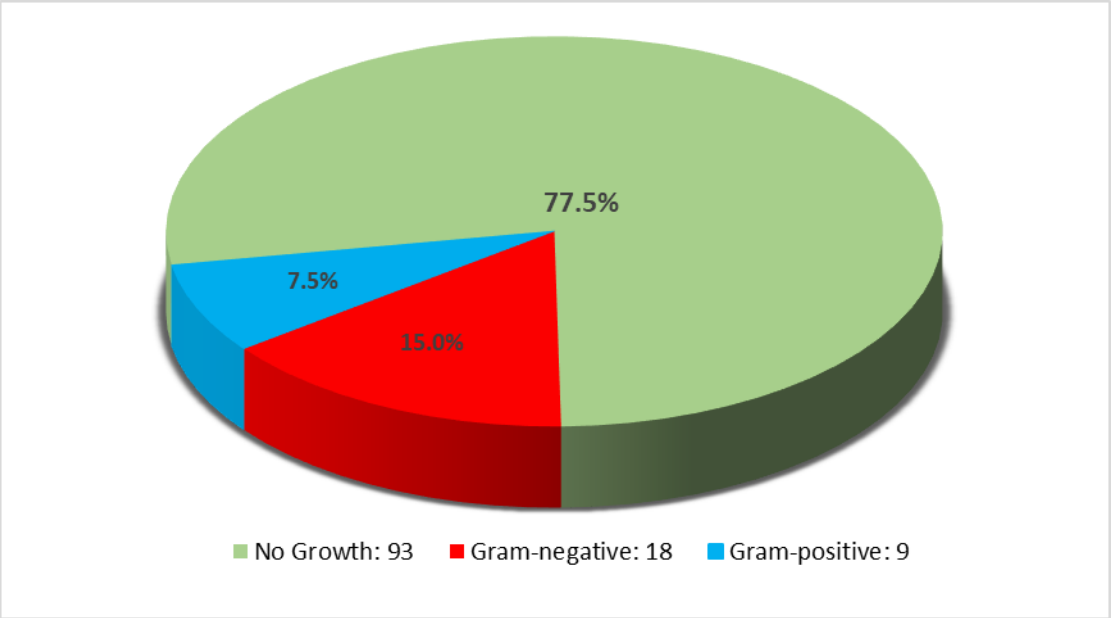


Figure 1: Distribution of isolates according to Gram staining reaction

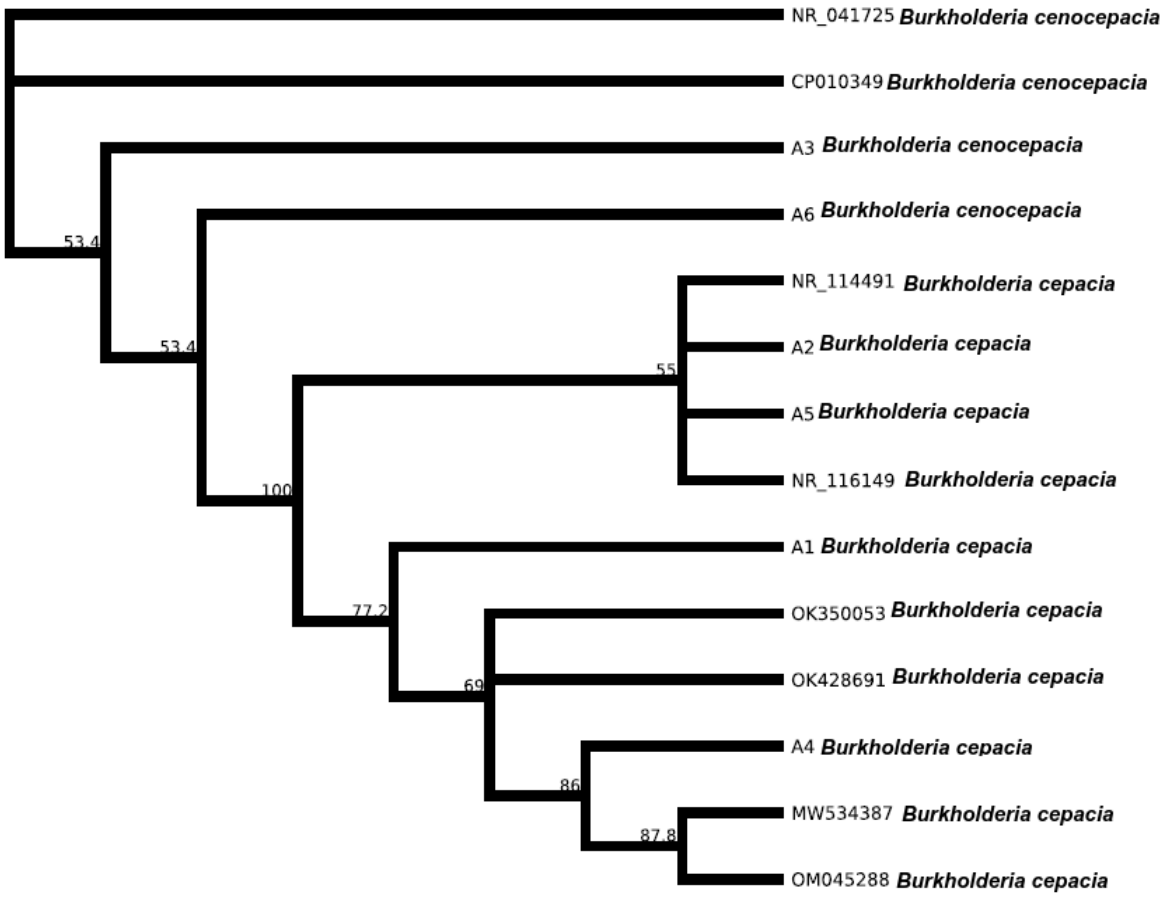


Figure 2: Evolutionary distances between six of the dominant bacterial isolates

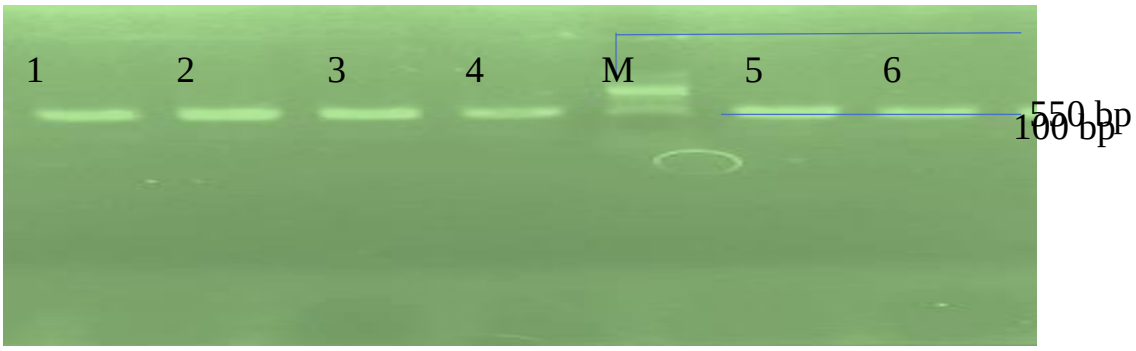


Figure 3: Detection of the CTX-M genes from six *B. cepacia* isolates

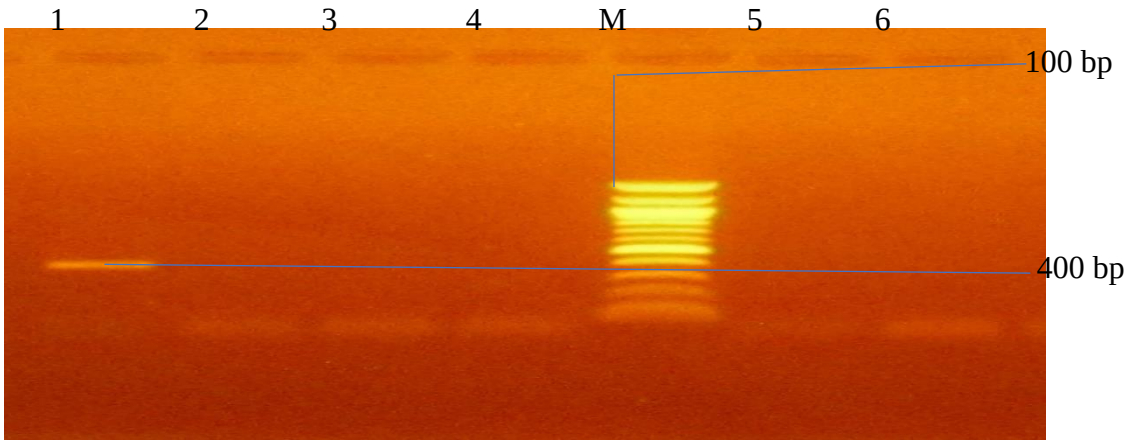


Figure 4: Detection of the TEM genes from six *B. cepacia* isolates

DISCUSSION

Out of the one hundred and twenty samples that were collected and processed, 27 isolates (22.5%) were obtained from culture; 9 (7.5%) were Gram-positive, 18 (15.0%) were Gram-negative, while 93 (77.5%) yielded no growth on culture (Figure 1). In Anejo-Okopi *et al.* (2015), of the 150 dental plaque samples obtained, 95 (63.3%) had dental caries. This shows that dental caries is more prevalent in Jos (Northern part of Nigeria) than in Uyo (Southern part of Nigeria), mostly due to the poor oral hygiene and nutritional status and/or habits of the participants.

The Gram-positive isolates comprised of various species such as; *Coagulase Positive Staphylococcus* 3 (2.5%), *Coagulase Negative Staphylococcus* 2 (1.7%), *Enterococcus faecalis* 1 (0.8%), Enterococcus *spp* (non-*E. faecalis)* 1 (0.8%), *Pediococcus pentosaceus* 1 (0.8%) and *Kocuria kristinae* 1 (0.8%). On other hand, the Gram-negative isolates included; *Burkholderia cepacia*

complex 7 (5.8%), *Proteus mirabilis* 4 (3.3%), *Serratia ficaria* 2 (1.7%), *Serratia marcescens* 2 (1.7%), *Klebsiella pnuemoniae* 1 (0.8%), *Acinetobacter spp* 1 (0.8%) and *Enterobacter cloacae ssp dissolvens* 1 (0.8%). These bacterial organisms ordinarily are oral flora which can become opportunistic pathogens when modifying factors are rife. This goes to confirm what other several researches have been reporting in which they have it that dental caries results from opportunistic bacterial oral flora (Subramonian *et al.*, 2016, Chandrabhan *et al.*, 2012 and Maripandi *et al.*, 2011).

In this study, *Burkholderia cepacia* group (also referred to as *Burkholderia cepacia complex* or *B. cepacia* or Bcc) was found to be the dominant bacteria causing dental caries 7 (25.9%). This is in contrast to reports from studies by Subramonian *et al.* (2016) and Jebashree *et al.* (2011), which had *S. mutans* as their dominant bacteria aetiology of dental caries. Furthermore, similar research by Corby *et* al. (2005), even revealed such bacterial species as *Streptococcus parasanguinis, Streptococcus mitis, Streptococcus oralis, Abiotrophia defectiva, and S. sanguinis* as the predominant indigenous bacteria flora causing dental caries. This notwithstanding, some previous studies, such as that by Anejo-Okopi *et al.* (2015) and Chhour *et al.* (2005), bacteria such as *Lactobacillus* species as their most prevalent isolate (28.4%).

Going forward therefore, it could be said that the finding of *Burkholderia cepacia* as the dominant bacteria in this research study, was not unconnected with the more sensitive and better molecular technique used Anejo-Okopi *et al.* (2015), as many routinely used none molecular method deplored by most laboratories in Nigeria, would have mistaken it for *Pseudomonas* species.

Worthy of note, is the fact that the dominant bacteria aetiology in this study - *B. cepacia* complex includes several species. Some of the common ones are; *Burkholderia cepacia, B. cenocepacia, B. multivorans, B. vietnamiensis, B. stabilis, B. pyrrocinia, B. ambifaria B. anthina, B. dolosa* and *B. ubonensis*. This species identification is usually achieved by carrying out 16S rRNA sequencing for molecular characterization/identification. Therefore, the agarose gel electrophoresis of the 16s gene of six of the dominant bacteria species (*B. cepacia complex*) of this study were used for 16S rRNA sequencing. The evolutionary distances which were computed using the Jukes-Cantor method, were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Burkholderia* sp (Figure 2).

It further revealed a closely relatedness to *Burkholderia cenocepacia* and *Burkholderia cepacia* - two *Burkholderia cenocepacia* and four *Burkholderia cepacia* (Figure 2). *Burkholderia cepacia* are opportunistic organisms (Mahenthiralingam *et al.,* 2008), while *B. multivorans* (genomovar II) and *B. cenocepacia* (genomovar III) are also common and may cause outbreaks (Drevinek and Mahenthiralingam, 2010 and Mahenthiralingam *et al.,* 2005). *B. cepacia* are commonly known to gain entry into the human body through cuts or openings such as the mouth, eyes, nose, ears and during the use of shared internal hospital equipment, such as those used for dental surgery, if adequate sterilization of the equipment was not achieved (as they have the ability to remain viable for a long period due to their minimal growth requirement).

Antibiotic susceptibility pattern of the isolates in this study revealed many isolates being resistant to at least two groups of antibiotics used. For Gram-positive isolates, resistance to Tetracycline, Quinupristin/Dalfopristin and Erythromycin were the highest with 77.8% for each of the tested antibiotic. On the other hand, the Gram-positive isolates were most sensitive to Levofloxacin with 77.8%. Gram-negative isolates showed most resistance to Ceftazidime with 66.7% resistance compared to other tested antibiotic. Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole were the antibiotic that the gram-negative bacteria were most sensitive to, with sensitivity of 33.3% for each of the three antibiotics used. The dominant isolates were most resistant to Ceftazidime 5 (71.4%), followed by other "Cef" antibiotics used (Table 1).

Six of the dominant bacteria species (*B. cepacia complex*) were further analysed for antibiotic resistance genes, due to their overwhelming level of antibiotic resistance compared to the other isolates in this research study. Six bla-CTX-M gene (100%) and One bla-TEM gene (16.7%) were seen, and no bla-OXA resistance gene present while viewing the electrophoresed agarose gels using a UV transilluminator. CTX-M resistance genes band were seen at 550 bp, TEM resistance gene band was found at 400 bp, while OXA resistance gene band was not found in the electrophoresed agarose gel of 100 bp molecular ladder used for its analysis (Figures 3 and 4).

The antibiotic resistance gene result obtained from this study is similar to some reports highlighted by some previous studies in which all the tested isolates had the bla-CTX-M resistance gene, some isolates were positive for the bla-TEM resistance gene while others were noted to have no or very few bla-OXA resistance gene in tested isolates.

Tuwaij *et al.* (2020), noted that all 16 (100 %) of *B. cepacia* carried bla-TEM and bla-CTX-M. 15 (93%) harboured bla-OXA gene, while 8 (50%) and 4 (25%) of isolates carried the bla-CTX-M-15 and bla-SHV-12 respectively, which helped in conveying antibiotic resistance on the organisms. According to Ogbolu *et al.* (2013), *B. cepacia* produce 100% beta-lactamase while 50% were ESBLs producers. 100% harboured bla-TEM gene and 50% carried bla-SHV and bla-CTX-M-15, while no isolate carried the bla-OXA gene (like in this study, with no bla-OXA gene).

CONCLUSION

The prevalence of dental caries, as well as the occurrence of *B. cepacia* complex as the dominant bacteria associated with caries among patients of the dental clinics of; University of Uyo teaching Hospital and Saint Luke's Hospital, Anua is worrisome especially as its occurrence seems to be higher than some of the documented instances. The bacteria being found in the mouth (in carious tooth) is a cause of more concern, as they can easily be transported to other parts of the body via the blood through the dental cavity. All the isolated bacteria, especially the Gram-negative isolates exhibited high levels of resistance against the commonly used antibiotics tested for, such as Ceftazidime and other tested cephalosporins. Mostly due to the fact that Gram-Negatives with ESBL resistance genes are capable of rapidly assuming varying forms. Although some researchers had stated that there is some degree of resistance conferred by bla-CTX-M gene, bla-TEM gene, bla-OXA genes, CTX-M-15, SHV-12 and VEB beta-lactamases.

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Author's Contributions in the Authorship of the Manuscript:

1. **Mary Athanasius Udoh**: substantial contributions to the conception and design of the work, patients' selection, acquisition of data, analysis and interpretation of data, drafting the work, final approval of the version to be published

2. **Ifeanyi Abraham Onwuezobe**: substantial contributions to the conception and design of the work, revising the work critically for important intellectual content, overall supervision, final approval of the version to be published

3. **Rasheedat Abdulkadir**: substantial contributions to design of the study, drafting the work, final approval of the version to be published

4**. Auwal Abubakar**: substantial contributions to design of the study, drafting the work, final approval of the version to be published.

5**. Musbau Adekunle Yahaya**: substantial contributions to design of the study, drafting the work, final approval of the version to be published.

6. **Daniel Oche Onah**: substantial contributions to design of the study, drafting the work, final approval of the version to be published.

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Issues This research study was approved by the Ethical Review Board of; Akwa Ibom State Ministry of Health and University of Uyo Teaching Hospital, all in Uyo Akwa Ibom State, Nigeria, and informed consent were given by participants in the study.

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