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Phenotypic Detection and Risk Factors of Bacteria Aetiological Agents of Dental Caries in Patients in Uyo Nigeria

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Abstract

Background. Dental caries is a highly prevalent oral disease that affects approximately 100% of individuals in the society. This disease causes severe damage to the teeth. Several acidophilic bacteria are implicated in the disease. This research was done to detect bacterial agents and risk factors implicated in caries in Uyo, Nigeria. **Methods**. A total of 120 dental plaque samples were cultured. Vitek 2 System (bioMe´rieux) was used for biochemical characterization and antibiotic susceptibility of isolates. **Results.** Several bacterial species (Thirteen) were obtained from significant culture growth of 27 (22.5%). *Burkholderia cepacia* group were 7(5.8%), and the most prevalent. *Enterococcus faecalis* 1(0.8%), *Enterococcus spp* (non *E. faecalis*) 1(0.8%), *Pediococcus pentosaceus* 1(0.8%), *Kocuria kristinae* 1(0.8%), *Serratia ficaria* 2(1.7%), *Klebsiella pneumoniae* 1(0.8%), *Acinetobacter spp* 1(0.8%) and *Enterobacter cloacae ssp dissolvens* 1(0.8%) were the least prevalent isolates. This study showed new bacteria

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species being implicated in caries, and high consumption of sugary foods being the main risk factor of caries. **Conclusions.** The identification of novel bacteria species in caries, and the attribution of staple foods as the lead risk factor of caries amongst dental caries patients in Uyo is a cause for alarm, as most of these people rely on carbohydrates for their daily food, as the soil of their farmlands are most favourable in yielding carbohydrate crops compared to other crops. Hence, there is need for frequent health enlightenment program, especially in the urban areas on adverse effect of frequent snacking and/or eating carbohydrates without rinsing the mouth frequently to rid it of food debris.

Keywords: Dental-Caries, Plaque, Risk-Factors, Sugars, Tooth

INTRODUCTION

Dental caries (also known to as cavities, tooth decay or caries) is an irreversible, non-communicable, multifactorial microbiological infectious disease of the calcified tissues of the tooth. This chronic oral infection (Chandrabhan *et al.,* 2012), is known to permanently damage previously healthy teeth through staining or development of holes (cavities), and may gradually lead to the inflammation of the gums if not detected and treated on time. This disease results most times due to poor oral hygiene, frequent snacking, frequent consumption of sugary foods or drinks, and deficiency in tooth minerals. This leads to the buccal cavity being acidic, and the oral microbiota becoming opportunistic pathogens.

As the cariogenic bacteria feed on sugary foods, they produce acid, and in turn gradually form deposits (plaque or biofilm) on the teeth, which eventually erodes the hard tissues of the teeth. In the newly created environment, they tend to flourish and build up biofilm on the susceptible tooth, causing the gradual breakdown of the teeth due to the acids produced by the bacteria during their metabolism. Caries usually cause mild to extreme discomfort or pain when hot or cold foods are consumed. The severe pain felt by patients make them to visit the dentist. Unfortunately, most times when severe tooth sensitivity is felt, tooth extraction is often advised, as the teeth are usually highly destroyed, and antibiotics would hardly have much impact on the highly decayed tooth.

Lamont and Egland (2015), confirmed caries to be one of the most ancient and frequent oral infections. They reported that its incidence sky rocked due to excess manufacturing of carbohydrates (sugars) products, especially by highly industrialized countries. It is also

reported that caries is less prevalent in children than adults. FDI (2017), Aas *et al.* (2008), Petersen *et al.* (2005), Kaste *et al.* (1991), Janakiram *et al.* (2018), and Udoh *et al.* (2025), are of the opinion that about 44 to 100% of adults are affected globally. Ritter *et al.* (2015), stated that various protective and modifying risk factors dictate caries onset and process. The risk/predisposing factors of caries are many, and are responsible for caries formation or its inhibition.

Keyes and Jordan (1963), reported that modifying factors that dictate caries formation are categorized into two; primary modifying factors and secondary modifying factors. The most important factors of caries are the primary modifying factors. This develop from interactions of cariogenic microbiota producing plaque/biofilm while feeding on fermentable carbohydrates on a tooth surface that is susceptible for a long time. pH in the biofilm becomes highly acidic and minerals (soluble calcium and phosphate) from the affected tooth is forced out (demineralization) to its enamel to try achieve equilibrium.

This process sometimes lasts for extended period of time, causing the destruction of the tooth. If the pH condition changes to neutral, then minerals are restored to the affected tooth (remineralization), that was partially demineralized (Featherstone 2003). Other factors that affects demineralization or remineralization process include; type and abundance of microbiota in the biofilm, oral hygiene, diet, genetics, anatomy of teeth, fluorides and other chemotherapeutic agents use, salivary flow and buffering capacity, among others. Equilibrium is reached if pathological factors (factors that lead to demineralization) is equal to protective factors (factors that lead to remineralization), as documented and illustrated in Featherstone (1999).

Anejo-Okopi *et al.* (2015), Chandrabhan *et al.* (2012), Maripandi *et al.* (2011), Tseng *et al.* (2014) among other researchers, had documented different species of microorganisms being implicated in dental caries. Some of the documented caries bacteria are; *Streptococcus species, Lactobacillus* species, *Enterobacter* species, *Bacillus subtilis, Staphylococcus aureus*, among others. In this study, *Burkholderia* cepacia complex (dominant species isolated), *Serratia species, Pediococcus pentosaceus*, *Kocuria kristinae*, *Enterobacter cloacae ssp dissolvens* were isolated among others. This shows that new species of bacteria keeps being implicated in caries, as shown in Udoh *et al.* (2025).

Researchers over the years documented that routine laboratory testing methods usually miss some bacteria implicated in caries (as seen in Udoh *et al.*, 2025), and that caries

bacteria are inherently resistant to commonly used antibiotics, such as Vancomycin, Chloramphenicol, Penicillin, Bacitracin, Streptomycin, Ampicillin, Ampicillin/Sulbactam, Piperacillin, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole, among others.

Therefore, there is need for improved laboratory testing methods and equipment, good oral hygiene practices coupled with fluoridation of the public water supply, along with the presence/use of fluoride in dentrifices, and the use of good mouth rinses which will help reduce prevalence of dental caries in populations.

MATERIALS AND METHODS

Study design and bacterial isolates

Study design used was descriptive cross-sectional hospital-based of 120 dental plaque samples of participants with caries in two government hospitals (Saint Luke's Hospital, Anua - General Hospital, and University of Uyo Teaching Hospital – UUTH) in Uyo, South-South, Nigeria. The ethical committee of; Akwa Ibom State Ministry of Health and UUTH, both in Uyo, Akwa Ibom, Nigeria, gave ethical approval for this study. Informed consent forms were issued to patients eligible for the study or their parents/guardians for patients below legal age prior to sample collection, for their written consent. Carefully structured questionnaires were made and given to participants to evaluate the risk factors of caries in Uyo. All personal data and results of participants were treated with confidentiality. The samples obtained were analysed/identified in Medical Microbiology and Parasitology Laboratory of UUTH using standard laboratory methods. Gram staining for primary categorization of isolates, and Vitek 2 System (bioMe´rieux) for biochemical test and antibiotic susceptibility test. Assessments of growth was initially done on Brain Heart Infusion (BHI) broth incubated for 24 hours at 37 ^{0}C, and the mixed isolates obtained were further sub-cultured into Nutrient Agar (NA), Blood agar (BA), MacConkey agar (MAC) and Cystine Lysine Electrolyte Deficient (CLED), and incubated at 37 ^{0}C over night to yield pure colonies and isolates differentiation (Cheesbrough, 2006).

Interpretation of antibiotic susceptibility testing

Antibiotic susceptibility results of isolates were obtained within 12-18 hours of insertion of the antibiotic susceptibility test kits into the Vitek 2 System (bioMe´rieux) as; 'Resistant' 'Intermediate' or 'Susceptible', based on their turbidity level. In this study, the isolates that were resistant to at least one agent in three or more antimicrobial categories were regarded as multiple-drug-resistance (MDR) (Magiorakos *et al.*, 2012). Control strains were used to test the performance of the methods used in this research. They are; *Escherichia coli* ATCC 25922 for Gram-negative isolates and *Staphylococcus aureus* ATCC 25923 for Gram-positive isolates (Cheesbrough, 2006).

Statistical analysis

Statistical analysis conducted was carried out 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

This study participants were 120 persons between the ages of 3 to 72 years. The 33-42 years (40; 33.3%) group had the highest number of participants. The female gender was 70 (58.3%). People in marital relationship were 84 (70%), while the employed ones were 80 (66.7%), and urban residents 90 (75%) area (Table 1). Lifestyle and oral hygiene of participants showed that they all experienced pain in the affected tooth before they sought dental care (Table 2). Table 2, further shows that people that brush their teeth daily can also be affected (can have tooth decay), if the teeth is not brushed at the right time and the right way. People that take sugary foods or drinks frequently were the most affected by caries as shown in Table 3. Of the 120 plaque samples cultured, there was no significant growth of 93 (77.5%), whereas there were 9 (7.5%) culture growth of Gram-positive bacterial isolates of 6 different species (Figure 1) and 18 (15.0%) Gram-negative bacterial isolates of 7 different species (Figure 2).

The Antibiotic susceptibility pattern showed Gram-positive isolates being mostly sensitive to Levofloxacin (7; 77.8%), and mostly resistant to Tetracycline, Quinupristin/Dalfopristin and Erythromycin with 77.8% each, while the Gram-negative isolates being mostly

sensitive to Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole, each having (6, 33.3%) and mostly resistant to Ceftazidime 12 (66.7%).

Table 1: R**isk factors of caries based on sociodemographic data (n = 120)**

Some Risk Factors of Caries	Groups Most Affected	Frequency	Percent (%)
Age	33-4	40	33.3
Gender	Female	70	58.3
Marital Status	Married	84	70.0
Employment Status	Employed	80	66.7
Residential Area	Urban	90	75.0
Level of education	Secondary	50	41.7

Table 2: **Lifestyle and oral hygiene history of participants (n = 120)**

History	**Frequency**	**Percentage (%)**
Do you have tooth cavity?		
Yes	120	100.0
No	0	0.0
Do you experience tooth pain?		
Yes	120	100.0
No	0	0.0
If yes, how long have you been having tooth pain?		
0-1 month	90	75.0
2-3 months	20	16.7
Above 3 months	10	8.3
Do you brush/clean your teeth?		
Yes	120	100.0
No	0	0.0
What do you use in brushing/cleaning your teeth?		
Toothbrush, toothpaste and water	90	75.0
Chewing stick only	5	4.2
Chewing stick and salt	10	8.3
Charcoal and water	2	1.7
Charcoal, salt and water	3	2.5
Salt and water	10	8.3
How often do you brush/clean your teeth?		
Once or Twice a day	87	72.5
Thrice a day	30	25.0
Sometimes	3	2.5

Table 3: **Lifestyle and nutritional/health history of participants (n = 120)**

History	Frequency	Percentage (%)
How often do you eat or take sugary foods?		
Everyday	40	33.3
More than thrice in a week	70	58.3
Rarely	10	8.3
How often do you drink alcohol?		
More than thrice in a week	24	20.0
Less than thrice in a week	96	80.0
How often do you take or smoke tobacco?		
More than thrice in a week	10	8.3
Less than thrice in a week	110	91.7
Do you have diabetes?		
Yes	8	6.7
No	112	93.3

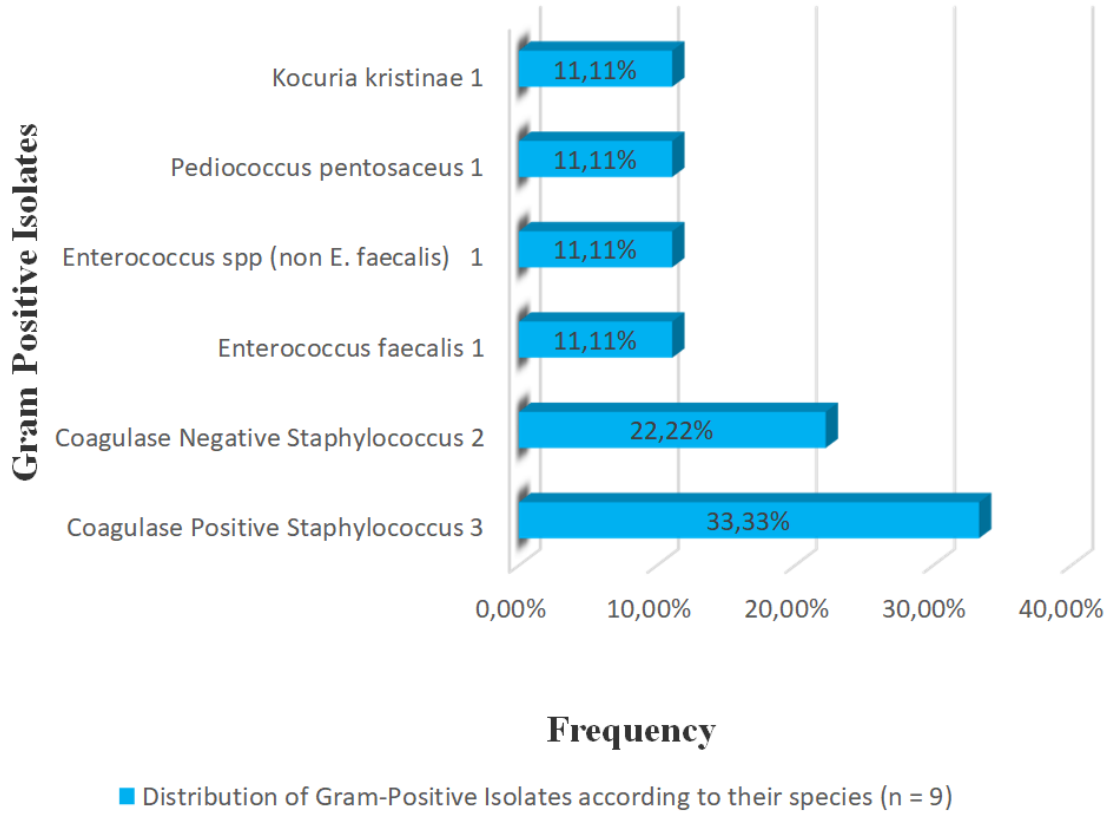


Figure 1: Distribution of Gram-positive isolates according to their species (n = 9)

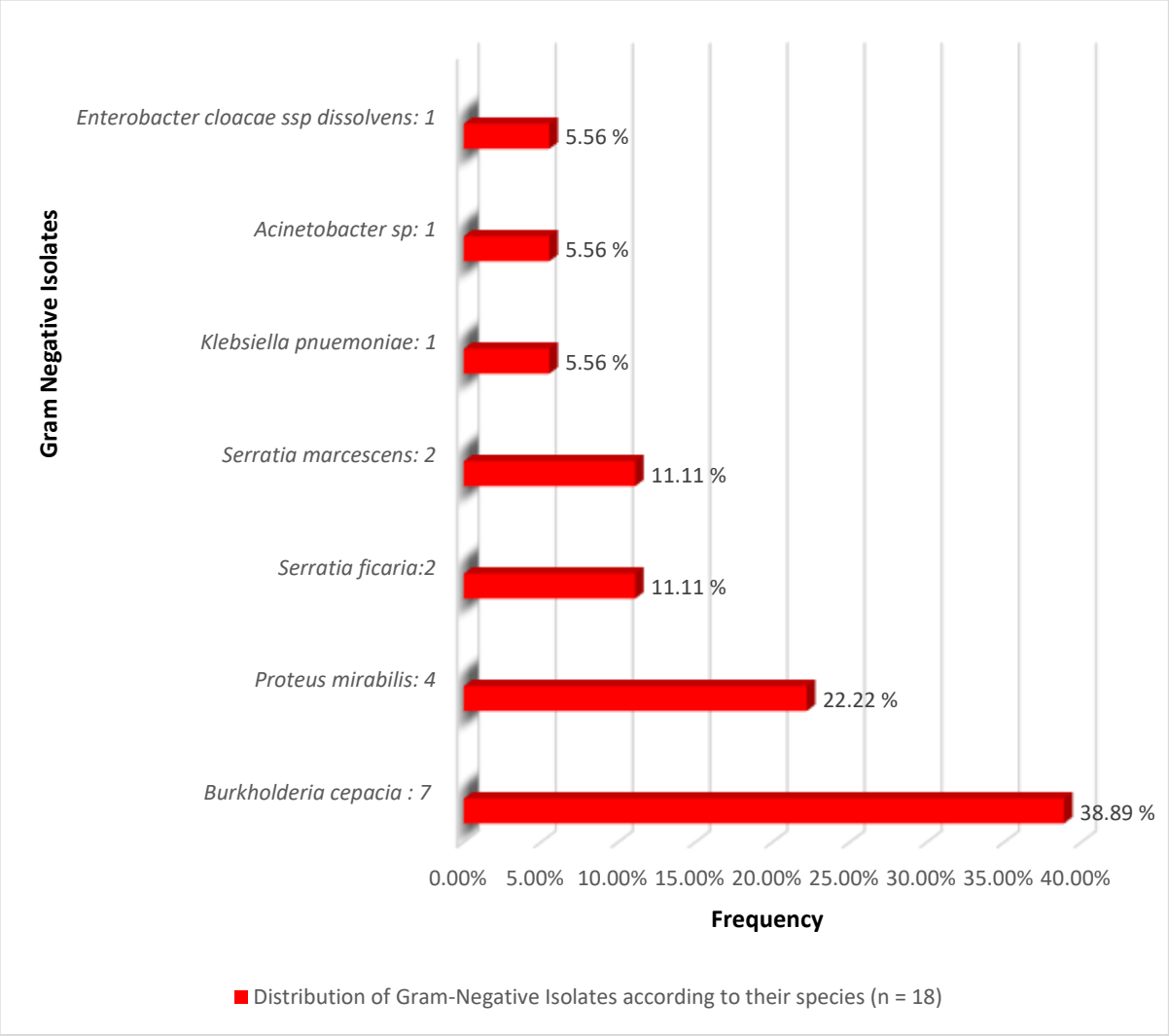


Figure 2: Distribution of Gram-Negative Isolates according to their species (n = 18)

DISCUSSION

Of the 120 samples (dental plaques) that were collected from participants in Uyo (Southern part of Nigeria) and cultured; 93 (77.5%) had no significant growth, and twenty-seven (22.5%) showed significant growth. Whereas in a similar research conducted by Anejo-Okopi *et al.* (2015) in Jos (Northern part of Nigeria) with 150 dental plaque samples, the total isolates obtained were 95 (63.3%). This proves that caries is more common in Jos than in Uyo. Several risks factors of caries were analysed and interpreted from the well-structured administered questionnaires given to participants. Excessive intake of carbohydrate rich foods/drinks, without the proper washing off of the leftovers (debris), was seen as the main cause of caries in this study. Phenotypic characterisations of the

bacterial growth from culture were studied and documented, and several laboratory tests and techniques were performed in the identification of the bacterial growth.

Gram staining was done, and isolates were of two Gram staining categories; 9 (7.5%) were Gram-positive and 18 (15.0%) were Gram-negative. Vitek 2 System machine was used for biochemical identification and antibiotic susceptibility testing. Gram-positive isolates identified were; *Coagulase Positive Staphylococcus* 3 (2.5%), *Coagulase Negative Staphylococcus* 2 (1.7%), *Enterococcus faecalis* 1 (0.8%), among others as shown in Figure 1, while Gram-negative isolates were; *Burkholderia cepacia complex* 7 (5.8%), *Proteus mirabilis* 4 (3.3%), *Serratia ficaria* 2 (1.7%), among others (Figure 2). This confirms the claim made by Maripandi *et al.*, 2011, Chandrabhan *et al.*, 2012, Subramonian *et al.*, 2016, among others, that bacteria implicated in caries keeps changing and increasing or declining due to several modifying factors that may enhance their abundance or inhibit their existence, and the laboratory techniques used for microbial detection.

In this study *Burkholderia cepacia* group is the dominant bacteria isolated (routine laboratory identification would have mistaken it for *Pseudomonas*), whereas researchers such as; Jebashree *et al.* (2011), Subramonian *et al.* (2016), among others documented *Streptococcus mutans* as bacteria of dominance. On the other hand, are researchers such as; Corby *et* al. (2005), Chhour *et al.* (2005), Anejo-Okopi *et al.* (2015) among others, who reported other species of bacteria to be dominant in caries. Anejo-Okopi *et al.* (2015) further stated that molecular techniques make it easier to identify more and less frequently identified groups of bacteria. Therefore, there is need for improved laboratory testing methods/techniques for appropriate and timely identification of bacteria, due to their ever-growing diverse nature.

In this study's antibiotic susceptibility testing, isolates showed great resistance to at least two groups of antibiotics used. Gram positive bacteria showed 77.8% resistance (highest) to Tetracycline, Quinupristin/Dalfopristin and Erythromycin, but most sensitive to Levofloxacin with 77.8%. whereas Gram-negative isolates showed 33.3% resistance (highest) to Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole, but most sensitive with 33.3% each to Tobramycin, Nitrofurantoin and Trimethoprim/Sulfamethoxazole.

CONCLUSION

Dental caries being a chronic disease that comes with so much pain, sometimes causes some patients to have low self-esteem due to teeth discolouration and/or tooth or teeth loss(es). The disease often comes with enormous financial burden. *B. cepacia* complex which is the dominant bacteria implicated in caries in Uyo, makes it more worrisome, as most routinely used laboratory techniques do not identify it. *B. cepacia* complex is also known to be highly resistant to commonly used antibiotics, which makes it worse. Frequent excessive consumption of sugary foods/drinks by people without immediate proper mouth rinsing or washing leads to caries formation. This can be avoided through frequent health awareness or campaigns on proper mouth washing and oral hygiene for the public. Government should provide improved standard laboratory testing equipment as the need arise, and train her laboratory health workers periodically to ensure that they are up to date with the use of the equipment, and the modern way of interpreting laboratory test results. Furthermore, due to the rise in antimicrobial resistance, self-medication is greatly discouraged to avoid more resistance of bacteria to antibiotics, as the process of formulating and producing new antibiotics takes a long time, and usually involves a lot of money.

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

2. **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
3. **Agantem Emmanuel Ekuma:** substantial contributions to the design of the work, revising the work critically for important intellectual content, final approval of the version to be published
4. **Mary Bondick Takon:** substantial contributions to the design of the work, revising the work critically for important intellectual content, final approval of the version to be published
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Declarations

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