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Research Article

Antibiotic Sensitivity Profiles of Bacteria Isolated from Decayed Teeth

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Abstract: Every tooth surface is susceptible to decay and the most vulnerable spots are the areas where bacteria can take refuge. This study was carried out to investigate the antibiotic susceptibility patterns of bacteria associated with tooth decay. Over a seven-week period (between April and June, 2012), twenty freshly removed decayed teeth from patients attending the dental clinic at Ijebu-Ode General Hospital, Ogun State, Nigeria were screened for dental pathogens. Teeth were removed from patients diagnosed for various dental ailments like dental caries, periodontitis, aveola abscess and gingivitis. Infected areas of teeth were swabbed and inoculated directly on different media by streaking method. Blood agar, centrimide agar, Simon's citrate agar, nutrient agar and nutrient broth were used for the isolation of associated dental pathogens. The isolates were further subjected to antibiotic susceptibility testing employing the disc diffusion technique. The bacterial isolates implicated were *Streptococcus mutans*, *Klebsiella pneumoniae*, *Staphylococcus albus*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The highest percentage frequency (53.13%) was shown by *Streptococcus mutans* while *Proteus vulgaris* and *Pseudomonas aeruginosa* showed the least percentage frequency of 6.25%. All isolates were sensitive to ciprofloxacin, gentamycin and norfloxacin while highest level of resistance was shown to chloramphenicol and tetracycline. Bacteria isolates were relatively susceptible to the antibiotics investigated. However, multiple drug resistance of some bacterial strains to chloramphenicol and tetracycline should be of interest.
Keywords: Bacteria, antibiotics, susceptible, tooth, decay.

INTRODUCTION

Tooth decay is caused by certain types of acid producing bacteria which cause damage in the presence of fermentable carbohydrates such as sucrose, fructose and glucose. Oral streptococci, which are the major members of oral flora, frequently cause bacteremia and infective endocarditis. In a healthy mouth, the pH is around 6.2 to 7.0. A pH of 7 is neutral. Thus, problem starts when the pH is less than 5.5. The tooth is now in an acid environment and starts to demineralise (lose calcium and minerals from the enamel). As the enamel loses its minerals, it starts to break down. In recent years, multiple drug resistance have been developed in both human and plant pathogenic microorganisms due to the indiscriminate use of commercial antibacterial drugs commonly used in the treatment of infectious diseases. It has been well established that oral health impacts overall health. As far back as 1926, Gies [1] described how "certain common and simple disorders of the teeth may involve prompt or insidious development of serious and possibly fatal ailments in other parts of the body", as causative bacterial pathogens could turn to be life-threatening. There is significant evidence that oral health complications have adverse health outcomes. Periodontal disease has been associated with low self-esteem, adverse pregnancy outcomes, increased risk of myocardial infarction, and other cardiovascular, respiratory, erectile, and diabetes complications [2-8]. Being able to recognize, prevent, and treat dental pathology, as well as improve access to preventive dental care, is paramount to improving the overall health of our nation, and this brings oral microbiology to a light of the day. Primary care health practitioners often encounter a variety of dental and oral complaints that can vary from minor annoyances to life-threatening emergencies. In order to appropriately treat and refer patients in both the primary and urgent care settings, it is essential for providers to have a solid understanding of basic dental anatomy. The basis for many dental complaints is dental caries or bacterial disease of the teeth. Teeth are made up of 3 layers: enamel, the hard outer layer; dentin, the core structure; and the pulp chamber at the center, which contains blood vessels and nerves [9]. After eating, a combination of bacteria, acid, food debris, and saliva, collectively referred to as plaque, begins to build up on the teeth. If not removed with regular brushing, flossing, and rinsing, the acidic nature of plaque begins to dissolve tooth enamel, creating dental caries [10]. Some of the symptoms of dental caries may be pain after consuming sweet, hot, or cold foods and drinks or visible holes in the teeth [11].Once the decay has reached the pulp, this is referred to as pulpitis. Douglass and Douglass [9] differentiate reversible from irreversible pulpitis. Reversible pulpitis is the result of mild tooth pulp inflammation. It is triggered by an offending stimulus (hot, cold, or sweet foods or drinks) and lasts only for a few seconds. If the carious lesion is left untreated, irreversible pulpitis will result. Once the progression to irreversible puplitis occurs, pain is persistent, poorly localized, and not just a response to noxious stimuli. The treatment for irreversible pulpitis is pain control and dental referral for root canal or extraction of the tooth. Apical periodonitis, or inflammation around the apex of the tooth, is the result of a severely inflamed pulp that has become necrotic. If apical periodonitis becomes localized and purulent, it is referred to as an apical abscess. The accurate description and ability to concisely describe dental and oral anatomy requires some basic knowledge of dental nomenclature. Providers should be familiar with the following terms [12].

- Mandibular—refers to the lower jaw or mandible
- Maxillary—refers to upper jaw or maxilla
- Primary or deciduous—can be used interchangeably to describe baby teeth
- Succedaneous—permanent teeth
- Labial—toward the lips
- Buccal—toward the cheek
- Facial—labial and buccal collectively
- Lingual—toward the tongue
- Mesial—Vertical tooth surface toward front of jaw and midline
- Occlusal—premolar and molar surfaces that come in contact with the teeth in the opposite jaw during closure
- Incisal—surfaces of incisors and canines that come in contact with the teeth in the opposite jaw

In addition to basic dental anatomy, health practitioners should be familiar with the names of different types of teeth and how they are arranged in both deciduous and permanent dentition. There are eight kinds of teeth: central incisors, lateral incisors, canines (cuspids), first premolars (first bicuspid), second premolar (second bicuspid), first molars, second molars, and third molars. There are twenty deciduous teeth: four central incisors, four lateral incisors, four canine (cuspids), four first molars, and four second molars. In permanent dentition, there are also four premolars (first bicuspid), four second premolars (second bicuspid), and four third molars, for a total of thirty-two teeth [12].This study, therefore, provides experimental information on the bacteria associated with tooth decay and the susceptibility of the oral pathogenic isolates to certain antibiotics.

MATERIALS AND METHODS

Isolation of bacteria

The test organisms were isolated directly from freshly removed infected teeth of twenty patients visiting the dental clinic at Ijebu-Ode General Hospital, Ogun State, Nigeria. They were diagnosed for various dental ailments like dental caries (ten), periodontitis (five), aveola abscess (three) and gingivitis (two). The infected areas of the teeth were swabbed with sterile cotton wools to remove debris and saliva, and were inoculated directly on different media by streaking method. *Streptococcus mutans*, *Pseudomonas aeruginosa, Staphylococcus albus, Proteus vulgaris* and *Klebsiella pneumonia* were isolated on blood agar, centrimide agar, Simon's citrate agar, nutrient agar and nutrient broth, respectively. The plates were incubated at 37°C for 24-48 hour. Characteristic colonies were picked from the plates and purified by repeated sub-culturing. Pure colonies were streaked on nutrient agar slopes in McCartney bottles incubated at 37^0C for 24 h. These slants were used as stock cultures and were stored in the refrigerator.

Characterization of bacterial isolates

Pure cultures of bacteria isolated were characterized and identified on the basis of their biochemical properties. Carbohydrates fermentation tests were also carried out on isolates according to the method of Benson [13].

Antibiotic Susceptibilty Testing

The susceptibility of isolates to various conventional antibiotics was determined using Mueller-Hinton agar (Oxoid Ltd, UK) while employing the disk diffusion method in accordance with the guidelines of the National Committee for Clinical Laboratory Standards[14]. The antibiotics used in this study include Amoxycillin (Amx), Chloramphenicol (Chl), Ciprofloxacin (Cpx), Cloxacillin (Clo), Cotrimoxazole (Cot), Erythromycin (Ery), Gentamycin (Gen), Norfloxacin (Nfx), Rifampicin (Rfp), Streptomycin (Str) and Tetracycline (Tet). The commercial antibiotic disks were introduced on to agar plates previously seeded with 18 h-broth cultures of the test organisms. The plates were incubated at 37^0C for 48 h. The inhibition zones were measured, scored as sensitive, intermediately susceptible and resistant according to the NCCLS recommendations.

RESULTS AND DISCUSSION

In this study, five genera of bacteria were implicated to be directly associated with the various dental diseases and these include *Streptococcus, Staphylococcus, Pseudomonas, Klebsiella* and *Proteus* (Table 1).

Streptococcus mutans, Klebsiella pneumoniae, Staphylococcus albus, Proteus vulgaris and *Pseudomonas aeruginosa* were directly associated with dental caries (Table 2). Furthermore, it was established that *Streptococcus mutans, Staphylococcus albus* and *Proteus vulgaris* were responsible for periontitis. *Streptococcus mutans* was the only implicated bacterium in the case of gingivitis while *Streptococcus mutans* alongside *Staphylococcus albus* were directly associated with *Aveola abscess* (Table 2).

Table 1: Biochemical characteristics of bacterial isolates from decayed teeth

Tests		Most probable isolates				
		Streptococcus mutans	*Staphylococcus albus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Proteus vulgaris*
Biochemical Reactions and	Gram's stain	+	+	-	-	+
	Shape	C	C	R	R	R
	Motility	-	-	+	-	+
	Catalase	-	+	+	-	+
	Oxidase	+	-	+	+	+
	Urease	-	-	-	+	+
	Coagulase	+	+	-		-
	Indole	+	-	-	-	+
	Citrate utilization	-	+	+	-	+
Sugar Utility	Fructose	A	NR	A	NR	NR
	Lactose	NR	AG	A	AG	AG
	Arabinose	A	AG	NR	A	A
	Sucrose	A	AG	A	AG	AG
	Malatose	A	A	A	NR	NR

Keys: A =Acid Production; R = Rods; G= Gas production; C = Cocci; A/G=Acid/Gas production, + = Positive, - = Negative, NR= No reaction

Table 2: Composite table relating the occurrence of dental diseases and the implicated bacteria

Dental diseases	Case number	Implicated organisms
Dental caries	10	*Streptocococcus mutans* *Klebsiella pneumonia* *Staphylococcus albus* *Pseudomonas aeruginosa*
Periontitis	5	*Streptocococcus mutans* *Staphylococcus albus* *Proteus vulgaris*
Gingivitis	3	*Streptocococcus mutans*
Aveola abscess	2	*Streptocococcus mutans* *Staphylococcus albus*

Table 3 shows the percentage frequency of the bacterial isolates with associated dental diseases. *Streptococcus mutans* was the common organism associated with the four dental diseases with the highest percentage frequency of 53.13%. This was followed by *Staphylococcus albus* (25%), *Klebsiella pneumoniae* (9.34%). However, *Proteus vulgaris* and *Pseudomonas aeruginosa* showed the same percentage frequency of 6.25% indicating that they are seldom associated with dental diseases (Table 3).

Table 3: Percentage frequency of bacterial isolates with associated dental diseases

Bacterial species	Dental diseases	Occurrence of the Bacteria	Percentage occurrence of the bacteria
Streptococcus mutans	dental caries, periodontitis and gingivitis	17	53.13%
Staphylococcus albus	dental caries and aveola abscess	8	25%
Proteus vulgaris	dental caries	2	6.25%
Klebsiella pneumonia	dental caries	3	9.34%
Pseudomonas. Aeruginosa	dental caries	2	6.25%

There were variations in the susceptibility patterns of the isolates to the various antibiotics. All the isolates were found to be sensitive to ciprofloxacin, gentamycin and norfloxacin. *Streptococcus mutans, Staphylococcus albus* and *Pseudomonas aeruginosa* were resistant to amoxicillin. *Proteus vulgaris* was extremely resistant to chloramphenicol and tetracycline as shown by no zones of inhibition. Only *Staphylococcus albus* was sensitive to tetracycline, *Klebsiella pneumoniae* was intermediately susceptible while others were found resistant. None of the isolates was sensitive to chloramphenicol; *Streptococcus mutans, Staphylococcus albus* and *Klebsiella pneumoniae* were intermediately susceptible while the other two were resistant (Table 4).

Table 4: Antibiotic susceptibility patterns of bacterial isolates associated with tooth decay

Isolates	Diameter of zones of inhibition (mm) to different antibiotics								
	Amx	Chl	Clo	Cot	Cpx	Ery	Gen	Nfx	Str	Tet
S. mutans	8± 0.5	12±1.0	16± 1.0	13±1.0	19±1.2	15±0.9	20±1.4	16±1.2	16± 1.0	6± 0.3
S. albus	7± 0.1	14±0.3	6 ± 0.2	8 ± 0.3	17±1.0	12±0.5	17± .5	15± 0.9	17± 1.2	16± 1.0
P. aeruginosa	6± 0.2	2 ± 0.0	14± 1.0	5 ± 0.2	16±1.2	17±1.4	21± .0	19± 2.0	20 ± 1.8	2 ± 0.1
K. pneumonia	23± 0.4	10± .7	13± 0.8	12±0.6	21±1.5	19±1.5	28±2.0	19± 1.4	20 ± 1.5	11± 0.7
P. vulgaris	18±1.0	0.0	15± 0.5	16±1.5	19±2.0	18±1.5	23±1.6	17± 1.6	12 ± 0.7	0.0

Keys: Amoxycillin (Amx), Chloramphenicol (Chl), Ciprofloxacin (Cpx), Cloxacillin (Clo), Cotrimoxazole (Cot), Erythromycin (Ery), Gentamycin (Gen), Norfloxacin (Nfx), Rifampicin (Rfp), Streptomycin (Str) and Tetracycline (Tet), ≤ 8 = Resistant, 9 to 14 = Intermediately susceptible, ≥ 15 = Sensitive

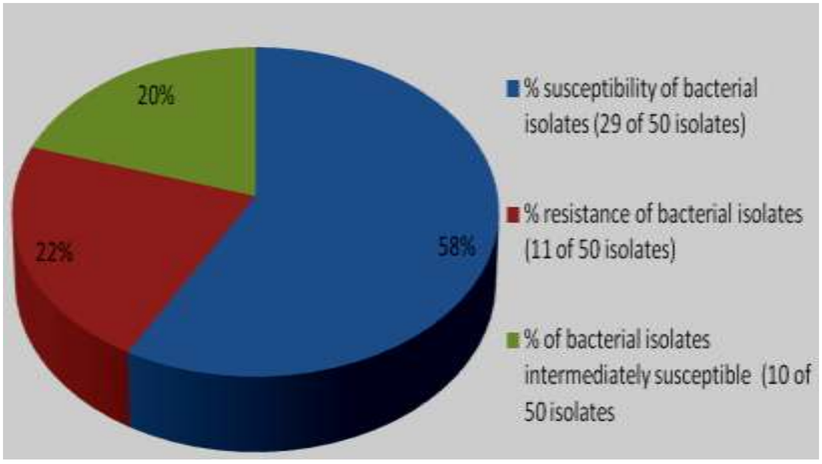

Fig. 1: Percentage antibiotic susceptibility profiles of bacterial isolates from decayed teeth in Ogun State, Nigeria

The percentage sensitivity of the isolates to the antibiotics was 58% (29 of 50 isolates), percentage resistance 22% (11 of 50 isolates) while the remaining 20% occupies intermediately susceptible isolates (10 of 50) (Fig. 1).

An investigation by Akinwande *et al.* [15] reported that in Nigeria out of 100% of cases, 63.7% were of carious origin while 26% and 10% were of periodontal and traumatic origin respectively. The impact of several different classes of medications and the effects that those medications had on oral health had been described [16]. A number of methods are used in oral hygiene to prevent and cure oral diseases. It is of pertinent importance to look at the role plants play in oral hygiene as a number of them have medicinal properties [17]. In many African homes, teeth are cleaned in the morning by chewing the root or slim stem of certain plants until they acquire brush-like ends [18, 19]. Bello *et al.* [10] in their study on used toothbrushes mentioned that sharing of toothbrushes could result in an exchange of body fluids and/or microorganisms between the users of the toothbrush, placing the individuals involved at an increased risk for dental infections. They mentioned that the practice should be of particular concern for persons with compromised immune systems or existing infectious diseases. Fluoride therapy is often recommended to prevent against dental caries [17]. It has been said that fluoride makes enamel more resistant to demineralization, and thus resistant to decay [19]. But according to Nagel [20], fluorides is a deadly poison, it makes the teeth brittle, as well as all the bones in the body, destroys liver over time and causes dental fluorosis. Reuniting the practice of mouth care with a body of scientific findings may be further accomplished by a shift towards realist-driven, experiential forms of inquiry [21-23].

CONCLUSION

It was concluded in this study that *Streptococcus mutans*, *Staphylococous albus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* were directly associated with the establishment of dental infections. It was further concluded that bacteria isolates were relatively susceptible to the antibiotics investigated in this study and could be adopted in the treatment of related infections. However, multiple drug-resistance patterns of some strains of bacterial isolates to chloramphenicol and tetracycline should be of interest.

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