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ORIGINAL ARTICLE

Susceptibility Pattern of Bacterial Isolates from Dental Caries Patients Attending Clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria

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ABSTRACT

This study was carried out to evaluate the antibiotic susceptibility pattern of bacterial isolates from dental caries patients attending the clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria. A total of 223 bacteria samples (*Streptococcus mutans* = 151; *Streptococcus sobrinus* = 36; *Lactobacillus acidophilus* = 22; *Streptococcus salivarius* = 10; *Streptococcus mitis* = 4) were collected from the patients. Antimicrobial sensitivity testing was done by single disc agar diffusion method on 24 antibiotics; selected into eight different groups of 3 according to action, community usage, and generation. The average group susceptibility of antibiotics to all bacterial isolates were 25.71%, 53.81%, 13.75%, 32.74%, 10.76%, 8.52%, 0.60% and 64.42% for group 1 to 8 respectively. Specifically, the most potent antibiotic in the different groups of antibiotics was **Amoxicillin (42.60%), Unasyn (78.03%),** Chloramphenicol (37.67%), Erythromycin (74.44%), Streptomycin (28.70%), Cefotaxime (18.39%), Pefloxacin (1.79%) and Clindamycin (96.41%). There was total resistance of all isolates to Cotrimoxazole, Neomycin, Ciprofloxacin, and Ofloxacin. The overall sensitivity of each isolated bacterial to the 24 antibiotics was 26.27%, 26.62%, 22.73%, 32.50%, and 28.13% for Strep. mutans, Strep. sobrinus, L. acidophilus, Strep. salivarius and Strep. mitis respectively. Considering the overall low sensitivity of dental caries isolates to the overall 24 antibiotics, there is a need for antibiotic susceptibility screening before an antibiotic prescription for the treatment of dental caries.

INTRODUCTION

Worldwide approximately 36% of the world population presents with dental caries in permanent teeth [1] and the World Health Organization estimated that about 60-80% of children and nearly all adults have dental caries at some point in time [2-4] and can affect 620 million baby teeth [1]. The treatment of dental caries is expensive; for both governments of developed and developing countries, exceeding the cost of treating cardiovascular disease, cancer, and osteoporosis [4]. Dental caries has become more common in both children and adults in recent years [5] and a person experiencing caries may not be aware [6]. Oral diseases are now public health problems worldwide and their impact in terms of pain and suffering, functional impairment and reduced quality of life is considerable [2,6]. In fact, in most industrialized countries, oral diseases have become the fourth most expensive to treat [2] as untreated dental caries results in worldwide productivity losses in an estimated size of about US $27 billion yearly [4].

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The chemoparasitic caries theory states that bacteria inhabit the mouth and produce acids that dissolve tooth structures in the presence of fermentable carbohydrates [7]. Cariogenic bacteria that can ferment carbohydrates to produce acid and further demineralize the tooth surfaces are the primary etiologic agents of dental caries [8-10]. The oral cavity is a unique ecological niche of microorganisms, most of which accumulate on dental surfaces to form dental plaque (oral biofilm) [11] and thus, bacteria (dental plaque) are considered the primary factor among caries aetiologic factors. Subsequently, antibiotics have been used to meet the challenges posed by bacterial infections in clinical and pharmacological research [12-15] and the prevention or treatment of dental caries [16,17]. Resistance of numerous bacterial pathogens to many antibiotics continues to increase worldwide [18] and the development of multi-drug resistance to antibiotics is a challenge in the use of antibiotics; their implication and their elimination is even a more difficult challenge to surmount [19]. Also further exacerbating the antibiotics challenges are the frequencies, pattern, and distribution of extensive resistant bacterial diversity with geographic regions and often reflect the patterns of antibiotics usage [20], antibiotic abuse by non-professionals, and its inappropriate application by professionals [19].

A shift to broad-spectrum antibiotics which are a decisive treatment for dental caries is preferred by Dentists which according to Al Haroni and Skaug [21] is due to the increase of bacterial isolates resistant to narrow-spectrum antibiotics. Although antibiotics susceptibility patterns of bacterial isolates are important for the determination of appropriate empirical treatment for dental caries, Roy [22] reported that culture and susceptibility testing to aid diagnosis and the rational choice of antibiotics for dental caries management often do not precede prescription for dental infection. This means that antibiotics are being prescribed for a range of dental infections for which they may not be required. The goal of antimicrobial susceptibility testing is to predict the *in vitro* success or failure of antibiotic therapy. Susceptibility testing is usually done or called for when there is a failure of prescribed drug therapy [22]. This study was carried out to evaluate the antibiotic susceptibility pattern of bacterial isolates from dental caries patients attending the clinic at Irrua Specialist Teaching Hospital, Irrua, Nigeria.

MATERIALS AND METHODS

Study population

Samples for the study were obtained from patients attending dental clinics in Irrua Specialist Teaching Hospital (I.S.T.H), Irrua in Esan Central Local Government Area of Edo State, Nigeria which lies at Latitude 6.45°N and Longitude 6.15°E. This hospital is a tertiary health facility.

Study samples

The bacteria samples were bacteria-positive samples of patients attending the clinic for dental problems at a health facility. Three hundred and forty samples (340) of suspected cases of dental caries were collected but 223 samples were positive for bacteria colonization.

Ethical clearance

Approval for the study was obtained from the Health Research Ethics Committee of the Ambrose Alli University, Ekpoma with the assigned number: 10/17 (001/17).

Antibiotic susceptibility test

The susceptibility of the bacteria isolates to antibiotics was determined by the single-disc agar diffusion method as previously described by Cruichank, et al. [23] and Ochei and Kolhatkar [24]. Briefly, the antimicrobial sensitivity test was carried out aseptically. Discrete colonies of test organisms were inoculated aseptically into numbered bijou bottles containing 4ml of nutrient broth. The straight wire was repeatedly sterilized for each test organism as well as for the control organism. Each bijou bottle was covered and shaken lightly for proper emulsification of the test organism. 0.5 Mcfarland standard was used to standardize the inoculum to the density of bacterial suspension of 1.5×10^8 (CFU/ml). Streptococci isolates were seeded on chocolate agar, while Lactobacilli species were seeded on Mueller Hinton agar for antimicrobial sensitivity testing. The emulsified and standardized test organism from each bijou bottle were then used to seed three sets of antimicrobial sensitivity plates per test organism. The plates were generally rocked with both hands, for proper seeding with the test organism. Excess broth from the plates was discarded into decontaminating jar. The Petri dishes were immediately covered and left for 30 minutes for excess broth to run out of the surface of the agar.

The test plates were inoculated anaerobically at 37°C for 24-48hrs, observed for their sensitivity patterns, and recorded. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) methodology. Furthermore, before the commencement of the experiment, control for the antibiotics was carried out against *Staphylococcus aureus* ATCC 25923 at 37°C for 24-48hours. The antimicrobial sensitivity plates were also controlled before usage by sterility test as described by Ochei and Kolhatkar [24].

The selection of antibiotics for this study was done by dividing 24 antibiotics into eight different groups of 3 antibiotics per group, according to their mode of action, similarities, community usage, and generation of discovery as previously documented in Orhue [25] and Orhue, et al. [26]. Group 1 (Ampicillin, Amoxicillin, and Imipenem), group 2 (Augmentin, Unasyn and Flucloxacillin), group 3 (Chloramphenicol, Tetracycline, and Cotrimoxazole), group 4 (Erythromycin, Clarithromycin, and Azithromycin), group 5 (Gentamycin, Streptomycin and Neomycin), group

6 (Cephalexime, Cefuroxime, and Cefotaxime), group 7 (Ciprofloxacin, Pefloxacin, and Ofloxacin) and group 8 (Metronidazole, Lincomycin, and Clindamycin). In cases where the commercially prepared antibiotic discs were not available, the discs were personally prepared following the procedures outline by Cruickshank, et al. [23].

Data analysis

Data were analyzed using the Statistical Package for Socio Sciences (version 17) and where applicable the simple descriptive statistics were carried out. Results were then presented in suitable tables and charts for summarization and simplicity.

RESULTS

Tables 1-4 are the antibiotics susceptibility pattern of bacterial isolates from dental caries patients attending the dental clinic at Irrua Specialist Teaching Hospital. Overall, the percentage average group susceptibility of antibiotics to all bacterial isolates were 25.71%, 53.81%, 13.75%, 32.74%, 10.76%, 8.52%, 0.60% and 64.42% for group 1 to 8 respectively. Specifically, the most potent antibiotic in the different groups of antibiotics was **Amoxicillin (42.60%) for group 1, Unasyn (78.03%) for group 2,** Chloramphenicol (37.67%) **for group 3**, Erythromycin (74.44%) **for group 4**, Streptomycin (28.70%) **for group 5**, Cefotaxime (18.39%) **for group 6**, Pefloxacin (1.79%) **for group 7** and Clindamycin (96.41%) **for group 8**. There was total resistance of all isolates to Cotrimoxazole, Neomycin, Ciprofloxacin, and Ofloxacin.

Strep. mutans isolates were most susceptible to Clindamycin (96.0%), Metronidazole (82.1%), **Unasyn (76.8%),** Erythromycin (72.8%), **Augmentin (66.2%), and Amoxicillin (55.6%) but were 100% resistant to** Cotrimoxazole, Gentamycin, Neomycin, Cephalexin, Ciprofloxacin, and Ofloxacin. *Strep. sobrinus* isolates were most susceptible to Clindamycin (100%), Metronidazole (94.4%), Augmentin (88.9%), Erythromycin (83.3%),

Table 1: Group 1 and 2 antibiotics susceptibility pattern of bacterial isolates from dental caries patients attending the dental clinic at Irrua specialist teaching hospital.

			Group 1			Group 2		
Bacterial isolates	No of isolates	% of isolates	Ampicillin	Amoxicillin	Imipenem	Augmentin	Unasyn	Flucloxacillin
			10µg/disc	25µg/disc	10µg/disc	30µg/disc	20µg/disc	10µg/disc
Strep. mutans	151	67.7	18(11.9%)	84 (55.6%)	42 (28%)	100 (66.2%)	116 (76.8%)	15 (9.9%)
Strep. Sobrinus	36	16.1	2 (5.6%)	8 (22.2%)	9 (25%)	32 (88.9%)	28 (77.8%)	4 (11.1%)
L. acidophilus	22	9.9	0 (0%)	0 (0%)	0 (0%)	18 (81.8%)	16 (72.7%)	2 (9.1%)
Strep.Salivarius	10	4.5	0 (0%)	2 (20%)	4 (40%)	8 (80%)	10 (100%)	2 (20%)
Strep. Mitis	4	1.8	0 (0%)	1 (25%)	2 (50%)	4 (100%)	4 (100%)	1 (25%)
			8.97%	42.6	25.56	72.65%	78.03%	10.76%
Mean %			25.71%			53.81%		

Table 2: Group 3 and 4 antibiotics susceptibility pattern of bacterial isolates from dental caries patients attending the dental clinic at Irrua specialist teaching Hospital.

			Group 3			Group 4		
Bacterial isolates	No of isolates	% of isolates	Chloramphenicol	Tetracycline	Cotrimoxazole	Erythromycin	Clarithromycin	Azithromycin
			30µg/disc	30µg/disc	30µg/disc	10µg/disc	15µg/disc	15µg/disc
Strep. mutans	151	67.7	48 (31.8%)	3 (1.98%)	0 (0%)	110 (72.8%)	22 (14.6%)	20 (13.2%)
Strep. sobrinus	36	16.1	18 (50%)	0 (0%)	0 (0%)	30 (83.3%)	3 (8.3%)	2 (5.6%)
L.acidophilus	22	9.9	12 (54.5%)	5 (22.7%)	0 (0%)	16 (72.7%)	0 (0%)	0 (0%)
Strep.salivarius	10	4.5	4 (40%)	0 (0%)	0 (0%)	8 (80%)	0 (0%)	6 (60%)
Strep. mitis	4	1.8	2 (50%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)
			37.67%	3.59%	0%	74.44%	11.21%	12.56%
Mean %				13.75%			32.74%	

Table 3: Group 5 and 6 antibiotics susceptibility pattern of bacterial isolates from dental caries patients attending the dental clinic at Irrua dpecialist teaching hospital.

			Group 5			Group 6		
Bacterial isolates	No of isolates	% of isolates	Gentamycin	Streptomycin	Neomycin	Cephaleximе	Cefuroximе	Cefotaximе
			10µg/disc	30µg/disc	30µg/disc	30µg/disc	30µg/disc	30µg/disc
Strep.Mutans	151	67.7	0 (0%)	36 (23.8%)	0 (0%)	0 (0%)	10 (6.6%)	25 (16.6%)
Strep.sobrinus	36	16.1	0 (0%)	14 (38.9%)	0 (0%)	0 (%)	0 (0%)	4 (11.1%)
L. acidophilus	22	9.9	8 (36.4%)	8 (36.4%)	0 (0%)	0 (0%)	0 (0%)	6 (27.3%)
Strep.salivarius	10	4.5	0 (0%)	6 (60%)	0 (0%)	2 (20%)	4 (40%)	4 (40%)
Strep. Mitis	4	1.8	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)
			3.59%	28.70%	0%	0.90%	6.28%	18.39%
Mean %				10.76%			8.52%	

Table 4: Group 7 and 8 antibiotics susceptibility pattern of bacterial isolates from dental caries patients attending the dental clinic at Irrua specialist teaching hospital.

			Group 7			Group 8		
Bacterial isolates	No of isolates	% of isolates	Ciprofloxacin	Pefloxacin	Ofloxacin	Metronidazole	Lincomycin	Clindamycin
			5µg/disc	10µg/disc	5µg/disc	25µg/disc	15µg/disc	10µg/disc
Strep.mutans	151	67.7	0 (0%)	4 (2.6%)	0 (0%)	124 (82.1%)	30 (19.9%)	145 (96%)
Strep.sobrinus	36	16.1	0 (0%)	0 (0%)	0 (0%)	34 (94.4%)	6 16.7%)	36 (100%)
L. acidophilus	22	9.9	0 (0%)	0 (0%)	0 (0%)	4 (18.2%)	3 13.6%)	22 (100%)
Strep.salivarius	10	4.5	0 (0%)	0 (0%)	0 (0%)	10 (100%)	0 0%)	8 (80%)
Strep. mitis	4	1.8	0 (0%)	0 (0%)	0 (0%)	4 (100%)	1 (25%)	4 (100%)
			0%	1.79%	0%	78.92%	17.94%	96.41%
Mean %			0.60%			64.42%		

and Unasyn (77.8%) **but were 100% resistant to** Tetracycline, Cotrimoxazole, Gentamycin, Neomycin, Cephalexime, Cefuroxime, Ciprofloxacin, Pefloxacin, and Ofloxacin. *L. acidophilus* isolates were most susceptible to Clindamycin (100%), Augmentin (88.9%), Unasyn (77.8%), Erythromycin (72.7%), and Chloramphenicol (54.5%) **but were 100% resistant to** Ampicillin, Amoxicillin, Imipenem, Cotrimoxazole, Clarithromycin, Azithromycin, Neomycin, Cephalexime, Cefuroxime, Ciprofloxacin, Pefloxacin, and Ofloxacin. *Strep. salivarius* isolates were most susceptible to Unasyn (100%), Metronidazole (100%), Augmentin (80%), Erythromycin (80%), Clindamycin (80%), Azithromycin (60%), and Streptomycin (60%) **but were 100% resistance to** Ampicillin, Tetracycline, Cotrimoxazole, Clarithromycin, Gentamycin, Neomycin, Ciprofloxacin, Pefloxacin, Ofloxacin, and Lincomycin. *Strep. mitis* isolates were most susceptible to Augmentin (100%), Unasyn (100%), Metronidazole (100%), Clindamycin (100%), Chloramphenicol (50%), Erythromycin (50%), Imipenem (50%), and Cefotaxime (50%) **but were 100% resistance to** Ampicillin, Tetracycline, Cotrimoxazole, Clarithromycin, Azithromycin, Gentamycin, Streptomycin, Neomycin, Cephalexime, Cefuroxime, Ciprofloxacin, Pefloxacin, and Ofloxacin.

Figure 1 shows the overall susceptibility of bacterial isolates from dental caries patients to all 24 antibiotics. The highest susceptibility was observed in *Strep. Salivarius* with a susceptibility percentage of 32.50% while the lowest was by *L. acidophilus* with a susceptibility percentage of 22.73%. The overall sensitivity of each isolated bacterial to the 24 antibiotics was 26.27%, 26.62%, 22.73%, 32.50%, and 28.13% for *Strep. mutans*, *Strep. sobrinus*, *L. acidophilus*, *Strep. salivarius* and *Strep. mitis* respectively.

DISCUSSION

In the present study, *Streptococcus* (90.1%) and *Lactobacillus* (9.9%) *species* were the bacteria isolates causing dental caries among dental caries patients attending the dental clinic in the area and *Streptococcus mutans* was the most prevalent isolates (67.7%). This finding agrees with the study by Jubair [27] who reported *Streptococcus* species to

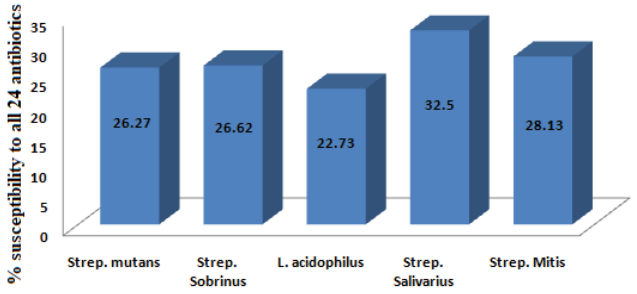

Figure 1 Overall susceptibility of bacterial isolates from dental caries patients to all 24 antibiotics.

account for 70% positive dental caries culture and the study by Enweani, et al. [28] who reported *Streptococcus mutans* to be the prevalence isolated bacteria (72%).

The findings of the present study showed that the Lincosamide and Metronidazole (group 8) were the most effective (group means frequency susceptibility of 64.42%) and followed by the anti-β-lactamase antibiotics (group 2; group mean frequency susceptibility of 53.81%). Unlike most other infections, the Fluoroquinolones (group 7; group mean frequency susceptibility of 0.60%) were not effective and this agrees with the study by Emmerson and Jones [29]. Also, the Cephalosporins and the Aminoglycosides (with a group mean frequency susceptibility of 8.52 % and 10.76% respectively) were not effective against the isolated bacteria. The low efficacy recovered for the most commonly used antibiotics (Tetracycline, Chloramphenicol, and Cetromoxazole), as well as other antibiotics such as Neomycin, Cephalexin, Ciprofloxacin, Pefloxacin, and Ofloxacin, is possible because their abuse rate is high in the community. For example, it is not uncommon to hear "native" saying buy me red capsule, white capsule, or M and B3. It is only logical and prudent to recommend the Lincosamides, Metronidazole, Augmentin, Unasyn, and Erythromycin for treatment of dental infections, especially because of their therapeutic efficacy, ease of administration, and availability.

As observed in the present study, Clindamycin (susceptibility of 96.41%) was the most effective antibiotic against all isolates while the least was Cephalexime (susceptibility of 0.9%). All the isolated bacteria were highly susceptible to Augmentin, Unasyn, Metronidazole, Clindamycin, and Erythromycin **but high resistance to** Ampicillin, Tetracycline, Cotrimoxazole, Clarithromycin, Neomycin, Cephalexin, Ciprofloxacin, Pefloxacin, and Ofloxacin. This is in contrast to the report of Mussrat, et al. [30] who reported *Streptococcus mutans* strains to be sensitive to Ofloxacin. However, the findings that all isolates were resistant to Gentamycin, Tetracycline, and Chloramphenicol are in line with the findings by Mussrat, et al. [30]. Also in line with the present study, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [31] has reported increasing resistance to penicillin among oral *streptococci*. In contrast to the findings of this study, Al-Shami, et al. [32] demonstrated *S. mutans* clinical isolates from dental patients to show significant levels of penicillin, erythromycin, amoxicillin, clindamycin, and lincomycin resistance but more susceptible to ampicillin, cefotaxime, and cefazolin than others tested antibiotics.

Based on the findings from this study, *Streptococcus mutans* is the main etiologic agent of dental caries and Clindamycin seems to be the best therapeutic agent for the management of dental caries and could be applied for blind treatment. Neomycin, Ciprofloxacin, Cotrimoxazole, and Cephalexime were not effective against etiologic agents isolated in this study, and may not be useful for the treatment of dental caries. Importantly, the observed resistance of the isolates to commonly used antibiotics is updated information for health workers and can be served as a notify pharmaceutical makers for the dentist and those concerned to design new strategies for effective prophylaxis against dental infections in the study area.

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