Susceptibility Pattern of Isolated Pathogenic Oral Bacteria to Some Commonly Prescribed Antibiotics in Dental and General Medical Practice

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ABSTRACT

The current global rise in the morbidity and mortality rates of infectious diseases is due in part, to the problem of antimicrobial resistance. The global threat of antimicrobial resistance is also partly a consequence of widespread inappropriate prescription and use of antibiotics, sometimes on empirical basis, without any established antimicrobial susceptibility data-based guidelines on such empirical use. In the present study, we re-examined the susceptibility pattern of organisms isolated from active carious lesions to some commonly prescribed antibiotics and suggest a comprehensive data generation and establishment of guidelines based on a holistic assessment of susceptibility patterns of different organisms to the different classes of antibiotics commonly prescribed in various branches of medicine. For instance, our results revealed significant resistance of cariogenic organisms such as Streptococcus mutans and Lactobacillus to Amoxicillin, Gentamicin, Tetracycline, Chloramphenicol and Sparfloxacin, with good susceptibility to Ciprofloxacin, Ofloxacin, Pefloxacin and Streptomycin. These findings suggest that the latter group of antibiotics could function better than Amoxicillin in the prophylactic and empirical management of dental caries and other oral infections prior to obtaining antimicrobial susceptibility test results.

Keywords: Antimicrobial, Susceptibility, Empirical, Resistance.

INTRODUCTION

The morbidity and mortality rate of infectious diseases have been on a steady increase globally, especially in developing countries. This increase in deaths from infectious diseases is not unconnected to the current global threat of antimicrobial resistance. Consequently, some common infections such as gastrointestinal, respiratory, hospital acquired and sexually transmitted infections have become leading causes of death in developing countries, due to increasing resistance of causative agents, especially bacteria, to commonly used antibiotics (1).

Several studies, including those by Loffler et al., (2) have identified inappropriate prescription of antibiotics by dentists as a major contributor to the increasing resistance of bacteria to commonly used antibiotics. This observation is highly significant, considering the fact that dental infections, especially dental caries, has been identified as the most common infectious disease of man (3), suggesting that the greater percentage of the global human population are dental patients, receiving one form of dental antibiotic prescription or the other. Some common antibiotics that have been widely used in dental practice include Amoxicillin, Metronidazole and Ciprofloxacin (4).

Unfortunately, most bacterial organisms involved in the etiology and pathogenesis of common infectious diseases in developing countries are increasingly developing resistance to these antibiotics commonly prescribed in dentistry. The condition is worsened by the fact that most of the causative bacteria for common gastrointestinal, respiratory, nosocomial and sexually transmitted infections are components of the normal oral flora. Although majority of these organisms are rarely involved in the pathogenesis of common oral infections, they however, become virulent, when disseminated or transmitted to other parts of the body, especially during periods of immunosuppression. In cases where there has been prolonged and inappropriate exposure of affected patients to the common broad spectrum antibiotics used in dentistry, such patients become resistant to these antibiotics hence susceptible to complications and possibly death from such common gastrointestinal, respiratory and sexually transmitted infections.
Urgent steps therefore need to be taken in terms of policy formulation and professional guidelines aimed at regulating the prescription and use of antibiotics in dentistry. As a first step towards achieving this goal, an assessment of the antibacterial effect of some commonly prescribed antibiotics on isolated pathogenic oral bacteria was carried out. Specifically, we isolated, characterized and identified some common oral pathogens, including Streptococcus mutans, Lactobacillus spp., Pseudomonas eruginosa and Candida albicans; and examined their susceptibility to commonly used antibiotics in dental and general practice.

MATERIALS AND METHODS
Collection of Bacterial Plaque Samples and Isolation of Plaque Bacteria

Informed consent was obtained and bacterial plaque samples collected from affected tooth surfaces of five patients presenting at a private dental practice in South-South, Nigeria, on account of dental caries, with or without other associated oral conditions. Samples were collected from within and around carious lesions, using sterile curettes and swabs (in cases of tenderness around the gums and affected tooth). All collected samples were immediately inoculated in nutrient broth contained in sterile sample bottles, sealed properly, labeled and taken to an incubator within one hour of collection. Using methods previously described by Onoriodie and Oshomoh, 2018 (5), samples were incubated at 37°C for 24 hours, after which they were observed for revival and growth of organisms, as indicated by attainment of stationary phase.

Primary cultures were sub-cultured into nutrient agar and blood agar and incubated at 37°C for 24 hours. Following growth of organisms, macroscopic features of colonies, including their sizes, shapes, color, elevation, surface moisture and margins were all observed and recorded.

Gram-Staining, Biochemical Analysis and Identification of Isolated Organisms

Gram-staining of colonies was done using Lugol’s iodine and slides observed under the 100X magnification of a light microscope, for detection of Gram-negative and Gram-positive organisms. Results of Gram-staining and other morphological features of colonies such as cell types and arrangements were observed and recorded (table 1). Other biochemical tests, including catalase, oxidase, dtrate, lactase, indole and urease tests were also done, as previously described (table 2). Following macroscopic and microscopic analysis, gram-staining and biochemical characterization, isolated bacterial strains were systematically identified according to guidelines stipulated in Bergey’s Manual of Systematic Bacteriology (table 2). Prevalence of isolated organisms among the five samples collected was also observed (figure 1) and slants prepared for all identified organisms and stored at 4°C until needed.

Antibiotic Susceptibility Tests

The antibiotic susceptibility test for isolated pathogenic oral bacteria was done, using the disc diffusion methods, as previously described by Reller et al., with slight modifications. Single colonies of pure bacterial strains from prepared slants were inoculated in fresh nutrient broth and incubated overnight at 37°C. Next day, cultures of identified pure bacterial strains were streaked on the surfaces of freshly prepared Mueller-Hinton nutrient agar plates in triplicates. Commercially available antibiotic paper disks containing fixed concentrations (30µg) of 11 different antibiotics, including Cotrimazole (Septin), Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Gentamycin, Perfloxacin, Streptomycin, Tarivid and Ofloxacin were placed on triplicate agar plates of each identified pure bacterial strain and incubated at 35°C for 24 hours.

After 24 hours of incubation assessment of growth inhibition or susceptibility of organisms to the different antibiotics used was done and zones of inhibition (indicated by clear areas around the paper disks) measured, using a pair of dividers and transparent meter rule. The average of triplicate zones of inhibition for each bacterial strain was calculated to the nearest mm (Fig. 2).

RESULTS

Table 1: Macroscopic and morphological features of bacterial isolates

<table>
<thead>
<tr>
<th>Serial Number of Isolate</th>
<th>Size (mm)</th>
<th>Shape</th>
<th>Color</th>
<th>Elevation</th>
<th>Surface</th>
<th>Margin</th>
<th>Gram Reaction</th>
<th>Shape of isolates (under light microscope)</th>
<th>Cell arrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1mm</td>
<td>Irregular</td>
<td>Cream</td>
<td>Flat</td>
<td>Moist</td>
<td>Rough</td>
<td>Negative</td>
<td>Rod</td>
<td>Cluster</td>
</tr>
<tr>
<td>2</td>
<td>0.1mm</td>
<td>Round</td>
<td>Pale cream</td>
<td>Flat</td>
<td>Moist</td>
<td>Smooth</td>
<td>Positive</td>
<td>Cocc</td>
<td>Grouped</td>
</tr>
<tr>
<td>3</td>
<td>0.1mm</td>
<td>Round</td>
<td>Pink</td>
<td>Raised</td>
<td>Moist</td>
<td>Smooth</td>
<td>Negative</td>
<td>Rod</td>
<td>Chain</td>
</tr>
<tr>
<td>4</td>
<td>1mm</td>
<td>Round</td>
<td>Pale green</td>
<td>Raised</td>
<td>Dry</td>
<td>Smooth</td>
<td>Negative</td>
<td>Rod</td>
<td>Singling</td>
</tr>
<tr>
<td>5</td>
<td>1mm</td>
<td>Irregular</td>
<td>Cream white</td>
<td>Flat</td>
<td>Moist</td>
<td>Rough</td>
<td>Negative</td>
<td>Rod</td>
<td>Singling</td>
</tr>
<tr>
<td>6</td>
<td>1mm</td>
<td>Oval</td>
<td>Cream</td>
<td>Raised</td>
<td>Moist</td>
<td>Smooth</td>
<td>Positive</td>
<td>Rod</td>
<td>Singling</td>
</tr>
<tr>
<td>7</td>
<td>0.1mm</td>
<td>Round</td>
<td>Cream</td>
<td>Raised</td>
<td>Moist</td>
<td>Smooth</td>
<td>Positive</td>
<td>Rod</td>
<td>Singling</td>
</tr>
</tbody>
</table>
Table 2: Biochemical characterization and identification of isolated oral bacteria

<table>
<thead>
<tr>
<th>Serial Number of Isolate</th>
<th>Citrate test</th>
<th>Indole test</th>
<th>Urease test</th>
<th>Oxidase test</th>
<th>Motility test</th>
<th>Lactase test</th>
<th>Glucose test</th>
<th>Catalase test</th>
<th>Identified organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Streptococcus mutans</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>E. coli</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Psuedomonas eruginosa</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Proteus mirabilis</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>7</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Lactobacillus spp.</td>
</tr>
</tbody>
</table>

Prevalence of identified organisms in collected samples

Figure 1: Prevalence of identified pathogenic oral bacteria in collected samples
Antibiotics are usually products of microorganisms, which can either inhibit (bacteriostatic) or kill (bactericidal) bacteria and other microorganisms, thereby ensuring survival of the host microorganism within a relatively competitive microbial environment. It is therefore reasonable to presume that the problem of antimicrobial resistance is as old as the discoveries of antibiotics, since the target microorganisms are also biological agents hence, possess some level of inherent resistance to substances that are toxic to them. Nevertheless, the rapid development of new and effective antimicrobial agents, including synthetic antibiotics for decades, ensured sustenance of susceptibility of microorganisms to different classes of antibiotics, beginning from the early 1940s, when they were first used for treatment of human diseases. However, in recent years, there has been a drastic decline in the rate of discovery and development of new antimicrobial agents, a situation that has provided room for an unprecedented and unhindered progress in the development of resistance to most commonly used antibiotics.

The contribution of dental practitioners to the current global threat of antimicrobial resistance through inappropriate prescription of antibiotics has been universally acknowledged. Across the globe, dentists are believed to be responsible for about 7 to 11% of all antibiotic prescriptions, with approximately 50% of such prescriptions being considered as inappropriate. For example, the two common conditions for which dentists should prescribe antibiotics include acute odontogenic infections such as active dentoalveolar abscess and some cases of periodontitis such as acute necrotizing ulcerative gingivitis (ANUG). However, most dentists have been observed to prescribe antibiotics, especially broad spectrum antibiotics, following minor procedures such as simple tooth extractions and cavity preparation/restorations in immune competent individuals, especially in developing countries where the sterility of such procedures cannot be guaranteed.

An ideal and appropriate antibiotic prescription should be guided by results of antibiotic susceptibility test, which is not only capable of identifying the most effective antibiotic but also the most effective dose to administer. However, most antimicrobial susceptibility test results are usually obtained after 24 to 48 hours, making it difficult for dentists to appropriately prescribe the correct antibiotics within the first few hours of presentation, which oftentimes, are the critical stages of infection.

Consequently, most early prescriptions made for acute infections are empirical and broad spectrum in nature, largely because the causative organisms involved and specific antibiotics to which they are susceptible are not initially known. Among the commonly prescribed broad spectrum antibiotics, Amoxicillin has been observed to be the most frequently prescribed antibiotic by dentists in many parts of the world, especially in penicillin-sensitive patients. Interestingly, results from several studies on the susceptibility of pathogenic oral organisms, including Streptococcus mutans, to Amoxicillin and other commonly used broad spectrum antibiotics in dentistry, are quite conflicting. For example, Devi and colleagues reported an 80% sensitivity of plaque bacteria to Amoxicillin, with resistance to Metronidazole and Ciprofloxacin (14). Similarly, Pranay and Ram observed that Amoxicillin and Penicillin G produced the maximum diameter of zone of inhibition (44mm-45mm) against Streptococcus mutans, when compared to Ofloxacin (32mm-37mm); Erythromycin (19mm-20mm) and Gentamycin (18mm-19mm). They also...
observed a resistance of Streptococcus mutans to Metronidazole, Ciprofloxacin and Rifampicin (15). In contrast, studies by Katsuhiko et al revealed a high degree of resistance of oral Streptococci obtained from dental plaque in healthy Japanese adults, against a group of common antibiotics, including Amoxicillin, Ampicillin, Erythromycin, Penicillin and Levofloxacin (16). These observations are in consonance with previous reports by Deepak et al, which observed a 90% resistance to Amoxicillin; 78% resistance to Cloxacin; 60 % resistance to Erythromycin and 48% resistance to Penicillin, by a group of pathogenic oral bacteria isolated from carious teeth of dental patients (17). Also, a more recent study by Ibrahim et al showed susceptibility of oral isolates selected from active carious lesions to Ampicillin, Cefotaxime, Cefazolin, Metillin and Clindamycin, with significant resistance against Erythromycin, Lincomycin, Penicillin and Amoxicillin (18).

Our results indicated that over 80% of isolated pathogenic oral bacteria including Streptococcus mutans, Lactobacillcus species, Proteus mirabilis, Pseudomonas aeruginosa, E. coli and Proteus mirabilis, are resistant to Amoxicillin, with only Candida albicans showing a high degree of sensitivity to the drug. On the contrary, 100% of the organisms isolated showed significant susceptibility to Ciprofloxacin, with the highest susceptibility being observed for Streptococcus mutans and Candida albicans (20mm diameters of zones of inhibition each), followed by Lactobacillus and Proteus mirabilis (16mm and 18mm diameters of zones of inhibition respectively), while E.coli and Klebsiella pneumonia showed moderate susceptibility to Ciprofloxacin (15mm and 12mm diameters of zones of inhibition, respectively). Apart from Amoxicillin, all isolated organisms showed complete resistance to Chloramphenicol and limited susceptibility to Sparfloxacin (Lactobacillcus and Proteus mirabilis); Gentamicin (Lactobacillcus, Pseudomonas aeruginosa and Streptococcus mutans); Tarivid (Pseudomonas aeruginosa and Klebsiella pneumonia) and Septrin (E.coli, Streptococcus mutans and Klebsiella pneumonia).

Similar to Ciprofloxacin, all organisms isolated showed some amount of sensitivity to Ofloxacin and Pefloxacin, with diameters of zones of inhibition ranging between 12mm and 14mm; and 13mm – 16mm, respectively. Over 85% of pathogenic oral bacteria isolated showed sensitivity to Augmentin, with diameters of zones of inhibition ranging between 12mm and 14mm; while 71% of these organisms showed sensitivity to Streptomycin, with average zones of inhibition of between 14mm and 16mm. These findings largely suggest an emergence of Amoxicillin resistant strains of Streptococcus mutans, similar to results of Deepak et al. and Katsuhiko et al. They are however in sharp contrast to results of Devi and colleagues; and Pranay and Ram, both of which observed susceptibility of Streptococcus species to Amoxicillin and resistance to Ciprofloxacin, as opposed to the significant susceptibility of Streptococcus mutans to ciprofloxacin that was observed by our group. A holistic and critical evaluation of these studies and indeed most other antimicrobial susceptibility studies on pathogenic oral bacteria reveals the possible reason for the conflicting results observed – source of bacterial sample and their previous exposure to Amoxicillin.

Bacterial samples studied by Pranay and Ram were procured from MTCC Institute of Microbial Technology Chandigarh, while Devi and colleagues collected a total of 150 plaque samples from a dental hospital and several dental clinics in Dehradun, with no information concerning the oral health status and pathological condition of associated teeth. It is common knowledge that some patients visit dental clinics, even tertiary dental facilities, for simple scaling and polishing or other periodontal problems such as chronic periodontitis that require deep scaling and likely prescription of other antibiotics such as Tetracycline and Doxyecycline but not Amoxicillin.

On the contrary, antimicrobial susceptibility experiments of Deepak et al, Ibrahim et al and the current study were all done on pathogenic oral organisms isolated from active carious lesions often associated with pain, which is the most common reason why patients visit the dentist. Most patients with active carious lesions probably had previous episodes of cavity restorations or tooth extraction, with post-operative exposure to Amoxicillin and Metronidazole. Consequently, these studies were carried out on a population of organisms with previous and repeated exposure to Amoxicillin, which could account for their resistance to the drug. On the contrary, studies with results indicating high susceptibility of pathogenic oral organisms to antibiotics commonly prescribed in dentistry were most likely carried out on bacteria obtained from individuals without any history of inappropriate exposures to such antibiotics, especially Amoxicillin.

This observation is in line with previous findings by Harrison et al, which suggested that repeated use of single-dose prophylactic regimen of Amoxicillin encourages emergence of more resistant strains of streptococci, compared to those generally encountered in the oral cavity (19). Similarly, Woodman et al had observed a significant increase in the number of resistant streptococcal species, following the second and third doses of Amoxicillin therapy in healthy adult volunteers (20). Consequently, repeated prescription of Amoxicillin and other broad spectrum antibiotics such as Metronidazole to dental patients following minor dental procedures could be doing more harm than good, especially in the face of current global threat of antimicrobial resistance.

Susceptibility of all isolated organisms to Ofloxacin, Pefloxacin and Ciprofloxacin also suggests that most of the organisms tested were obtained from patients with minimal previous exposures to the Quinolones although gradual emergence of resistance can be observed, especially against Sparfloxacin. For example, although 100% of the organisms studied showed susceptibility to Ciprofloxacin, Pefloxacin and Ofloxacin, the degree of susceptibility to these antibiotics decreased in that order. Furthermore, each of the organisms exhibited varying degrees of susceptibility to the quinolones, with Lactobacillcus, Candida albicans and Streptococcus mutans generally showing more impressive susceptibility levels to the quinolones than other organisms. The gradual emergence of antimicrobial resistance against the quinolones could be attributed to the concept of cross resistance. Christine Sanders and colleagues had previously observed similar cross resistance to the Quinolones, which were observed to result from “accumulation of mutations in chromosomal genes responsible for the drug targets, permeability, or active efflux.” (21)

The spectrum of activity of Aminoglycosides against Gram-positive and Gram-negative organisms has been extensively studied, with significant activity seen against E. coli, Klebsiella pneumonia and Proteus species (22) and Pseudomonas aeruginosa (23), (24). However, in a study done by Podnecky and colleagues (25), no significant activity of Aminoglycosides was observed against Streptococcus species. In the present study, the Aminoglycosides Streptomycin showed significant activity against Lactobacillcus species, Proteus mirabilis, Pseudomonas
aeruginosa, Candida albicans and Klebsiella pneumoniae. These findings were largely in concurrence with previously reported results, except for E. coli, against which Streptomycin and Gentamycin showed no significant activity in our study. Mechanisms of resistance of organisms against Aminoglycosides have been identified to include enzymatic drug modifications, 16S rRNA methylation and efflux-mediated resistance (26).

Although the problem of antimicrobial resistance is a global one, the pattern of resistance of microorganisms, including pathogenic oral bacteria against commonly used antibiotics, is far from being universal. Unfortunately, current attention is focused on isolating or investigating mechanisms of antimicrobial resistance without considering the variations in pattern of resistance among different populations or groups of patients within the same population, who are frequently and most often, inappropriately exposed to different classes of antibiotics commonly used in the various fields of medical practice. For example, while Amoxicillin and Metronidazole are widely prescribed in Dentistry, this is usually not the case in General Surgery, Orthopedic Surgery, Traumatology, Ophthalmology or other areas of specialty where other classes of antibiotics are often times also inappropriately prescribed. Apart from primary resistance arising from inappropriate prescription of specific classes of antibiotics, limited attention is also paid to patterns of cross-resistance developed by different groups of patients to other classes of antibiotics, due to previous exposures to the initial class of antibiotics. Most of the resistance encountered among patients is due to previous inappropriate use of antibiotics, yet there are no well characterized patterns of antimicrobial resistance for any group of patients or antibiotics.

A holistic evaluation of variations in pattern of resistance and cross-resistance is important, especially in developing countries where some common antibiotics such as penicillin are readily available and easily purchased without the doctor’s prescription. While the search for new antimicrobial agents is on, efforts must also be made to prevent unnecessary deaths resulting from resistance to commonly used antibiotics especially by Gram-negative nosocomial organisms. The way forward in understanding the variations in antimicrobial resistance among vulnerable populations both in the hospital environment and the general public is to encourage and facilitate regular and more frequent assessment of antimicrobial susceptibility patterns for various populations exposed to inappropriate prescriptions and indiscriminate use of common antibiotics. If widely implemented, results from such studies could potentially guide medical personnel in the prescription of the correct antibiotic, especially in cases of life threatening emergencies requiring the use of antibiotics.

For instance, results of the present study largely suggest that dental patients who had previously been inappropriately exposed to Amoxicillin could benefit more from Ciprofloxacin, in cases of life-threatening infections such as Ludwig’s Angina, where continued prescription of Amoxicillin or Metronidazole is not likely to produce any positive results, due to resistance developed overtime. Ideally, identification of such specific antibiotics to which causative organisms would be susceptible should be based on results of antimicrobial susceptibility tests done for individual cases. However, since most antimicrobial susceptibility test results are usually not ready within the first 24 to 48 hours, establishment of prescription guidelines based on well characterized susceptibility patterns for the different specialties of medicine could be the way to go in the prevention of unnecessary deaths resulting from the current widespread antimicrobial resistance. It is worth emphasizing that such guidelines cannot function as substitutes for the standard practice of antimicrobial susceptibility tests for individual cases but are rather better options that should guide empirical prescriptions within the first 24 to 48 hours prior to availability of individual susceptibility test results; when compared to the current practice of unguided empirical use of broad spectrum antibiotics.

REFERENCES


