Antibiotic sensitivity pattern of Streptococcus mutans against commercially available drugs

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ABSTRACT

Streptococcus mutans is the leading cause of dental caries (tooth decay) worldwide and is considered to be the most cariogenic of all of the oral streptococci. In the present investigation, the evaluation of current efficacy of 15 commercially available antibacterial drugs in India was carried out against three strains of S. mutans by Kirby-Bauer disc diffusion method and for also determining which drug should be prescribed by the dentists exhibiting minimal side effects and maximum inhibitory activity. Of the 15 antibacterial drugs evaluated against all the three strains of S. mutans, amoxicillin and penicillin G were highly effective in terms of maximum diameter of growth inhibition zones followed by chloramphenicol. Nine drugs namely, ofloxacin, doxycycline, tetracycline, chlorotetracycline, erythromycin, vancomycin, clindamycin, methicillin and gentamycin were found to be moderately effective against the three strains of S. mutans. Metronidazole, ciprofloxacin and rifampicin were not effective against the bacteria as they did not show any inhibitory activity.

Keywords: Dental caries, antibiotic resistance, Kirby-Bauer Disc Diffusion method, antibacterial activity

INTRODUCTION

Dental caries is one of the most important problems in public health because of its ubiquitousness in civilized population. The prevalence of dental caries in industrialized countries like India is on the rise. As the treatment is very costly and requires a lot of manpower, the prevention at the primary level is the solution of the choice [1-2]. The disease is marked by a localized progressive demineralization of the hard tissues of the crown and root surfaces of the tooth. The demineralization is caused by acids produced by bacteria, particularly, Streptococcus mutans that ferment dietary carbohydrates [3]. S. mutans is gram-positive cocci, non-motile facultative anaerobic microorganism which can metabolize carbohydrates and is considered to be the principle etiological agent of dental caries [4-5]. The demineralization occurs within a bacteria-laden gelatinous material called dental plaque that adheres to the tooth surfaces and become colonized by bacteria [6]. Secondary infections are caused by Lactobacillus species, and yeasts such as Candida albicans [7-8].

In the present clinical scenario globally, there is a great interest in the use of antimicrobial agents for prevention and treatment of dental caries due to the spread of antibiotic resistance [9]. The widespread concern about the increasing problem of antibiotic resistance has emphasized the need for rationalization of antibiotic use in the treatment of dental caries [10]. Inappropriate antibiotic prescribing and use have been identified as major factors in the emergence of antibiotic resistance in S. mutans. Consequently, modification and surveillance of prescribing attitudes have become crucial [11].

In the recent years, a shift from narrow-spectrum antibiotic prescriptions which included penicillins to broad-spectrum aminopenicillins with which include amoxicillin by dental professionals has been reported and the increase of bacterial isolates resistant to the former antibiotics is blamed for such a shift in prescription practices [11]. The paper is aimed towards the evaluation of current efficacy of commercially available drugs in India against three strains of S. mutans which is primarily responsible for causing dental caries and for also determining which drug should be prescribed by the dentists that exhibits minimal side effects and maximum inhibitory activity.

MATERIALS AND METHODS

I. Procurement of microbial cultures and antibiotic discs

Microbial cultures, three strains of S. mutans, namely S. mutans I (MTCC 1943), S. mutans II (MTCC 890) and S. mutans III (MTCC 497) were procured from MTCC, Institute of Microbial Technology, Chandigarh. The agar slants of Brain Heart Infusion agar (HiMedia, Mumbai) were used for maintaining S. mutans. The culture slants were stored at 4°C in the refrigerator. The minimum inhibitory concentration (MIC) of 15 antibacterial antibiotic discs having diameter 6mm (HiMedia, Mumbai) were as follows: clindamycin (2µg), metronidazole (5µg), methicillin (5µg), rifampicin (5µg), ciprofloxacin (5µg), ofloxacin (5µg), gentamycin (10µg), penicillin G (10µg), erythromycin (15µg), clindamycin (30µg), doxycycline (30µg), vancomycin (30µg), chloramphenicol (30µg), tetracycline (30µg) and amoxicillin (30µg).

II. Inoculum standardization

The inocula of different strains of S. mutans were adjusted according to 0.5 McFarland standard which was prepared by adding 0.05ml of barium chloride (BaCl2) (1.17% w/v) to 9.95ml of 0.18M H2SO4 (1.0% w/v) with constant stirring. The McFarland standard tube was slightly sealed to prevent loss by evaporation and was stored for 6 months to protect from light at room temperature. The inoculum of test strains was adjusted to 1.5 x 108 CFU/ml equal to that of the 0.5McFarland standard by adding sterile distilled water. To aid com-
The antibacterial activity of 15 commercial drugs was assayed by Kirby-Bauer disc diffusion method following the recommendations of CLSI [17]. Metronidazole, ciprofloxacin and rifampicin showed no inhibition zones against the growth of *S. mutans* strains, hence considered to be resistant against these drugs.

Among the antibacterial drugs tested, amoxicillin, penicillin G and chloramphenicol showed maximum zone of inhibition against all the three strains of *S. mutans*. *S. mutans* strains have been found to be most susceptible against amoxixillin as revealed by the data, the maximum zone of inhibition was found in amoxicillin (44mm to 45mm).

Our results from the present study substantiate the frequent use of broad spectrum amoxicillin in dental practice by Al-Harooni and Skoog [11]. This antibiotic is routinely prescribed as prophylaxis to the patients prior to massive dental procedures. It has been reported that the introduction of penicillin in the prophylactic treatment has reduced the infection, but the long-term use of penicillin could be compromised by the emergence of resistant strains [5]. Erythromycin and clindamycin have been recommended as an alternative options for patients who are allergic to penicillin and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures [18-19]. These two antibiotics have not developed resistance against the three strains of *S. mutans* as revealed by the zone of inhibition that varied between 25mm and 28mm in the present study. Ofloxacin, doxycycline, tetracycline and chlorotetracycline exert their side effects mainly on the digestive system which include mild stomach pain or upset, nausea, vomiting and diarrhoea. They are however effective in inhibiting the growth of *S. mutans* and hence should be recommended for use. Tetracyclines have few side effects but are not recommended for children or pregnant women because they can discolor developing teeth and alter bone growth [20]. Gentamycin, an aminoglycoside, may lead to side effects which include damage to the ears and kidneys. Metronidazole has been most frequently prescribed by the dental professionals. However, the emergence of resistance to this drug may be slower than if it were used alone, because in order to target both aerobic and anaerobic organisms, metronidazole is used empirically in combination with one or more antibiotics, although the resistance to the drug may be associated with mobile genetic elements, aiding spread [9].

It may be suggested from the present study that due to lack of appropriate knowledge of prescribing antibiotics for the treatment of dental caries on part of dental professionals, the microbial flora responsible for causing dental caries has developed resistance to the commercially available drugs. It may also be recommended that amoxicillin and penicillin G are the most effective antibacterial drugs for the treatment of dental caries. Further investigation and education are required to attempt to slow resistance development and lessen the future impact on antibiotic prescribing in dentistry.

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**REFERENCES**


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### Table 1: Antibiotic sensitivity pattern of *Streptococcus mutans* against 15 commercial drugs by Kirby-Bauer disc diffusion method

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Antibacterial drug</th>
<th>Concentration (µg)</th>
<th>Mean Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. mutans I</em></td>
</tr>
<tr>
<td>1</td>
<td>Amoxicillin</td>
<td>30</td>
<td>48±0.37</td>
</tr>
<tr>
<td>2</td>
<td>Penicillin G</td>
<td>10</td>
<td>45±0.57</td>
</tr>
<tr>
<td>3</td>
<td>Chloramphenicol</td>
<td>30</td>
<td>36±0.57</td>
</tr>
<tr>
<td>4</td>
<td>Doxycycline</td>
<td>30</td>
<td>34±0.57</td>
</tr>
<tr>
<td>5</td>
<td>Ofloxacin</td>
<td>5</td>
<td>32±0.37</td>
</tr>
<tr>
<td>6</td>
<td>Tetracycline</td>
<td>30</td>
<td>32±0.57</td>
</tr>
<tr>
<td>7</td>
<td>Chlorotetracycline</td>
<td>30</td>
<td>29±0.81</td>
</tr>
<tr>
<td>8</td>
<td>Erythromycin</td>
<td>15</td>
<td>27±0.37</td>
</tr>
<tr>
<td>9</td>
<td>Vancomycin</td>
<td>30</td>
<td>25±0.57</td>
</tr>
<tr>
<td>10</td>
<td>Clindamycin</td>
<td>2</td>
<td>25±0.57</td>
</tr>
<tr>
<td>11</td>
<td>Methicillin</td>
<td>5</td>
<td>20±0.81</td>
</tr>
<tr>
<td>12</td>
<td>Gentamycin</td>
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<td>18±0.57</td>
</tr>
<tr>
<td>13</td>
<td>Metronidazole</td>
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</tr>
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<td>14</td>
<td>Ciprofloxacin</td>
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</tr>
<tr>
<td>15</td>
<td>Rifampicin</td>
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<td>Control</td>
<td>-</td>
<td>NA</td>
</tr>
</tbody>
</table>

± Standard deviation; NA – No activity, No drug


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