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Research Article

Efficacy of some Antibiotics against Streptococcus Mutans Associated with Tooth decay in Children and their Mothers

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Abstract

Background: Dental caries is recognized as one of the most infectious diseases worldwide and *Mutans streptococci* (MS) have been commonly associated as major cariogenic bacteria.

Objectives: The objective of this study was to identify and determine the antibiogram profile of *Streptococcus mutans* associated with tooth decay in children and their mothers.

Methods: The dental plaque samples were collected from caries active subjects children group (aged 2-5 years) and mother group (aged 35-44 years) at dental clinics of Sana'a University in Sana'a city, Yemen. *S. mutans* identified by standard bacteriological methods and 87 clinical isolates *S. mutans* form mothers and 87 clinical isolates *S. mutans* from children were tested for antibiogram profile. Antibiogram profiling was performed to determine the susceptibility of 6 *β*-Lactam antibiotics (penicillin, ampicillin, cefotaxime, amoxicillin, cefazolin and methicillin) and 4 non *β*-Lactam antibiotics (erythromycin, lincomycin, clindamycin and vancomycin) by disc diffusion method.

Results: Ampicillin, cefotaxime cefazolin, methicillin and clindamycin were the most effective antibiotics against *S. mutans* isolates and resistance rate for them do not exceed 2.3%. The highest resistance rates were against erythromycin (24.1%), lincomycin (28.7%) followed by penicillin (14.9% in children *S. mutans* isolates) and amoxicillin (14.9% in mother *S. mutans* isolates).

Conclusion: The study demonstrates significant levels of penicillin, erythromycin, amoxicillin, clindamycin and lincomycin-resistance in *S. mutans* clinical isolates in dental patients. Further study is required to know the minimum inhibitory concentration of *β*-Lactam and non *β*-Lactam antibiotics. These results also, call for improved the assessment of antibiotic susceptibility testing during prophylaxis. The alternative of antibiotic such as herbal extract is most likely preferable for the coming years to avoid the upcoming bacterial resistance to the antibiotics.

Keywords: Antibiotic susceptibility testing; *Streptococcus mutans*; Dental caries; Children; Mothers

Introduction

Dental caries is recognized as one of the most infectious diseases worldwide [1,2]. *Mutans streptococci* (MS) have been commonly associated as major cariogenic bacteria. *S. mutans* is present in oral flora and has been demonstrated to be a causative specialist for dental caries because of its capacity to metabolize fermentable carbohydrate into organic acids. These acids can cause a fall in pH, which can lead to an increase of enamel solubility that is dental caries [3]. Expanding resistance of bacterial pathogens to regularly utilize antibiotics has turned into general human concern. The spread of antibiotic resistance is causing fatalities, as well as a high financial inconvenience. In low economic nations as Yemen, antibiotic resistance is more prevalent than in the developed countries [4].

S. mutans is also included as a causative agent of endocarditis. Information about the antibiogram profile of *S. mutans* is of significance for prescribing the appropriate treatment in the case of endocarditis [5]. One-hour prior dental procedure, the American Heart Association suggests antimicrobial prophylaxis for high-risk cardiovascular patients, such as amoxicillin (2g) as first choice and clindamycin (600mg) as a second choice [6]. Production of

β-lactamase is, however, unusual for most of *streptococci,* where resistance is happening by slightly altered of penicillin binding proteins [7-9]. In 2012 investigators have accounted a significant level of penicillin resistance 13.4% of 550 oral streptococcal clinical isolates, out of 50 isolates of *S. mutans* 14% were resistant to penicillin [10]. Consistent with the study performed in 2014, 38 isolates of *S. mutans* showed a complete resistance to penicillin and ampicillin [11]. Bacterial resistance to antibiotics such as penicillin and other β-lactam is a health issue in numerous parts of the world. Thus, the objective of this study was to identify and determine the antibiogram profile of *Streptococcus mutans* associated with tooth decay in children and their mothers.

Materials and Methods

The present study was conducted in the Department of Conservative Dentistry and Oral Health, Faculty of Dentistry, Sana'a University, Republic of Yemen. The study protocol was approved by the ethics committee of Sana'a University. A written informed consent was obtained from the selected participants. An eighty-seven plaque dental samples were collected from caries active mothers and 87 plaque dental samples were collected from their caries active children.

Microbiological procedure

The sample was transported to the laboratory immediately after collection using Thioglycollate broth and processed on same day. The sample was vortexed (15sec,) and diluted 1:1000 in isotonic saline solution prior to inoculation. One loop ($1/1000^{th}$ml of sample) was inoculated on the Mitis Salivarius agar with potassium tellurite medium, bacitracin and 20% sucrose. The plates were incubated at 37 °C anaerobically. After 72-hour, colony characteristics were studied and identified. Then antibiogram profiling was performed to determine the susceptibility of 6 *β*-Lactam antibiotics (penicillin, ampicillin, cefotaxime, amoxicillin, cefazolin and methicillin) and 4 non *β*-Lactam antibiotics (erythromycin, lincomycin, clindamycin and vancomycin) by disc diffusion method.

Antibiogram

The antibiotic susceptibility profile was determined by disc diffusion method. The inoculums were adjusted to match the turbidity of 0.5 McFarland standards, and was swabbed on Brian heart infusion agar and allowed to dry for 10min [12]. The antibiotics employed in this study were: penicillin-G (P) 10 units, ampicillin (AMP) 10μg, cefotaxime (CTX) 30μg, erythromycin (E) 15μg, cefazolin (CZ) 30μg, methicillin (MET) 5μg, lincomycin (L) 2μg, clindamycin (CC) 2μg and vancomycin V (30μg) (Oxide, USA). Inhibition zone was measured after 24h of anaerobically incubation at 37 °C. The experiments of each antibiotic were performed in triplicate. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) methodology [13].

Results

Table 1 presented the pattern of antibiotic sensitivity of *S.mutans* isolates from carries mother patients and caries children patients. Ampicillin, cefotaxime cefazolin, methicillin and clindamycin were the most effective antibiotics against *S. mutans* isolates and resistance rate do not exceed 2.3%. The isolated *S.mutans* shows a high resistance rate against erythromycin (children isolates= 24.1%), and against lincomycin (mother isolates 28.7%). The resistance rate against penicillin was 14.9% in children *S.mutans* isolates and 8% in *S.mutans* mother isolates. The resistance rate against amoxicillin was 14.9% in mother *S.mutans* isolates and 12.6% in *S.mutans* children isolates.

Discussion

Most of the antibiotics employed in this study are commonly prescribed by dentists [2,14]. The number of resistant of oral *mutans streptococci* is greater in people frequently exposed to antibiotics, although the resistant bacteria may also be found in healthy subjects who have not been recently treated with antibiotics [2]. β-lactam antibiotics are the most commonly prescribed chemo prophylactic agent's in general dental practices. However, resistance to penicillin among oral *streptococci* is increasing [15]. The number of resistant oral *streptococci* is greater in people frequently exposed to antibiotics [16], although these bacteria may also be found in healthy subjects who have not been recently treated with an antimicrobial [17].

Bacterial resistance to antibiotics such as penicillin and other β-lactam is a health issue in numerous parts of the world. In our study we observed a significant level of penicillin resistance (14.9%) in children *Strept mutans* clinical isolates. The high prevalence of resistance to penicillin in *Strept. mutans* in our study is like that previously observed in South Africa and Spain in oral *streptococcus viridans* [18,19]. Several in-vitro studies have demonstrated the capability to transfer penicillin resistance determinants among related species [20]. These mechanisms, together with selective antibiotic pressure, may play an important role in the emergence and spread of penicillin resistance in oral *streptococci.*

Also, the significant level of penicillin resistance (14.9%) in *Strept. mutans* clinical isolates in our study is similar to Pasquantonio *et al.* [10] study that reported a significant level of penicillin resistance: 13.4% of 550 oral *streptococcal* clinical isolates, out of 50 isolates of *S. mutans* 14% were resistant to penicillin [10]. However, our result is lower than the rate of a study conducted in 2014 by Dhamodhar *et al.* [11] in which 38 isolates of *S. mutans* showed a complete resistance to penicillin and ampicillin. One-hour prior dental procedure, the American Heart Association suggests antimicrobial prophylaxis for high-risk cardiovascular patients, such as amoxicillin (2g) as first choice and clindamycin (600mg) as a second choice [6]. Production of β-lactamase is, however, unusual for most of *streptococci,* where resistance is happening by slightly altered of penicillin binding proteins [7-9].

However, in our study we observed a significant level of amoxicillin resistance [14.9%) in mother isolates and (12.6%) in children isolates of *Strept. mutans;* and 8% for clindimycin in children isolates. Thus, in this condition first choice should be go to third generation of cephalosporin's and ampicillin in which resistance to cephalosporins and ampicillin is less than 2%

(Table 1). The children isolate of *S. mutans* showed slightly more susceptibility against tested antibiotics employed in this study than the mother isolates of *S. mutans*. This different might be due to long exposure of mothers to these antibiotics than their children. However, a significant level of erythromycin resistance (24.1%) in children isolates than mother isolates (10.3%).

Table 1: The antibiotic sensitivity test for S. mutans clinical isolates from mothers and their children.

Antibiotics	Mother isolates n=87			Children isolates n=87		
	Sensitive No %	Moderate No %	Resistant No %	Sensitive No %	Moderate No %	Resistant No %
Ampicillin	76 (87.4%)	9 (10.3%)	2 (2.3%)	80 (92%)	6 (6.9%)	1 (1.1%)
Amoxicillin	51(58.6%)	23 (26.4%)	13 (14.9%)	57 (65.5%)	19 (21.8%)	11 (12.6%)
Penicillin	52 (59.8%)	28 (32.2%)	7 (8%)	50 (57.5%)	24 (27.6%)	13 (14.9%)
Cefotaxime	72 (82.8%)	14 (16.1)	1 (1.1)	74 (85.1%)	12 (13.8%)	1 (1.1%)
Cefazolin	71(81.6%)	14 16.1%)	2 (2.3%)	75 (86.2%)	10 (11.5%)	2 (2.3%)
Methicillin	69 (79.3%)	13 (14.9%)	4 (4.6%)	72 (82.8%)	13 (14.9%)	2 (2.3%)
Erythromycin	67 (77.6%)	11 (12.6%)	9 (10.3)	43 (49.4%)	16 (18.4%)	21 (24.1%)
Lincomycin	42 (48.3%)	20 (23.6 %)	26 (28.7%)	67 (77%)	13 (14.9 %)	7 (8%)
Clindamycin	69 (79.3%)	14 (16.1%)	4 (4.6%)	67 (77%)	13 (14.9%)	7 (8%)
Vancomycin	48 (55.2%)	37 (42.5%)	2 (2.3%)	48 (55.2%)	37 (42.5%)	2 (2.3%)

The high rate of erythromycin resistance in children isolates can be explained by selective antibiotic pressure, in which erythromycin is used more common in children than in adults; and this may play an important role in the emergence and spread of erythromycin resistance in children isolates. Ultimately, the resistant developed by *S. mutans* is obscure. Updated information on antibiotic susceptibility testing such as reported in the present study helps to notify pharmaceutical makers to design new strategies for effective prophylaxis against dental infections. This result also gives an ideal choice to the dentist to prescribe a suitable antibiotic in Yemen.

Conclusion

Our study demonstrates significant levels of penicillin, erythromycin, amoxicillin, clindamycin and lincomycin-resistance in *S. mutans* clinical isolates in dental patients. Isolates were more susceptible to ampicillin, cefotaxime, and cefazolin than others tested antibiotics. Further study is required to know the minimum inhibitory concentration of β-Lactam and non β-Lactam antibiotics. These results also, call for improved the assessment of antibiotic susceptibility testing during prophylaxis. The alternative of antibiotic such as herbal extract is most likely preferable for the coming years to avoid the upcoming bacterial resistance to the antibiotics. In addition, the rise in the rate of antibiotic resistance in *S. mutans* clinical isolates suggested taking extra precaution while prescribing antibiotics will maintain the bacteria with less resistance.

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Conflict of Interest

No conflict of interest associated with this work.

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