



## Antibiotic susceptibility of *Streptococcus pyogenes* isolated from throat cultures of healthy children aged between 5-15 years

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### ABSTRACT

*Streptococcus pyogenes* is one of the most important causes of bacterial pharyngitis. Asymptomatic carriage of this organism especially among schoolchildren is a common issue. Study of the prevalence and antimicrobial susceptibility pattern of the flora strains, as clinical indicators, are useful for treatment of streptococcal infections. The aim of this study was to determine the prevalence and resistance pattern of *S. pyogenes* isolates detected from throat of healthy children in Tehran. After filling a questionnaire including general information, from preschoolers, primary school students and school age children referring to the follow up center of the Ali-Asghar hospital of pediatrics, throat samples were collected from 5-15 year old eligible children by a sterile cotton swab. Then the samples were immediately seeded onto %5 sheep blood agar media. The plates were streaked and incubated appropriately after transferring to the laboratory. Biochemical and serological identification of isolates were done and then Antimicrobial susceptibility pattern of all identified isolates was determined by the disk diffusion method. Finally, Penicillin, Erythromycin and clindamycin MICs were determined by the E-test method for all isolates. The total number of 423 sample swabs were collected during a period of 6 months, showing the carriage rate of %5.7 (n=24). Using chi-square test showed that there were no significant differences in carriage rates between the age sub groups (p=0.095). All isolates were sensitive to Penicillin, Cefotaxime, Erythromycin, Vancomycin, Azithromycin and Clindamycin. The rate of intermediate sensitivity and resistance to Tetracycline was 25 and 12.5%, respectively. Two isolates had intermediate sensitivity to each of the agents Ofloxacin and Chloramphenicol. The MIC level of Penicillin for all isolates were  $\leq 0.016$   $\mu\text{g/ml}$ , and MIC level of clindamycin and erythromycin for all isolates were  $\leq 0.25$   $\mu\text{g/ml}$ , which were in sensitive range. It is concluded that in contrast to the published reports about rising penicillin MIC and resistance to erythromycin, Penicillin remains the first choice of drug for streptococcal infections and also macrolides and lincosamides can be considered as the alternative choice of drug in allergic patients.

### 1. Introduction

*Streptococcus pyogenes* (*S.pyogenes*) is annually responsible for about 619 million cases of throat infections and 111 million cases of skin infections in the worldwide (Bessen, 2009). This

organism is the most common cause of bacterial pharyngitis and also subsequent acute post streptococcal glomerulonephritis and rheumatic fever especially among children (Brunton and Pichichero 2006). Depending on the type of the organism, up to 15-20 % of schoolchildren are

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considered as the main asymptomatic carriers of the organism which varies with geographical location and season of the year (Mandel, 2009). The transmission of the infection mostly occurs via respiratory droplets of cough or sneezing from an infected person to a healthy one (Bessen, 2009). Since the bacteria existing in the throat of healthy children reflects the organisms responsible for respiratory infections in the society, therefore the information about the prevalence and antimicrobial resistance patterns of these organisms can be more helpful for successful treatment of streptococcal infections than invasive strains (Herruzo et al., 2002). Published data about rising Minimum Inhibitory Concentration (MIC) of penicillin in this organism has led the scientists to monitor the MIC level of penicillin for this organism, however, penicillin G is the drug of choice for treatment and there has not been any report about the resistance to this antibiotic until now (Ray et al., 2010). Moreover, failure of penicillin to eliminate *S.pyogenes* from the throat of patients, resulted to *S.pyogenes* carriers, has been increasingly reported. Alternatively, macrolides and lincosamides are used as the second choice of drug for the treatment in these and allergic patients (Richter et al., 2005). In this study, we investigated the colonization rate and sensitivity pattern to penicillin clindamycin and several antimicrobials among *S.pyogenes* isolates detected from healthy preschool and primary school age children in Tehran, Iran.

## 2. Materials and Methods

### 2.1. Study population

The study included all primary school age children referring to the follow up center of Ali-Asghar pediatric hospital and students of two schools, aged between 5-15 years during a 6 months period, from the January to July 2011. The total numbers of 423 non duplicate sample swabs were collected.

### 2.2. Exclusion criteria

Children who exhibited any signs of acute or chronic inflammatory disease, those who have been recorded for an underlying disease, or benefited from any antimicrobial therapy during the past 3 months, or have been hospitalized

during the last year or had shown any signs of upper respiratory tract infections, were excluded from the study (Ioannidou, 2001).

### 2.3. Sample collection

Demographic information of the students was obtained. This information were gender, age, family history of an acute rheumatic fever, a history of antibiotic usages and their living place. Throat samples were taken by a sterilized cotton swab, from the oropharynx of students and seeded immediately onto a sector of a sheep blood agar medium, and then transferred to the laboratory within a maximum time of 2 hours.

### 2.4. Samples preparation

Samples were streaked on 5% sheep blood agar medium (Himedia) and then placed into a candle jar containing 5 to %10 CO<sub>2</sub> and then incubated at 37°C for an overnight. Beta hemolytic colonies were selected for identification by gram staining and Catalase test, sensitivity to bacitracin (0.04U) and cotrimoxazole test (Mast Diagnostics, UK) (Ioannidou, 2001). Then, the Pyrrolidonyl Arylamidase (PYR) Test (PYR Rosco Taastrup, Denmark) was done for bacitracin sensitive and cotrimoxazole resistant isolates. PYR positive results were identified as *S. pyogenes* (Murray et al., 2003) and latex agglutination test (Avipath-Strep Omega Diagnostics-United Kingdom) was also performed in order to serologically confirmation of the *S.pyogenes* identification test results.

### 2.5. Antimicrobial susceptibility testing

Antimicrobial sensitivity testing was done by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines on Muller Hinton agar medium supplemented with 5% sheep blood (CLSI, 2013). *Streptococcus pneumonia* ATCC 49619 was used as a quality control strain. The antibiotic disks (Mast Diagnostics, UK) used in this study were penicillin G 10 unit, Cefotaxim 30µg, Vancomycin 30µg, Tetracycline 30µg, Chloramphenicol 30µg, Erythromycin 15µg, Azithromycin 15µg, Clindamycin 2µg, Ofloxacin 5µg.

## 2.6. MIC determination

The MIC of Penicillin was measured for all isolates to monitor MIC levels and also to confirm the disk diffusion results by the E-test method using E-test strips (Liophilchem, Via Scozia, Roseto d. A. Italy) (0.016-256µg/ml). Also the MIC level of erythromycin and clindamycin were determined by using E-test strips (0.016-256µg/ml) for all isolates according to the manufacturer recommendations (AB Biodisk, genetix, Biotech Asia Pvt. Ltd).

## 2.7. Statistical analysis

Data collected from the epidemiological surveys and throat swab samples were recorded using Microsoft excel program version 2010, and the statistical analysis of the population was done by the SPSS version21 (IBM SPSS, Chicago, IL, USA) and p-values of  $\leq 0.05$  were considered as significant.

## 3. Results

### 3.1. Sample collection

A total number of 423 sample swabs were obtained which 5.7% (n=24) of isolates were identified as Group A streptococci. This study

population was sub grouped to 5-8, 9-12, and 13-15 year old in order to evaluate the influence of the age on the carriage rate. According to the given information, 9, 12 and 3 isolates were related to 3 age sub groups respectively, which are shown in table 1. Chi-square test showed that there were no differences of carriage rates between the age sub groups (P=0.095).

### 3.2. Antimicrobial susceptibility testing results

All isolates were sensitive to penicillin, cefotaxime, erythromycin, vancomycin, azithromycin and Clindamycin. The prevalence of Intermediate sensitivity and resistance to tetracycline was 25 and 12.5%, respectively. Two isolates had intermediate sensitivity to ofloxacin and chloramphenicol. Antimicrobial susceptibility testing results are shown in Table 2.

### 3.3. MIC level measurement:

Penicillin MICs for all isolates were in the range of  $\leq 0.016$  µg/ml. MIC levels of clindamycin and erythromycin also were in sensitive range  $\leq 0.25$ µg/ml.

**Table 1.** prevalence of the positive cultures for *S. pyogenes* cases between age subgroups

age sub group	positive cultures for <i>S.pyogenes</i> cases %(n)	Total % (n)
5-8	37.5 (9)	36 (153)
9-12	50 (12)	52 (219)
13-16	12.5 (3)	12 (51)
Total	100 (24)	100 (423)

**Table 2.** Antibiotic susceptibility results for *Streptococcus pyogenes* isolates

	Susceptible % (n)	Intermediate % (n)	Resistant % (n)
penicillin G 10 unit	100 (24)	0 (0)	0 (0)
Cefotaxim 30µg	100 (24)	0 (0)	0 (0)
Vancomycin 30µg	100 (24)	0 (0)	0 (0)
Tetracycline 30µg	62.5 (15)	25 (6)	12.5 (3)
Chloramphenicol 30µg	92 (22)	8 (2)	0 (0)
Erythromycin 15µg	100 (24)	0 (0)	0 (0)
Azithromycin 15µg	100 (24)	0 (0)	0 (0)
Clindamycin 2µg	100 (24)	0 (0)	0 (0)
Ofloxacin 5µg	92 (22)	8 (2)	0 (0)

## 4. Discussion

Streptococcal pharyngitis is one of the most common bacterial infections among children aged between 5 to 15 years old that has the most

prevalence in the primary years of the school and will gradually decrease by aging (Shulman et al., 2012). 5.7% of the studied population in this research were identified as asymptomatic carriers, slightly similar to that of some other

cities in Iran such as %4.86 (Tavakkoli et al., 2003), and 4.3 % (Kheshabi et al., 2003), or 6.96% (Nowroozi et al., 2009), but different from a northern city of Iran which is 11.8 % (Ghaemi et al., 2000) and Tehran which is 21.4% (Jasir et al., 2000a). This variation in the rates of carriage between different studies could be due to the differences in geographical locations (Mandel et al., 2009). Different geographical locations reflect the different demographic situation and regional climate. For example the occurrence of pharyngeal carriage is more common in a city with moderate climate, while in tropical areas skin infection caused by this organism is more common. Additionally the mode of the transmission of the organism which is transmitted by respiratory droplets from an infected person to a healthy one is affected by the population of the area. For example in crowded societies, the occurrence rate of infection and subsequently carriage of this organism is more than less crowded areas (Mandell, 2009; Ghaemi et al., 2000).

Also the source of the sampling in the throat (tonsils, oropharynx, nasopharynx), is an effective factor in the differences while our samples were mostly taken from the oropharynx.

No difference of carriage rates between the three age subgroups were seen in this study. According to Rijal et al, 11.8 % of the carriers belonged to 5-8 year olds that were higher than of the two other sub groups 9-12 (7.8%), and 13-15 (8.2%) year olds (Rijal et al., 2009).

In comparison to the results of this study, the study by Jasir et al demonstrated that penicillin MICs for all isolates were less than  $\leq 0.25\mu\text{g/ml}$  proving all isolates were sensitive to penicillin (Jasir et al., 2000b). In another study in Iran the levels of Penicillin MICs were measured in the sensitive range (0.02-0.0025 $\mu\text{g/ml}$ ) (Kamaly et al., 2001). Although there are some reports primarily about the rising of penicillin MICs (Ray et al., 2010; Capoor et al., 2006), but there is not any reports proving resistance to the penicillin, proving that the antibiotic has been remained as the first choice for treatment.

Resistance to erythromycin as the second choice of drug, especially in allergic patients has been reported in several countries, particularly in those the drug has been over used (Jasir et al., 2000b). Jasir et al reported that 0.2% of resistance to erythromycin among isolates which is lower than other studies done in some other

parts of the world (Jasir et al., 2000b) but it should be noted that there is a wide heterogeneity of resistance in different parts of the world (Nielsen et al., 2004). The lack of resistance to erythromycin in our study may be due to a low isolation rate. Although there is a low ratio of resistance to erythromycin reported in Iran at this time (Tavakkoli et al., 2003; Kheshabi et al., 2003; Nowroozi et al., 2009; Ghaemi et al., 2000; Kamaly et al., 2001). It have been shown that Iran is one of the countries with a high rate of antibiotic consumption, which in turn leads to the risk of antibiotic resistance in the near future.

The tetracycline and erythromycin co-resistance occurs via the transposons carrying both tetracycline and erythromycin resistant genes, suggesting that resistance to one of the agents enhances development of resistance to the other one (Pavlovic et al., 2010). Therefore, 12.5% (n= 3) of tetracycline resistant isolates in this research may be an alarm for developing resistance to erythromycin in the future. In the study of Jasir et al, two isolates of the three erythromycin resistant isolates were also resistant to tetracycline with the rate of erythromycin resistance level being at 0.2%, while 42% of all *S.pyogenes* isolates were resistant to tetracycline (Jasir et al., 2000b). According to a study performed in Serbia, nine isolates from the total of 216 (6.8%) erythromycin resistant isolates, showed co-resistance to tetracycline (Pavlovic et al., 2010). According to a study investigating on macrolide and tetracycline co-resistance in *S.pyogenes* in Denmark, Tetracycline resistance was found in 52% of the total of macrolide-resistant *S.pyogenes* (Nielsen et al., 2004). Another study in Italy demonstrated simultaneous erythromycin and tetracycline co-resistance in clinical isolates of *S. pyogenes* (Giovanetti et al., 2003).

## 5. Conclusion

This study showed that Although penicillin, macrolides and lincosamides can be considered as the useful drugs for the treatment of streptococcal infections, but it is advisable to organize these researches more routinely in Iran to illustrate the demographic and microbiological changes about the rate of

carriage, and resistance patterns of the organism in order to monitor the resistance levels in the society.

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