

ANTIBIOTIC SUSCEPTIBILITY OF STREPTOCOCCUS PYOGENES STRAINS ISOLATED FROM THROAT CULTURES OF CHILDREN WITH TONSILLOPHARYNGITIS

Ergin Çiftçi* ❖ Ülker Doğru* ❖ Haluk Güriz* ❖ Ahmet Derya Aysev* ❖ Erdal Ince*

SUMMARY

Streptococcus pyogenes is the most important causative agent of tonsillopharyngitis. Although penicillin is drug of choice, and macrolide antibiotics are recommended drugs in patients who have penicillin allergy, various antibiotics other than penicillin and macrolide antibiotics are also used in the treatment of streptococcal tonsillopharyngitis. In addition, resistance to macrolide antibiotics is an important problem in some regions of the world. For these reasons, we designed a study to determine the antibiotic susceptibility of *S. pyogenes* strains isolated from children with tonsillopharyngitis. Two hundred and sixty three *S. pyogenes* strains were examined for penicillin, ampicillin, cefazolin, cefuroxime, ceftriaxone, erythromycin, clarithromycin and azithromycin, clindamycin, ofloxacin, and vancomycin susceptibility. Tolerance against penicillin was also investigated. All *S. pyogenes* strains were determined to be susceptible to penicillin, ampicillin, cefazolin, cefuroxime, ceftriaxone, ofloxacin, and vancomycin. Resistance to erythromycin, clarithromycin, azithromycin, and clindamycin were detected as 3.8%, 4.2%, 4.2%, and 3.0%, respectively. Penicillin tolerance wasn't determined. These data indicate that antibiotic resistance of *S. pyogenes* strains is not a clinically significant problem in Turkey.

Key Words: *Streptococcus Pyogenes*, Tonsillopharyngitis, Antibiotic Susceptibility.

ÖZET

Tonsillofarenjitli Çocukların Boğaz Kültürlerinden İzole Edilen Streptococcus Pyogenes Suşlarının Antibiyotik Duyarlılığı

Streptococcus pyogenes tonsillofarenjitin en önemli etkenidir. Tedavide birinci seçenek penisilin ve penisilin allerjisi olanlarda makrolid antibiyotikler önerilen ilaçlar olmasına karşın streptokoksik tonsillofarenjit tedavisinde penisilin ve makrolid antibiyotikler dışında antibiyotikler de kullanılmaktadır. Ayrıca dünyanın bazı bölgelerinde makrolid antibiyotiklere direnç önemli bir sorundur. Bu nedenle tonsillofarenjitli çocukların boğaz kültürlerinden izole edilen *S. pyogenes* suşlarında antibiyotik duyarlılığını saptamayı amaçlayan bir araştırma planladık. Toplam 263 *S. pyogenes* suşu penisilin, ampisilin, sefazolin, sefuroksim, seftriakson, eritromisin, klaritromisin, azitromisin, klindamisin, ofloksasin ve vankomisin duyarlılığı açısından araştırıldı. Penisiline karşı tolerans da araştırıldı. Bütün *S. pyogenes* suşları penisilin, ampisilin, sefazolin, sefuroksim, seftriakson, ofloksasin ve vankomisine karşı duyarlı bulundu. Eritromisin, klaritromisin, azitromisin, ve klindamisine karşı sırasıyla %3.8, %4.2, %4.2 ve %3.0 oranında direnç saptandı. Penisilin toleransı saptanmadı. Bu bulgular *S. pyogenes* suşlarında antibiyotik direncinin Türkiye'de klinik olarak önemli bir sorun olmadığını göstermektedir.

Anahtar Kelimeler: *Streptococcus Pyogenes*, Tonsillofarenjit, Antibiyotik Duyarlılığı.

Tonsillopharyngitis is one of the most common infections encountered in children. Most bacterial infections are caused by *Streptococcus pyogenes* (1). In addition, resurgence of severe forms of disease caused by

S. pyogenes has been detected in various parts of the world (2, 3). Although all *S. pyogenes* strains remain exquisitely sensitive to penicillin, erythromycin has been the drug of choice for individuals who cannot take penicillin. However,

* Ankara University Medical School, Department of Pediatrics, ANKARA, TURKEY

there is a concern that a significant incidence of erythromycin resistance of *S. pyogenes* has been reported from the various regions of the world (4-10). Because the nonsuppurative sequels of *S. pyogenes* infections are still an important problem in Turkey and invasive infections due to *S. pyogenes* have increased recently, the treatment of infections due to *S. pyogenes* is very important in our country (11). Because of these observations, it is important to determine the current status of antibiotic susceptibility of *S. pyogenes* strains.

Methods

Children with tonsillopharyngitis in Ankara University Faculty of Medicine, Department of Pediatrics between December 2000 and March 2001 were enrolled in the study. All children were examined by a pediatrician. Tonsillopharyngitis diagnosis was established based on symptoms such as fever, sore throat, headache and abdominal pain, and signs such as pharyngeal and tonsillar hyperemia, exudate and painful cervical lymphadenopathy.

Throat swabs were obtained from the children, and immediately cultured in Mueller-Hinton blood agar. After 24 hour incubation at 35°C, colonies surrounded by beta hemolysis were selected for grouping and antimicrobial susceptibility procedures. Strains were grouped by Streptococcal Grouping Kit (Oxoid, Diagnostic Reagents, Hampshire, UK). Group A beta hemolytic streptococci were analysed with respect to ampicillin, cefazolin, cefuroxime, ceftriaxone, erythromycin, clarithromycin, azithromycin, clindamycin, ofloxacin, and vancomycin susceptibilities using the agar dilution method. Penicillin G susceptibility was determined by broth dilution method. The minimal inhibitory concentration (MIC) limits for selected were determined according to the values determined by National Committee for Clinical Laboratory Standards (12). For cefazolin and cefuroxime for which there are no established NCCLS break points for *Streptococcus pyogenes*, NCCLS break points established for *Streptococcus pneumoniae* were used (12). Resistance limits determined as: penicillin G \geq

4.0 $\mu\text{g/ml}$, ampicillin \geq 8.0 $\mu\text{g/ml}$, cefazolin \geq 2.0 $\mu\text{g/ml}$, cefuroxime \geq 2.0 $\mu\text{g/ml}$, ceftriaxone \geq 2.0 $\mu\text{g/ml}$, erythromycin MIC \geq 1.0 $\mu\text{g/ml}$, clarithromycin MIC \geq 1.0 $\mu\text{g/ml}$, azithromycin MIC \geq 2.0 $\mu\text{g/ml}$, clindamycin \geq 1.0 $\mu\text{g/ml}$, ofloxacin \geq 8.0 $\mu\text{g/ml}$, and vancomycin \geq 2.0 $\mu\text{g/ml}$. Minimal bactericidal concentrations (MBC) for penicillin G were also determined (13). Penicillin tolerance was defined as an MBC-MIC ratio of greater than or equal to 32 (13).

Results

During the study period, 3127 children were diagnosed as tonsillopharyngitis. *S. pyogenes* were isolated from 345 throat swabs (11.0%). Because of contamination or missing of isolated strains, 263 *S. pyogenes* strains were found eligible.

Penicillin G susceptibility. All *S. pyogenes* strains were found to be susceptible to penicillin G. MIC values of *S. pyogenes* strains for penicillin G were determined as 0.0004-0.03 $\mu\text{g/ml}$ (Table 1). Tolerance to the penicillin G was not determined in any of the isolates (Table 2, and Figure 1).

Macrolide resistance in *S. pyogenes* strains. Out of 263 *S. pyogenes* strains, 10 (3.8%) were resistant to erythromycin, 11 (4.2%) were resistant to both clarithromycin and azithromycin (Table I).

Susceptibility to other antibiotics. All *S. pyogenes* strains were found to be susceptible to ampicillin, cefazolin, cefuroxime, ceftriaxone, ofloxacin and vancomycin. Eight of the 263 *S. pyogenes* strains (3%) were resistant to clindamycin (Table 1).

Discussion

Antimicrobial resistance is an important problem in the management of patients with infectious diseases. Interestingly, *Streptococcus pyogenes* remains susceptible to penicillin during the past 70 or 80 years. The reason for this unique lack of development of resistance to penicillin is unknown (14). On the other hand, numerous reports have demonstrated a significant prevalence of erythromycin resistant *S. pyogenes*

Table 1: Resistance to eleven antibiotics, MIC₉₀ values, and MIC ranges of 263 *S. pyogenes* strains.

Antibiotic	Resistant strains (n/%)	MIC ₉₀ (µg/ml)	MIC ranges (µg/ml)
Penicillin G	0/0	0.0075	0.0004-0.03
Ampicillin	0/0	≤ 0.125	≤ 0.125-0.25
Cefazolin	0/0	≤ 0.125	≤ 0.125-1.00
Cefuroxime	0/0	≤ 0.125	≤ 0.125-0.50
Ceftriaxone	0/0	≤ 0.125	≤ 0.125-≤ 0.25
Ofloxacin	0/0	1.0	≤ 0.50-1.0
Vancomycin	0/0	≤ 0.25	≤ 0.25-0.50
Clindamycin	8/3.0	0.25	≤ 0.25->2.0
Erythromycin	10/3.8	≤ 0.125	≤ 0.125-2.0
Clarithromycin	11/4.2	0.25	≤ 0.125-2.0
Azithromycin	11/4.2	0.25	≤ 0.125->2.0

Table 2: Penicillin G MBC/MIC ratios of *S. pyogenes* strains.

MBC/MIC	Number of strains
1	246
2	15
4	2
8	0
16	0
32	0
Total	263

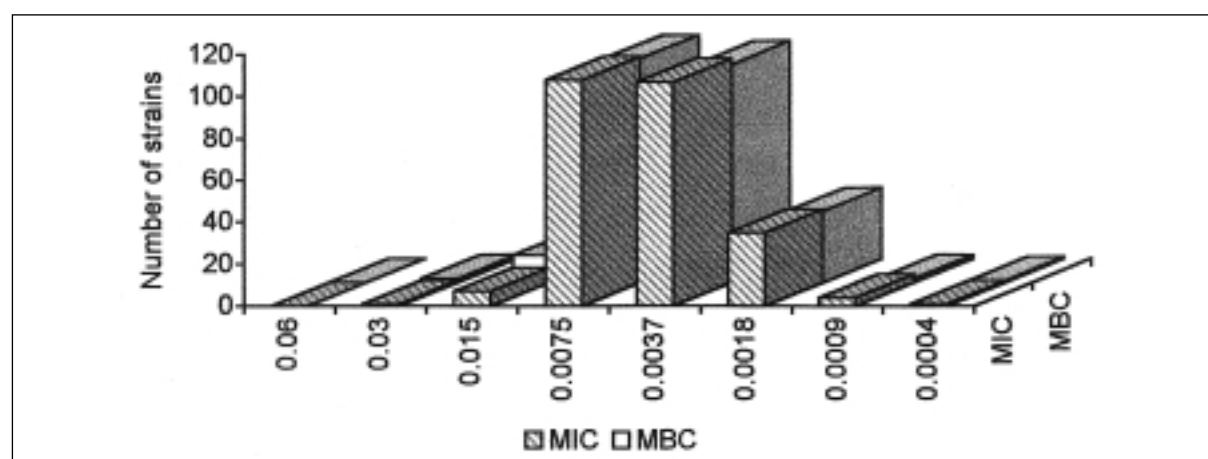


Figure 1: Penicillin G MIC and MBC values of *S. pyogenes* strains.

around the world during the past three decades (4-10). This resistance has been temporally related to increased or excessive use of macrolide antibiotics. Because of this relation, it is important to determine the geographic

prevalence of resistant *S. pyogenes* to facilitate clinical care and to address public health concerns.

Our study shows that penicillin is active for *S. pyogenes* in low MIC values. This finding is

concordant with other studies from around the world (15-17). We also could not determine any *S. pyogenes* strain tolerant to penicillin G. Penicillin G MIC and MBC values of *S. pyogenes* were similar in our study. Penicillin failure in streptococcal tonsillopharyngitis reported as 10-25% (18, 19). Some authors blamed penicillin tolerance in patients with penicillin failure (20, 21). Although we could not determine clinical response of our patients, our results do not support penicillin tolerance theory for penicillin failure.

Like various parts of the world, invasive infections due to *S. pyogenes* have increased recently in Turkey (unpublished data). Causative agents sometimes cannot be determined, and various antibiotics are chosen in these patients. Thus, it is important to know the current status of antibiotic susceptibility in *S. pyogenes* strains. Our study shows that many antibiotics are active for *S. pyogenes* strains. Although these antibiotics are not first choice, they can be used in invasive streptococcal infections.

Resistance to erythromycin, clarithromycin, azithromycin, and clindamycin were detected as

3.8%, 4.2%, 4.2%, and 3.0%, respectively in this study. During the past three decades, numerous reports have demonstrated a significant prevalence of erythromycin resistant *S. pyogenes* around the world (4-10). Furthermore, several reports have demonstrated that the increase in the incidence of erythromycin resistant *S. pyogenes* strains is related to increased macrolide consumption in the community (22-26). Macrolide antibiotics, especially new ones such as clarithromycin and azithromycin, are widely selected for the treatment of upper respiratory tract infections such as sore throat in our country. In a recent study from Ankara, erythromycin resistant *S. pyogenes* strains were increased from 3.29% to 15.74% in a 7-year-period²⁶. The authors emphasized that a substantial increase in erythromycin resistance was associated with the increase in the consumption of macrolide antibiotics.

Our results demonstrate that antibiotic resistance of *S. pyogenes* strains is not a clinically significant problem in Turkey. However, the susceptibility pattern of *S. pyogenes* strains must be monitored.

REFERENCES

1. Bisno AL, Gerber MA, Gwaltney JM, Kaplan EL, Schwartz RH. Diagnosis and management of Group A streptococcal pharyngitis: a practice guideline. *Clin Infect Dis* 1997; 25: 574-583.
2. Givner LB, Abramson JS, Wasilaukas B. Apparent increase in the incidence of invasive group A beta-hemolytic streptococcal disease in children. *J Pediatr* 1991; 118: 341-346.
3. Stevens DL. Invasive group A streptococcal infections: the past, present and future. *Pediatr Infect Dis J* 1994; 13: 561-566.
4. Maruyama S, Yoshioka H, Fujita K, Takimoto M, Satake Y. Sensitivity of group A streptococci to antibiotics: prevalence of resistance to erythromycin in Japan. *Am J Dis Child* 1979; 133: 1143-1145.
5. Zackrisson G, Lind L, Roos K, Larson P. Erythromycin resistant α -hemolytic streptococci group A in Göteborg, Sweden. *Scand J Infect Dis* 1988; 20: 419-420.
6. Trallero EP, Arenzana JMG, Egana MU. Erythromycin resistance in streptococci. *Lancet* 1989; ii: 444-445.
7. Linares J, Pallares R, Alonso T, Perez JL, Ayats J, Gudiol F, Viladrich PF, Martin R.. Trends in antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitge Hospital, Barcelona, Spain (1979-1990). *Clin Infect Dis* 1992; 15: 99-105.
8. Hsueh PR, Chen HM, Huang AH, Wu JJ. Decreased activity of erythromycin against *Streptococcus pyogenes* in Taiwan. *Antimicrob Agents Chemother* 1995; 39: 2239-2242.
9. Wu JJ, Lin KY, Hsueh PR, Liu JW, Pan HI, Sheu SM. High incidence of erythromycin-resistant streptococci in Taiwan. *Antimicrob Agents Chemother* 1997; 41: 884-886.
10. Cornaglia G, Ligozzi M, Mazzariol A, Masala L, Lo Cascio G, Orefici G, Fontana R. Resistance of *Streptococcus pyogenes* to erythromycin and related antibiotics in Italy: The Italian Surveillance Group for Antimicrobial Resistance. *Clin Infect Dis* 1998; 27: S87-S92.
11. Karademir S, Demirçeken F, Atalay S, Demircin G, Sipahi T, Teziç T. Acute rheumatic fever in children in the Ankara area in 1990-1992 and comparison with a previous study in 1980-1989. *Acta Paediatr* 1994; 83: 862-865.
12. National Committee for Clinical Laboratory Standards. *Minimum inhibitory concentration (MIC) interpretive standards (μ G/ML) for Streptococcus Spp.* National Committee for Clinical Laboratory Standards, Vol. 17, No. 2. M100-S7, Villanova, PA, 1997.
13. Wittler RR, Yamada SM, Bass JW, Hamill R, Wiebe RA, Ascher DP. Penicillin tolerance and erythromycin resistance of group A beta hemolytic streptococci in Hawaii and Philippines *AJDC* 1990; 144: 587-589.
14. Horn DL, Zabriskie JB, Austrian R, Cleary PP, Ferretti JJ, Fischetti VA, Gotschlich E, Kaplan EL, McCarty M, Opal SM, Roberts RB, Tomasz A, Wachtfogel Y. Why have group A streptococci remained susceptible to penicillin? Report on a symposium. *Clin Infect Dis* 1998; 26: 1341-1345.
15. Bass JW, Weisse ME, Plymyer MR, Murphy S, Eberly BJ. Decline of erythromycin resistance of group A beta-hemolytic streptococci in Japan: comparison with worldwide reports. *Arch Pediatr Adolesc Med* 1994; 148: 67-71.
16. Betriu C, Sanchez A, Gomez M, Cruceyra A, Picazo JJ. Antibiotic susceptibility of group A streptococci: a 6-year follow-up study. *Antimicrob Agents Chemother* 1993; 37: 1717-1719.
17. Kaplan EL, Johnson DR, Del Rosario MC, Horn DL. Susceptibility of Group A beta-hemolytic streptococci to thirteen antibiotics: examination of 301 strains isolated in the United States between 1994 and 1997. *Pediatr Infect Dis J* 1999; 18: 1069-1072.
18. Stillerman M. Comparison of oral cephalosporins with penicillin therapy for group A streptococcal pharyngitis. *Pediatr Infect Dis* 1986; 5: 649-654.
19. Holm S, Henning C, Grahn E, Lomberg H, Staley H. Is penicillin the appropriate treatment for recurrent tonsillopharyngitis? Results from a comparative randomized blind study of cefuroxime axetil and phenoxymethylpenicillin in children. The Swedish Study Group. *Scand J Infect Dis* 1995; 27: 221-228.
20. Grahn E, Holm SE, Roos K. Penicillin tolerance in beta streptococci isolated from patients with tonsillitis. *Scand J Infect Dis* 1987; 19: 421-426.
21. Kim KS, Kaplan EL. Association of penicillin tolerance with failure to eradicate group A streptococci from patients with pharyngitis. *J Pediatr* 1985; 107: 681-684.

22. Phillips G, Parratt D, Orange GV, Harper J, McEwan H, Young N. Erythromycin-resistant *Streptococcus pyogenes*. J Antimicrob Chemother 1990; 25: 723-724.
23. Seppala H, Klaukka T, Lehtonen R, Nenonen E, Huovinen P. Outpatient use of erythromycin: link to increased erythromycin resistance in group A streptococci. Clin Infect Dis 1995; 21: 1378-1385.
24. Seppala H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, Huovinen P. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland: Finnish Study Group for Antimicrobial Resistance. N Engl J Med 1997; 337: 441-446.
25. Baquero F, Garcia-Rodriguez JA, de Lomas JG, Aguilar L. Antimicrobial resistance of 914 beta-hemolytic streptococci isolated from pharyngeal swabs in Spain: results of a 1-year (1996-1997) multicenter surveillance study: The Spanish Surveillance Group for Respiratory Pathogens. Antimicrob Agents Chemother 1999; 43: 178-180.
26. Kürekçi AE, Baysallar M, Karaarslan A, Emekdaş G, Köseoğlu V, Akın R, Özcan O. The frequency of resistance of erythromycin in group A streptococci in Ankara. Eur J Pediatr 1996; 155: 780-782.