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Erythromycin Resistance Group A Streptococcus Associated with Acute Tonsillitis and Pharyngitis

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Abstract: Group A streptococci is the most important causative agent of tonsillo-pharyngitis. Erythromycin is drug of choice inpatients who are allergic to β-lactams. The aim of this study was to determine the prevalence of streptococcus pyogenes resistant to erythromycin and other commonly prescribed antimicrobial agents. This prospective study conducted in Mazandaran province teaching hospitals. Patients with fever and tonsillo-pharyngitis were enrolled: Throat culture was obtained at the time of admission. Antimicrobial susceptibility test was done. All 85 isolates of streptococcus pyogenes were sensitive to penicillin, amoxicillin, cephalexin. and clindamycin however 3 cases had poor response to penicillin therapy. The resistance rate to erythromycin, tetracycline and ceftriaxone were 28, 46 and 3.5%, respectively.

Key words: Pharyngitis, Group A streptococci, erythromycin resistance, acute tonsillitis

INTRODUCION

Group A β-Haemolytic Streptococci (GABHS) are the most frequent and important cause of bacterial pharyngitis in children and adults Gwaltney and Bisno (2000). Since, fifty years ago GABHS remained highly susceptible to penicillin. Although in vitro penicillin resistance has not yet been described in GABHS (Betriu *et al*., 1993) recently there are increasingly reports of treatment failures of up to 30% in some studies (Pichichero *et al*., 2002) causing uncertainties in the use of penicillin as empiric treatment in recurrent GABHS infections . Some studies showed that the majority of GABHS isolates were susceptible to erythromycin, which was the main alternative to penicillin in treatment of the GABHS tonsillo-pharyngitis inpatients who are allergic to β-lactams(Bass, 1991). In vitro resistance to macrolides was not uncommon (Sauermann *et al*., 2003). It was described in the UK in the late 1950 (Lowbury and Hurst, 1959) since then, resistance rates of 5% to near 50% have been reported in different studies(Sauermann *et al*., 2003; Ciftci *et al*., 2002; Weiss *et al*., 2001). In the majority of these studies, resistance has been linked to erythromycin consumption (Martin *et al*., 2002; Seppala *et al*., 1997). Antibiotics not subjected to inactivation by β-lactamases, i.e., amoxicillin clavulanate, oral cephalosporin, as well as erythromycin, are preferred by some clinicians in the treatment of GABHS, however reports of the emergence of erythromycin resistance GABHS through past decades and lack of comprehensive erythromycin resistant GABHS in our country alerts consideration on the antibiotic susceptibility patterns of these pathogens. The aim of this study was to determine the prevalence of streptococcus pyogenes resistant to erythromycin and 6 other commonly prescribed antimicrobial agents namely penicillin, cephalexin, amoxicillin, clindamycin, tetracycline and ceftriaxone.

MATERIALS AND METHODS

This prospective study carried out from November 2003 to April 2005 involved 2 teaching hospitals that provided pediatric and adult infectious disease tertiary care of Mazandaran province. Patients were enrolled at the time of admission to the emergency departments as well as out patient departments if they were aged 2 years and older with a clinical presentation consistent with clinical criteria for tonsillo-pharyngitis including fever>38°C and pharyngeal erythema and absence of cough.

Data collected at the time of enrolment included: age, sex, antibiotic therapy before admission, symptoms, recurrence of tonsillo-pharyngitis and history hospital admission.

Patients who received antibiotic therapy for current tonsillo-pharyngitis and those who were hospitalized within 30 days prior to admission were excluded.

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Throat culture was obtained at the time of admission by swabbing both tonsils and the posterior pharynx with a rayon tipped swab. The swab then placed in a medium consist of nutrient broth, transported to the laboratory and cultured within 2 h. The swab was plated onto 5% sheep's-blood agar.

The agar was tabbed and a bacitracin disc was placed in the primary streak. The plate was incubated at 37°C in 5% carbon dioxide and was examined at 24 and 48 h. B-Hemolytic colonies were sub cultured, isolated and then typed and confirmed by standard methods. Isolates of group A streptococci cultured on müller hinton agar and antimicrobial susceptibility test was done by using kerby Bauer disc diffusion test based on NCCLS criteria (NCCLS, 2002) with current disc(supplied with padtan teb company): Erythromycin (15 μg) penicillin (100 μg)-tetracycline (30 μg)-Amoxicillin (10 μg)-cephalexin (30 μg) -ceftriaxone (30 μg) and clindamycin (2 μg). Antimicrobial resistance pattern determined based on inhibition zone diameter by using NCCLS criteria (2003). The Minimal Inhibitory Concentrations (MICs) were then determined by a broth micro dilution method using Mueller-Hinton broth in 12 tube of mentioned antibiotics. MICs were recorded as the lowest concentration permitting no visible growth. Testing and quality control were carried out following NCCLS criteria (2002).

The double disk-diffusion method using erythromycin (15μg) and clindamycin (2μg) was used to identify the mechanism of resistance of all isolates of erythromycin resistant GABHS.

All throat swabs were obtained by trained laboratory technicians and the processing of throat cultures was overseen by a microbiologist.

Patients with erythromycin resistance were treated with penicillin, amoxicillin or clindamycin depending on susceptibility pattern and body weight.

RESULTS AND DISCUSSION

The study population of 400 consecutive patients with tonsillo-pharyngitis consisted of 252 male (63%) and 148 female (37%) ranging in age from 2-56 years with mean, of16 years. More than two-third of the patients were 5-25 years. Eighty-five (21.3%) were positive for group A streptococci., only 4 patients hospitalized due to sever illness diagnosed by emergency physician. Latter was found that 3 of them were erythromycin resistant. Fifty-two (61.2%) patients presented with bilateral spotty exudates on tonsils and 22 (25.9%) patients presented with lymphadenopathy. Clinically patients with erythromycin-resistant GABHS isolates were indistinguishable from those with erythromycin susceptible.

In 24/85 (28%) patients erythromycin-resistant GABHS (MIC:.032-64 μg mL^{-1}) were isolated from throat cultures, All strains were susceptible to penicillin (MIC:0.002-0.032 μg L^{-1}) .The MICs of the different antimicrobial agents are given in Table 1. Nevertheless 4 patients had clinical failure after treatment with intramuscular injection of benzathine penicillin with appropriate adjusted dose. Three out of 4 patients with poor response to penicillin therapy had erythromycin resistant GABHS throat culture. Double diffusion test indicated the presence of the M phenotype of all erythromycin resistance GABHS.

Group A β-Hemolytic Streptococci (GABHS) are in the main list of pathogens, causing an extensive range of clinical manifestations above all tonsillo-pharyngitis. Prompt initiation of proper and effective antibiotic therapy will prevent suppurative and some non-suppurative complications of tonsillo-pharyngitis with group A streptococci. It will also reduce the pool of patients from which adult and other children acquire their infection. One of the important points in the treatment of pharyngitis is, to identify the susceptibility pattern of streptococci group A to commonly prescribed antibiotics.

In a study of 500 throat cultures which isolated from patients with acute pharyngitis Kamali *et al.* (2001) noted all 44 GABHS were susceptible to penicillin, amoxicillin and erythromycin. Kohanteb *et al.* (2004) studied sensitivity pattern of GABHS isolated from patients with various streptococcal infection to penicillin and other commonly used antibiotics in Shiraz and noted that 63(15.4%) out of 410 children with acute pharyngitis had positive throat culture of GABHS which all were susceptible to penicillin, 58 (92%) were susceptible to erythromycin and 49 (77.8%) to tetracycline (Kamali *et al.*, 2001; Kohanteb *et al.*, 2004). Although studies in our country have carried out in different provinces it is useful to compare the result of the current study with those of two other studies. First study conducted 6 years ago with 100% susceptibility of GABHS to erythromycin, second study carried out 2 years ago showed 8% resistance of GABHS to erythromycin and current study is shown more than 20% resistance. Although these studies including current study couldn't be generalized, conducted in different regions of country and are unrelated studies, but they show an increasing trend of erythromycin resistance in the country.

Erythromycin resistant GABHS frequency varies notably in different studies from different countries, ranging from 0.45% (Lazarevic *et al.*, 2004) to 48% (Martin *et al.*, 2002). Even the frequencies vary in the same country in different periods of study. In Italy, the resistance rate to erythromycin was less than 10% until 1993, but it increased up to 30.7% in 1995 and 35.8% in

Table 1: Susceptibility pattern of 85 isolates of GAS obtained from pharyngeal samples

Anti-microbial agent	Range of MICs ($\mu g\ mL^{-1}$)	No.(%) of strains		
		Susceptible	Intermediate	Resistant
Penicillin	0.002-0.032	85(100)	0(0)	0(0)
Erythromycin	0.064-64	58(68)	3(3.5)	24(28)
clindamycin	0.064-128	85(100)	0(0)	0(0)
ceftriaxone	0.25-4	52(96.5)	3(305)	0(0)
Amoxicillin	0.008-0.016	58(100)	0(0)	0(0)
cephalexin	0.01-0.2	85(100)	0(0)	0(0)
tetracycline	0.128-64	50(59)	5(6)	30(35)

2002 (Dicuonzo *et al*., 2002). Bingen *et al*. (2004) in a recent study of the emergence of macrolide-resistant streptococcus pyogenes strains in French children noted that 72/322 (22.4%) of GABHS isolated from throat culture of children 2-16 years of age with pharyngitis, were resistant to erythromycin. This study showed a threefold increase in the prevalence of erythromycin resistance among GABHS in France compared to the result of their previous study in 2000 (Bingen *et al*., 2000). The prevalence of erythromycin resistant GABHS (22.4%) in recent French study (Bingen *et al*., 2004) is comparable with our study frequency rate of (21.3%), as is sensitivity pattern that all strains in both studies were susceptible to penicillin and amoxicillin, however in our study all isolated organisms were sensitive to clindamycin whereas in French study there were 16% resistance rate to clindamycin. The major limitation of our study was that we couldn't able to determine genotyping or Pulsed-Field Gel Electrophoresis (PFGE) however we were able to indicate the M phenotype (Sauermann *et al*., 2003) of erythromycin resistance.

In French study investigations showed that the main resistance mechanism was gene *erm* B (Bingen *et al*., 2004) Although in USA (Martin *et al*., 2002) Canada (Weiss *et al*., 2001), Italy (Dicuonzo *et al*., 2002). Genes that were leading cause of GABHS resistance were different types from what in French observed, but the emergence of macrolide resistance were due to dissemination of a limited number of clones in all studies.

Martin *et al*. (2002) in a study of erythromycin resistant group A streptococci in school children in Pittsburgh noted 153/318 (48%) GABHS isolated were resistance to erythromycin, belonged to M phenotype and all were susceptible to clindamycin as we found in current study. They noted the emergence of erythromycin resistant GABHS correlated with increasing use of macrolide antibiotics and specifically with the wide use of short courses of azithromycin for respiratory tract infections in both children and adults.

All of the GABHS isolates in this study were susceptible to penicillin *in vitro*, but in four patients we noticed clinical failure of penicillin treatment. This phenomenon has been attributed in some studies (Pichichero *et al*., 2002; Bass, 1991) to co-pathogenicity with β-lactamase producing microorganism and also penicillin tolerance, whereby streptococcal bacteria frequently exposed to sub-lethal concentrations of antibiotics become increasingly resistant to eradication. The susceptibility of all isolates to penicillin in the current study validate the practice of using this drug as a first line therapy for streptococcus pyogenes infections and not performing routine in virto susceptibility testing unless a patients is allergic to β-lactam agents. The strength of the current study is that at least in authors knowledge it is the first report of high rate of erythromycin resistance GABHS in our country. It warns the necessity of close monitoring of macrolide resistance in the country. We recommend that erythromycin not be used for the routine treatment of pharyngitis and/or tonsillitis due to group A streptococci until more epidemiological information is available or unless susceptibility testing is first performed. The result of present study showed that clindamycin could be a suitable alternative for treatment of acute GABHS tonsillo-pharyngitis in penicillin allergic patients. Longitudinal surveillance study is needed to determine the trend of such resistance pattern and physicians must know the prevalence of resistance in their local area.

CONCLUSION

The frequency of erythromycin resistant Group A β-haemolytic streptococci is high enough to concern about substitution of erythromycin to penicillin in patients who are allergic to β-lactams. It is necessary to do culture and susceptibility tests before using erythromycin

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