



Prevalence of Group A beta-haemolytic *Streptococcus* pharyngitis and drug resistance in community-acquired pharyngitis in Pakistan

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ABSTRACT

Streptococcus pyogenes infections have increased significantly in the last few decades. This increase in the infection rate is primarily due to development of resistance against macrolides and tetracyclines. A total of 250 pharyngeal swab samples were collected during four months time period. Samples were divided in to four groups; age group 1-4 years, 5-12 years, 13-18 years and ≥ 18 years. Isolates obtained were run for the evaluation of antibiotic resistance against ten antibiotics by using Kirby-Bauer's method/disc diffusion method. Out of 250 samples, 71 were confirmed as *S. pyogenes*. The prevalence of GAS pharyngitis in different age groups was; 16.0% in age group 1-4 years, 48.8% in age group 5-12 years, 14.8% in age group 13-18 years and 20.4% in age group ≥ 18 years. All the isolates were completely sensitive to penicillin, amoxicillin-clavulanic acid, ceftriaxone and vancomycin whereas erythromycin, azithromycin, tetracycline, clindamycin, chloramphenicol and levofloxacin resistance was recorded as 42.42%, 39.43%, 54.92%, 4.22%, 14.08% and 1.40% respectively. Data suggested that GAS pharyngitis was most prevalent in the age group 5-12 years and *S. pyogenes* was completely sensitive to penicillin and other cell wall synthesis inhibitors. However, higher resistance rates against macrolides (erythromycin and azithromycin) present an alarming situation in Pakistani population.

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Introduction

Pharyngitis is an inflammation of throat due to any infection which may result in fever, joint pain, muscle aches, skin rash and headache (1). Most of the acute pharyngitis cases are caused by viral infections whereas bacterial pharyngitis varies from 5%-17% of all the cases presented at health care facilities (2).

Patient's complaints in pharyngitis may include sore throat, fever, redness of pharynx and enlarged tonsils (1). The patients suffering from combination of four symptoms including fever, adenopathy of anterior cervical lymph nodes, tonsillar exudates and no cough are more likely to be suffering from GAS infection (3).

S. pyogenes is a gram positive, Lancefield group A Streptococcus (GAS) which causes acute pharyngitis, with estimate of about 600 million cases annually worldwide. The disease occurs in individuals of all ages but noted more frequently in school going children (4). Other causes of bacterial pharyngitis include Group C Streptococcus; 5% of the total cases, anaerobic species; about 1% and *Chlamydophila pneumonia* and *Mycoplasma pneumoniae* have a combined prevalence of 1%. Viral causes include Rhinovirus, Adenovirus and Coronavirus which account for about 30% of all the cases, Para-influenza and Influenza viruses account for 4% and Epstein Barr virus accounts for 1% of the total (1). GAS pharyngitis not only causes pain and discomfort in throat but also leads to the certain secondary conditions like peri-tonsillitis and otitis media (5).

GAS pharyngitis has a very high rate of person to person spread via direct contact and through aerosol transmission. It has high rate of spread from infected individual to other family members. It may also become a cause of outbreaks in nursing homes and even spread to healthcare professionals treating the patients with GAS pharyngitis (6). *S. pyogenes* may spread, by contamination of food at its final stage of preparation is of great importance. Food handlers having sore throat, skin infection and even asymptomatic carriers may transmit these bacteria to precooked and ready to eat food. GAS infections may also spread due to the insufficient refrigeration of raw food. Unrefrigerated or poorly refrigerated salad is more frequently associated with the spread of the disease (7).

S. pyogenes infection is treated with penicillins (penicillin G and amoxicillin) which are mostly seems an effective therapy. For individuals allergic to penicillin, macrolides are recommended including erythromycin and azithromycin while clindamycin is mostly recommended in patients suffering from recurrent infections of GAS (2).

S. pyogenes infections have increased significantly in the last few decades, which are primarily due to development of resistance against macrolides, and tetracyclines (8). There is very limited data related to prevalence of *S. pyogenes* associated pharyngitis in Pakistani population as well as antimicrobial resistance rates. The study was conducted in order to find out the prevalence of GAS pharyngitis in population and the assessment of most suitable antibiotic for its treatment based on obtained local resistance data. The

study also determined the prevalence of different phenotypic patterns of macrolide resistance in the pathogen.

Materials and Methods

Throat swab sample were taken from four different healthcare facilities of Faisalabad from May 2014 to August 2014. From all the four locations, individuals of all ages presenting with sore throat, fever and tonsillitis were enrolled. Patients using any antibiotic for the last two weeks, patients with history of previous rheumatic heart disease or rheumatic fever and patients with history of hospitalization were excluded from study. Exclusion criteria were intended to exclude patients who have a history of antibiotic use and who may not have a streptococcal pharyngitis but a carrier of *S. pyogenes*.

The ethics of the study were approved by the Pakistan Medical Research Council (PMRC), Research Centre Punjab Medical College, Faisalabad (Registration# 443/2014) (9,10). A total of 250 throat samples were collected by using sterile throat swabs. Prior to the enrolment of patients, informed consent was given by patients or by a parents/guardian for patients under the age of 18.

Standard posterior pharyngeal throat swab collection technique involving vigorous swab sampling of the posterior pharynx and bilateral tonsillar tissue, avoiding contact with the tongue, buccal surfaces, and lips was used (11,12,13). These throat swabs were then transported dry (not in a liquid medium) to Epidemiology and Public Health Laboratory, Institute of Microbiology, University of Agriculture, Faisalabad. Swab samples were inoculated on blood agar plates and incubated at 37°C for 24-48 hrs (14). The isolates were confirmed as *S. pyogenes* by colony morphology, microscopic characteristics, beta-hemolysis on blood agar, catalase test, PYR kit Test and bacitracin (0.04U) susceptibility test (15). All GAS isolates were then maintained as axenic cultures.

To perform antimicrobial susceptibility testing, rapidly growing colonies of *S. pyogenes* were used to prepare suspension solution of *S. pyogenes* colonies with comparable density to 0.5 MacFarland standard which contain approximately 10^8 CFU/ml, recommended for inoculation of growth on Mueller Hinton (5% Sheep Blood) agar for the performance of antimicrobial susceptibility test. Antibiotic susceptibility testing was performed by using Kirby-Bauer disk-diffusion method (16).

Antibiotic disks including Penicillin (10 units), Amoxicillin- Clavulanic acid (20/10 µg), Vancomycin (30 µg), Ceftriaxone (30 µg), Erythromycin (15 µg), Azithromycin (15 µg), Tetracycline (30 µg), Levofloxacin (5 µg) Chlarmphenicol (30 µg) and Clindamycin (2 µg) were purchased from HiMedia, India.

After the spreading 0.1ml of 0.5 MacFarland standard *S. pyogenes* suspension on Mueller-Hinton (5% sheep blood) agar, antibiotic discs were placed on the surface of the agar plate using a sterile forceps. When all the disks were placed, the lid was replaced and the plates were placed inverted in an incubator at 37°C for 16-18 hours. The zones of inhibition were measured after 18 hours of incubation at 37°C (16).

Discs containing erythromycin (15 µg) and clindamycin (2 µg) were used to identify phenotypic pattern of macrolide resistance in *S. pyogenes*. For finding the phenotypic basis of macrolide resistance the antibiotic discs were placed on blood agar at a distance of 20mm and the zone of inhibitions around the discs were measured for determining the macrolide resistance phenotype.

Results

A total of 250 patients were enrolled in the study. Patients were first examined and recommended by the physicians at all health care facilities. Patients of all age were included in the study, after which the data was divided in to four groups. The groups were made on the basis of age. The grouping criteria were followed from different research publication related to the topic of research. Four age groups were; Age group 1-4 years (age 1 ≤ to <5 years), Age group 5-12 years (age 5 ≤ to <13 years), Age group 13-18 years (age 13 ≤ to <18) and age group ≥18 Years. Overall prevalence of GAS pharyngitis was recorded as 28.8%. Out of 250 samples proportion of patients with GAS pharyngitis differ significantly among different age groups; 16.0% in age group 1-4 years, 48.8% in age group 5-12 years, 14.8% in age group 13-18 years and 20.4% in age group ≥18 years with an The data suggest highest proportion of cases; 48% in age group 5-12 years children. The proportion of female patients also differ in different age groups; 50.5% in age group 1-4 years, 44.3% in age group 5-12 years, 48.7% in age group 13-18 years and 42.7% in age group ≥18 years. The proportion of patients with culture positive GAS pharyngitis varied significantly among different age groups; 21.05% in age group 1-4 years, 36.88% in age group 5-12 years, 24.32% in age group 13-18 years and 18.36% in age group ≥18 years ($X^2=8.91$, $p=0.0305$).

Antimicrobial susceptibility testing was performed against ten antibiotics including penicillin, co-amoxiclave, vancomycin, ceftriaxone, erythromycin, azithromycin, tetracycline, levofloxacin, chloramphenicol and clindamycin. All the isolates were completely sensitive against penicillin, co-amoxiclave, ceftriaxone and vancomycin. Highest antibiotic resistance was noted against erythromycin, azithromycin and tetracycline; 42.42%, 39.43% and 54.92% respectively. It's also one of highest reported resistance GAS against these antibiotics. Resistance against other antibiotics were; levofloxacin 1.40%, chlarmphenicol 14.08% and clindamycin 4.22%. All the isolates which were resistant against erythromycin did not cause any change in the clindamycin zone of inhibition on double disk diffusion test. This suggests M phenotype for the pattern of resistance.

Prevalence of GAS Pharyngitis in different age groups is given in Table 1

Table 1. Prevalence of GAS Pharyngitis in different age groups

	Age Group 1-4 Years	Age Group 5-12 Years	Age Group 13-18 Years	Age Group ≥18 Years
Total No. of Samples	40	122	37	51
No. of Negative Samples	32	77	28	42
No. of Positive Samples	8	45	9	9
Percentage Prevalence	21.05%	36.88%	24.32%	18.36%

Percent prevalence of *S. pyogenes* resistance against different antibiotics used in the study is described in Table 2.

Table 2. Percent prevalence of *S. pyogenes* resistance against different antibiotics used in the study.

Drugs	Disc Content	Total Isolates Tested	No Of Isolates Resistant	Percentage Prevalence
Penicillin	10 units	71	0	0.00%
Amoxicillin-Clavulanic acid	20/10 µg	71	0	0.00%
Vancomycin	30 µg	71	0	0.00%
Ceftriaxone	30 µg	71	0	0.00%
Erythromycin	15 µg	71	30	42.42%
Azithromycin	15 µg	71	28	39.43%
Tetracycline	30 µg	71	39	54.92%
Levofloxacin	5 µg	71	1	1.40%
Cholarmphenicol	30 µg	71	10	14.08%
Clindamycin	2 µg	71	3	4.22%

Discussion

The study focused on finding the prevalence of GAS pharyngitis in patients presenting with the complain of upper respiratory tract problems. The prevalence of GAS pharyngitis was least studied in Pakistan and there are only few publication on the topic (9). Group A beta hemolytic streptococci responsible for upper respiratory tract infection and skin and soft tissue infection are not routinely tested for antimicrobial susceptibility. Due to increasing resistance to routinely prescribed antibiotics for different gram positive organisms, it was necessary to conduct surveillance studies of *S. pyogenes* to determine antibiotic susceptibility status of the organism.

The patients were divided in to four different age groups to find out the prevalence of GAS pharyngitis at different age levels (17). Four divisions in the age; age group 1-4 years, age group 5-12 years, age group 13-18 years and age group ≥18 years is in agreement with the previous studies. (10,18,19).

Prevalence of GAS pharyngitis in age group 1-4 years (21.05%) and age group 5-12 years (36.88%) are parallel with the present finding where the authors have reported high prevalence of GAS pharyngitis in young children (18,21).

While the prevalence of GAS pharyngitis in age group 13-18 years and in age group ≥18 years were not found in agreement with the previous study the difference in the results can be explained on the basis of geographical and environmental differences and on the basis of maintain poor hygienic conditions . (10,19). In the present study prevalence of GAS pharyngitis in age group 5-12 years is significantly higher as compared to the overall prevalence of the GAS pharyngitis in the population. This high in the GAS prevalence is mainly due to the direct contact and carelessness in maintaining hygienic conditions i.e. hand washing in children (2,18)

According to the guidelines of the Pakistan Antimicrobial Resistance Network (PARN) penicillin is the drug of first choice for the treatment of GAS pharyngitis. The present study suggests that the organism is completely sensitive against penicillin and other cell wall synthesis inhibitors including co-amoxiclave, ceftriaxone and vancomycin.(9,21)

Individuals allergic to penicillin are treated with macrolides (erythromycin and azithromycin) which are second line antibiotics for GAS pharyngitis. Present study has reported that macrolide resistance has reached to an alarming level; erythromycin 42.42%, and azithromycin 39.43%. Excessive unnecessary prescribing and noncompliance of the prescribed antibiotics may be the cause of this high spread of resistance in the community. (22). Furthermore over the

counter selling of the antibiotics at all community pharmacies are promoting the self medication a great.

All the isolates were also tested against different antibiotics effective against gram positive microorganisms including tetracycline, levofloxacin, chloramphenicol and clindamycin. The organism has also acquired varying degree of resistance against these antibiotics due to the irrational and excessive use of the antibiotics (23, 24, 25,).

In the present study resistance of clindamycin was not affected by any of the erythromycin resistant isolate on double disc susceptibility test. The isolates were identified as having M phenotype which suggests the isolates could have *mef* (macrolide efflux pump) genes of macrolide resistance (26).

There were certain limitations in our study. As a resource limited study we didn't employ serological technique to distinguish between active infected patient and a carrier. Another limitation of the study was that the genetic basis of the antibiotic resistance was not identified.

Conclusion

On the basis of the present study we can conclude that the overall prevalence of GAS pharyngitis in Pakistan was 28.4%. It can be recommended on the basis of local resistance study that penicillin is still effective as a drug of first choice. Macrolides (erythromycin and azithromycin) could only be used with great caution and according to the recommendation of physician to confirm its rational use. There is a great need of extensive surveillance of GAS pharyngitis country wide. This would help us in understanding the resistance status and help to make strategies to reverse the macrolide resistance back as many of the European countries have done.

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