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Brief communication (Original)

The emergence of lincosamide and macrolide resistance in *Streptococcus pyogenes* from Pakistan

Rao Muhammad Abid Khan[a], Sana Anwar[b], Zaid Ahmed Pirzada[a]
[a]*Department of Microbiology, University of Karachi, Karachi 75270, Pakistan*
[b]*Liaquat National Hospital, Karachi 75270, Pakistan*

Background: Many reports from developing and developed countries have shown increased resistance against macrolides and other antibiotics among *Streptococcus pyogenes* or group A streptococci (GAS).
Objectives: To study the current resistance pattern of *S. pyogenes* in Pakistan to commonly prescribed antibiotics.
Materials and Methods: Altogether, 85 (53%) of *S. pyogenes* strains were isolated and collected from 160 various clinical specimens from patients in Pakistan.
Results: Among other strains, 51 (32%) group D streptococci, 15 (9%) group B streptococci, 7 (4%) group G streptococci, and 2 (1%) group C streptococci were also identified. Predominantly, *S. pyogenes* were isolated from throat swabs (55%), followed by pus (17%), tissues (12%), and blood or wound swabs (7%). The majority of the *S. pyogenes* isolates were collected during the rainy season (55%) followed by cool season (40%), while merely 5% strains were isolated during the hot season, indicating a correlation of GAS incidence with seasonal changes. The highest rate of resistance was observed against clindamycin (29%), followed by macrolides (20%), and ciprofloxacin (14%). However, all strains of GAS were sensitive to penicillin and co-amoxiclav.
Conclusions: The emergence of lincosamide and macrolide resistance among GAS is a major problem worldwide which is probably due to misuse of antibiotics, self-medication, or frequent use of these antibiotics.

Keywords: Bacteria, drug resistance, GAS, pathogen, prevalence

Streptococcus pyogenes or group A streptococci (GAS) are among the most common pathogens, and are considered a globally important cause of morbidity and mortality [1]. The range of infections caused by GAS varies from superficial common to severe and life threatening illnesses. Common clinical infections include sore throat, impetigo, erysipelas, tonsillitis, otitis media, cellulitis, and scarlet fever; while severe and sometimes life threatening infections include puerperal sepsis, myositis, necrotizing fasciitis (NF), and streptococcal toxic shock syndrome (STSS) [2, 3]. These infections are often followed by poststreptococcal sequelae, including acute glomerulonephritis, rheumatic heart disease (RHD), and rheumatic fever [4, 5]. Underlying predisposing factors such as diabetes mellitus, immunological disorders, viral infections, and immunosuppressive therapy are often responsible for invasive GAS infections [5, 6]. GAS infections are more common in developing countries than developed countries and are the most frequent cause of bacterial pharyngitis/tonsillitis (strep throat) in school age children and adults [4, 7]. STSS was first reported in the late 1980s and has high mortality rate ranging from 30% to 70% [2]. NF, also called "flesh-eating" disease, is a disease of the deeper skin layers and tissues, while rheumatic fever is responsible for about half of all cardiovascular disease in India [8].

GAS possess a number of virulence factors including M protein, F protein, hyaluronic acid, lipoteichoic acid, and extracellular products, such as streptococcal pyrogenic exotoxins and erythrogenic toxins (Spe), Streptolysin S and O, C5a peptidase, DNases, and streptococcal inhibitor of complement-mediated lysis (SIC) [9, 10]. Peptides from M antigen and Spe have been reported to have super-antigenic (SAg) activity [11, 12]. Interestingly, the correlation of *emm* types with certain SAgs have been reported [11, 13].

Penicillin is the drug of choice for infections caused by *S. pyogenes*. For patients who are allergic to penicillin, macrolides are suggested as the

Correspondence to: Zaid Ahmed Pirzada, Department of Microbiology, University of Karachi, Karachi 75270, Pakistan. E-mail: zaahmed@uok.edu.pk

alternative treatment [8, 14, 15]. Severe and invasive cases of GAS infections are treated with combination therapy, including penicillin and clindamycin, which has demonstrated good results in experimental models of necrotizing fasciitis [2]. Fortunately, all the *S. pyogenes* strains are susceptible to penicillin, while clindamycin has been effective for invading GAS infections.

Many reports from developing and developed countries have shown increase in resistance against macrolides and other antibiotics in GAS. However, very limited data are available from Pakistan. The objective of this study was to gain knowledge about current resistance pattern of *S. pyogenes* against commonly prescribed antibiotics. To our knowledge this is the first comprehensive report of the prevalence and the antibiotic resistance pattern of GAS from Pakistan. Furthermore, this study may help in discouraging the misuse of antibiotics that leads to the emergence in drug resistance.

Materials and methods

Collection of S. pyogenes strains

This study was performed in the Department of Microbiology, University of Karachi from July 2013 to June 2014. To obtain strains and basic patient information such as sex, age, specimen and disease, assistance was obtained from laboratories, tertiary care hospitals, and medical institutes in Karachi. Altogether 85 *S. pyogenes* strains were isolated and collected from various clinical specimens such as throat swabs, pus, blood, wounds, and tissues. All samples were collected anonymously and no patients could be identified from the data.

Purification and confirmation of *S. pyogenes* strains

All of the collected strains of *S. pyogenes* strains were purified (to obtain pure cultures) and were systematically identified first by the routine variables according to diagnostic tests described in Bergey's Manual of Determinative Bacteriology, including the catalase, β hemolysis, bacitracin-sensitivity tests. ABIS (online-advanced bacterial identification software) www.tgw1916.net/bacteria_logare.html) was also used. Confirmation of the strains was also made using a Lancefield Grouping kit (Oxoid, Basingstoke, Hants, UK).

Antibiotic resistance pattern

The antibiotic resistance pattern of collected *S. pyogenes* strains was performed using 5% blood agar plates by Kirby–Bauer disc diffusion in triplicate and the average value was taken for interpretation against following groups of antibiotics, penicillins (penicillin G (10 μg), amoxicillin-clavulanic acid (30 μg)), macrolides (erythromycin (15 μg), azithromycin (15 μg), clarithromycin (15 μg)), lincosamide (clindamycin (2 μg)), cephalosporins (cefotaxime (30 μg), and quinolones (ciprofloxacin (5 μg)). CLSI guidelines were used to assist with interpretation of the results.

Results

For this study we collected a total of 160 β-hemolytic streptococci cultures from various clinical specimens. After the initial purification and preservation of these 160 cultures we identified prevalence of Lancefield's groups as 85 (53%) *S. pyogenes* or GAS, 51 (32%) group D streptococci (GDS), 15 (9%) group B streptococci (GBS), 7 (4%) group G streptococci (GGS), and 2 (1%) group C streptococci (GCS).

The majority of the GAS were isolated from throat swabs (55%), followed by pus (17%), tissues (12%), blood and wound swabs (7% each). Recovery from other specimens like urine and synovial fluid was just 1% each.

The majority (55%) of the GAS isolates were collected during rainy season, followed 40% collected in the cool season, while only 5% isolates could be collected during hot season. This data indicates a co-relation of GAS incidence with the seasonal changes.

The highest rate of resistance was observed against clindamycin (29%), followed by macrolides (20% each) and ciprofloxacin (14%), and cefotaxime (1%). However, all strains of GAS were sensitive to penicillin, and amoxicillin-clavulanic acid (**Figure 1**).

Discussion

S. pyogenes is responsible for more than 600 million cases of pharyngitis and more than a half million deaths because of complications of autoimmune rheumatic heart disease and invasive infections worldwide [16]. The epidemiology of GAS infections changes rapidly. Therefore, it is important to characterize isolates from time-to-time for epidemiological data and infection control. *S. pyogenes* or GAS was the most common isolate found in this study followed by GDS, GBS, GGS, and GCS. The incidence of *S pyogenes* infections was higher in rainy and cold seasons.

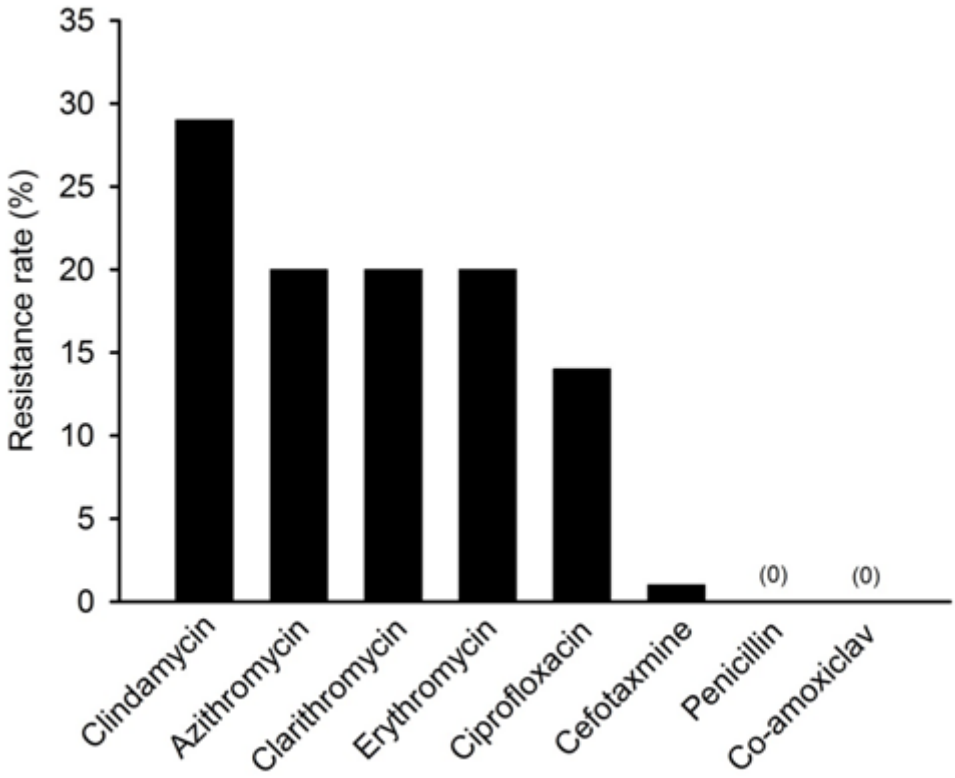

Figure 1. Antibiotic resistance pattern of group A streptococci (*Streptococcus pyogenes*) by Kirby–Bauer disc diffusion. Co-amoxiclav = amoxicillin-clavulanic acid

GAS has remained an important human pathogen for centuries and is thought to be susceptible to large numbers of antibiotics. Penicillin has been the drug of choice for the treatment of GAS infections; while macrolides are used as alternative treatment for patients allergic to penicillin. In addition, clindamycin is effective for invasive and severe streptococcal infections, and fluoroquinolones can be used with promising results [1]. Recently, drug resistance has emerged against macrolides worldwide, especially in Europe and Asia [8, 17-19]. GAS resistance to other antibiotics such as tetracycline and fluoroquinolones is also being reported [20, 21].

In the present study, none of the *S pyogenes* strains were found resistant to penicillin. These findings are consistent with studies conducted in several other countries. Similarly, all strains were sensitive to amoxicillin-clavulanic acid (co-amoxiclav). Results of the present study showed that 20% *S pyogenes* strains were erythromycin resistant. A similar level of resistance to erythromycin has been reported in India (16%–29%), Korea (20%), and Berlin (13%) [8, 22-24]. Drug resistance against erythromycin has also been reported in other studies from Pakistan; one having resistance of 30% [25], while the other showed an alarmingly high rate of 95% [26]. Yet another study indicated that 28% of *S pyogenes* isolated were resistant to macrolides [27].

In the present study, highest rate of resistance was observed against clindamycin. Emergence of clindamycin resistance has also been reported from China (98%) and Japan (15%) [2, 19]. Clarithromycin resistance has been reported in Berlin (13%). However, in the present study clarithromycin resistance has been observed at 20%, which is lower than found in China (97%) [19].

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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