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Antibiotic resistance pattern of *streptococcus pyogenes* isolated from clinical samples with special reference to quinolone resistance

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Abstract

Background & Objectives: *Streptococcus pyogenes* producing quinolone resistance are an increasing cause of concern in the hospitals as they produce a therapeutic dilemma for the treating physician. The present study was undertaken to know the prevalence of quinolone resistant *streptococcus pyogenes* from clinical isolates and their antibiotic resistance pattern.

Methods: A total of 28 *streptococcus pyogenes* were recovered from various clinical specimens. All the samples were processed for routine bacterial culture and antimicrobial susceptibility test as per standard protocol (CLSI, Koeneman's Text Book).

Results: Of 28 *streptococcus pyogenes* isolates 8(34.78%) were showing Quinolone resistance. In this study, we investigated antimicrobial activity of 20 widely used antibiotics against *S. pyogenes*isolates. Tetracycline (53.57%), Ampicilin (32.14%), Erythromycin (32.14%) Cotrimoxazole (39.28%), Levofloxacin (28.57%) and Ciprofloxacin (28.57%) were resistant. Cephalosporins like Ceftazidime (21.42%), Cefotaxime (21.42%) and Azithromycin (21.42%) were less resistant. Cefepime (10.71%), Linezolid (7.14%) and Vancomycin (3.57%) were least resistant. All the isolates were sensitive to Meropenem. Majority of Quinolone resistance isolates were resistant to tetracycline.

Conclusion: Of 28 *streptococcus pyogenes* isolates 8(34.78%) were showing Quinolone resistance. Cefepime (10.71%), Linezolid (7.14%) and Vancomycin (3.57%) were least resistant. All the isolates were sensitive to Meropenem

Keywords: *Streptococcus Pyogenes.* Quinolone resistance, GAS Infection, Therapeutic options.

Introduction

*Streptococcus pyogenes*is responsible for high rates of morbidity due to an increase in invasive group A streptococcal infections and bacteremia worldwide during the last decade.[1,3]

*Streptococcus pyogenes*is the etiologic agent of a wide range of human infections, including streptococcal sore throat, skin and soft tissue infections, and the post infectious syndromes of glomerulonephritis and acute rheumatic fever. Penicillin remains the drug of choice for the treatment of these infections because *S. pyogenes* remains susceptible to this antibiotic despite its intensive use. By contrast, an increasing frequency of *S. pyogenes* isolates that are resistant to macrolides, probably due to the increasing use of these antibiotics for the treatment of other bacterial respiratory pathogens, i.e., *Streptococcus pneumoniae*,[2] has been reported in different countries.[3,4,5] Similarly, the increasing use of fluoroquinolones, due to their excellent activities against some bacterial pathogens, has led to the emergence of *S. pyogenes*isolates with reduced susceptibilities to these antibiotics.[6,7,8,9] Increased resistance of *S. pyogenes*to ciprofloxacin has been reported in Spain at the highest rates ever published.[10]

The present study is carried out at Shivamogga Institute of Medical Sciences, Shivamogga, to know the prevalence of Quinolone Resistant strains of *Streptococcus Pyogenes* from clinical isolates.

Aims & Objectives

1. Detection of Quinolone resistant *Streptococcus Pyogenes.*
2. Study of antibiotic resistance pattern of *Streptococcus Pyogenes.*
3. Risk factors associated with the Quinolone resistant *Streptococcus Pyogenes* infection.

Materials & Methods

The present study was undertaken at the Department of Microbiology, Shivamogga Institute of Medical Sciences (SIMS), Shivamogga from JAN 2016 to DEC 2016.

Source of data: Clinical samples such as pus, urine, blood, body fluids etc. Obtained from patients admitted in Shivamogga Institute of Medical Sciences hospital and received at the department of Microbiology.

Inclusion criteria: Non repetitive, consecutive *Streptococcus Pyogenes* isolated from clinical samples obtained from hospitalised patients (IPD) received during study period.

Sample processing:

All the samples were processed for routine bacterial culture as per standard protocol.[18]

Microscopy: Smears were prepared on clean glass slides. Gram stain performed and observed for the presence of any gram positive cocci in chains.

Culture: Samples were inoculated into Thio-glycollate broth, chocolate agar, MacConkey's agar and Blood agar. All organisms that grew on blood agar and produced betahemolysis were provisionally considered

to be *Streptococcus Pyogenes* and identified further by using a standard protocol for identification.[18]

Antimicrobial susceptibility test:[2,19] Antimicrobial susceptibility test was carried out with modified Kirby-Bauer disk diffusion method using current CLSI[9] recommendations on blood agar. Commercially available antibiotic disks (Himedia, Mumbai) were used. The antibiotic susceptibility profile against Ampicillin, Daptomycin, Ciprofloxacin, Erythromycin, Azithromycin, Clindamycin. Linezolid, Teicoplanin, Vancomycin, Chloamphinicol, Tetracycline, Gentamicin, Amikacin, Levofloxacin, cotrimoxazole Cephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime) and Meropenemwere studied. *Staphylococus aureus* ATCC 29213 was used as control strain.[18,19]

Statistical analysis: Chi square test was used with appropriate correction to see the significance of difference between the sensitivity of various drugs in quinolone resistant strains using SPSS software. $p \leq 0.05$ was considered significant.

Ethical consideration: The protocol for this study was approved by the institutional Ethical Committee. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice, quality control ensured and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for Human Experimentation in Biomedical Research.[10] Approval was obtained from the subjects by taking informed consent.

Results & Observations

A prospective study was conducted to know the prevalence of different quinolone resistance among *streptococcus pyogenes* isolated from various clinical specimens received at the Department of Microbiology, Shivamogga Institute of Medical Sciences, Shivamogga, during the period JAN 2016 to DEC 2016.

Of 2269 bacterial isolates 28 (1.23%) were *streptococcus pyogenes* recovered from various clinical specimens like pus (683/8), sputum(35/9), urine(829/3), blood (985/3), throat swab (16/3) and pleural fluid (49/2).

Table 1: *Streptococcus Pyogenes* isolated from different clinical samples

Organisms	Pus No. (%)	Sputum No. (%)	Urine No. (%)	Blood No. (%)	Throat swab No. (%)	Pleural fluid (%)	Total No. (%)
Streptococcus Pyogenes	8(28.57)	9(32.14)	3(10.71)	3(10.71)	3(10.71)	2(7.14)	28(1.23)
Other	675(30.12)	26 (1.16)	826(36.85)	982(43.81)	13(0.58)	47(2.09)	2241(98.76)
Total	683(30.1)	35(1.54)	829(36.53)	985(43.41)	16(0.7)	49(2.15)	2269

Among the 28(1.23%) total isolates of *streptococcus pyogenes* 8(28.57%) were resistant to Quinolone. Maximum number of *streptococcus pyogenes* organisms were isolated from sputum 9(32.14%) and pus 8(28.57%) followed by urine 3(10.71%), blood 3(10.71%), throat swab 3(10.71%) and pleural fluid 2 (7.14%).

Table 2: Age and Sex distribution of the patients in the study group n=28

Age (yrs)	*streptococcus pyogenes*			
	Males		Females	
	Number	%	Number	%
0—10	1	3.57	1	3.57
11-20	1	3.57	1	3.57
21-30	2	7.14	3	10.71
31-40	4	14.28	2	7.14
41-50	2	7.14	2	7.14
51-60	2	7.14	2	7.14
61-70	2	7.14	1	3.57
>70 yrs.	1	3.57	1	3.57
Total(28)	15	53.57	13	46.42

Male to female ratio was 1.15:1.

Mean age in the study group was 28.1± 18.48 years.

Table 3: Antibiotic resistance pattern of *streptococcus pyogenes*

Antibiotics tested	*streptococcus pyogenes* (n=28)	
	No	(%)
Ampicillin,	9	32.14
Daptomycin,	6	21.42
Ciprofloxacin,	8	28.57
Erythromycin,	9	32.14
Azithromycin,	6	21.42
Clindamycin.	5	17.85
Linezolid,	2	7.14
Teicoplanin,	3	10.71
Vancomycin,	1	3.57
Chloamphinicol,	4	14.28
Tetracycline,	15	53.57
Gentamicin,	3	10.71
Amikacin,	2	7.14
Levofloxacin,	8	28.57
Cotrimoxazole	11	39.28
Ceftazidime,	6	21.42

Cefotaxime,	6	21.42
Ceftriaxone,	5	17.85
Cefepime	3	10.71
Meropenem	0	0

All the isolates were sensitive to Meropenem and least resistance was observed towards Vancomycin 3.57%.

Table 4: Distribution of *streptococcus pyogenes* isolates in the hospital ward

Wards	***Streptococcuspyogenes* no (%)**
Surgery	12(42.85)
Medicine	10(35.71)
Orthopedics	2(7.14)
ENT	2(7.14)
OBG	1(3.57)
Pediatrics	1(3.57)
Total(2269)	28(1.23)

Maximum number of the *streptococcus pyogenes* isolates were obtained from the Surgery, Medicine and Orthopedics wards.

Table 5: Comparison of Antibiotic resistance pattern of Quinolone resistant and Quinolone sensitive *streptococcus pyogenes isolates*

Antibiotics	**Quinolone sensitive *streptococcus pyogenes* n=20**		**Quinoloneresistant *streptococcus pyogenes* n=8**		**p value**
	Resistant	**%**	**Resistant**	**%**	
Ampicillin,	1	5	8	100	0.05
Daptomycin,	0	0	6	75	0.05
Ciprofloxacin,	0	0	8	100	0.05
Erythromycin,	1	5	8	100	0.05
Azithromycin,	1	5	5	62.5	0.05
Clindamycin.	1	5	4	50	0.05
Linezolid,	0	0	2	25	0.01
Teicoplanin,	0	0	3	37.5	>0.05
Vancomycin,	0	0	1	12.5	0.05
Chloamphinicol,	1	5	3	37.5	0.05
Tetracycline,	7	5	8	100	0.05
Gentamicin,	0	0	3	37.5	0.05
Amikacin,	0	0	2	25	0.05
Levofloxacin,	2	5	6	75	>0.05
Cotrimoxazole	3	5	8	100	0.05
Ceftazidime,	0	0	6	75	0.05
Cefotaxime,	0	0	6	75	0.05
Ceftriaxone,	0	0	5	62.5	0.01
Cefepime	0	0	3	37.5	>0.05
Meropenem	0	0	0	0	0.0001

Quinolone resistant *streptococcus pyogenes* were more drug resistant, difference was statistically significant towards all the antibiotics used in the study.

Table 6: Analysis of the risk factors for *streptococcus pyogenes*infection and Quinoloneresistant *streptococcus pyogenes* infection

Risk factors	***streptococcus pyogenes* infection No.(n=28) (%)**	**Quinolone resistant streptococcus pyogenes infection No. (n=8) (%)**
Pharyngitis(16)	3 (10.71)	2 (25)
Glomerulonephritis (9)	3 (10.71)	2 (25)
Carditis (RHD)(5)	3 (10.71)	1 (12.5)
Pneumonia (35)	8 (28.57)	1 (12.5)
Diabetis mellitus. (63)	1 (3.57)	0
Sepsis (85)	8 (28.57)	1 (12.5)
Cellulitis (83)	9 (32.14)	1 (12.5)

The major risk factors for infection with *streptococcus pyogenes* infection were cellulitis, sepsis followed by pharygitis, glomerulonephritis and carditis. But the risk factors for infection with Quinolone resistant *streptococcus pyogenes* infection were Pharyngitis, Glomerulonephritis followed by Pneumonia, Sepsis and carditis.

Discussion

Of 2269 bacterial isolates 28 (1.23%) were *streptococcus pyogenes* recovered from various clinical specimens like pus (683/8), sputum (35/9), urine (829/3), blood (985/3), throat swab (16/3) and pleural fluid (49/2). Among the 28(1.23%) total isolates of *streptococcus pyogenes* 8 (28.57%) were resistant to ciprofloxacin and levofloxacin (Quinolone resistant).

Maximum number of *streptococcus pyogenes* organisms were isolated from sputum 9 (32.14%) and pus 8 (28.57%) followed by urine 3(10.71%), blood 3(10.71%), throat swab 3(10.71%) and pleural fluid 2 (7.14%). Male to female ratio was 1.15:1.

All the isolates were sensitive to Meropenem and least resistance was observed towards Vancomycin 3.57%. Maximum number of the *streptococcus pyogenes* isolates were obtained from the Surgery (42.85%), Medicine (35.71%) and Orthopedics (7.14%) wards. Quinolone resistant *streptococcus pyogenes* were more drug resistant, difference was statistically significant towards all the antibiotics used in the study.

In this present study, we investigated antimicrobial activity of 20 widely used antibiotics against *S. pyogenes* isolates. Tetracycline (53.57%), Ampicilin (32.14%), Erythromycin (32.14%) Cotrimoxazole (39.28%), Levofloxacin (28.57%) and Ciprofloxacin (28.57%) were resistant. Cephalosporins like Ceftazidime (21.42%), Cefotaxime (21.42%) and Azithromycin (21.42%) were less resistant. Cefepime (10.71%), Linezolid (7.14%) and Vancomycin (3.57%) were least resistant. All the isolates were sensitive to Meropenem.

KR Rijal et al[16] described that Group A beta Hemolytic Streptococci (GABHS) is among the most prevalent bacterial childhood infection and constitutes 20.0%-40.0% of all cases of exudative pharyngitis. The condition is most prevalent in the age group of 5 to 15 years, the highest prevalence occurring in 7 years old children and rarely occurring in those under 3 years of age. Males are equally affected by GABHS as females. GABHS frequently colonizes the pharynx of asymptomatic individuals, as 15.0%-20.0% of school age children are asymptomatic carriers.

In study KR Rijal et al,[16] 9.2% of school children were colonized by GAS in their throat. Similar study done by Tavakkoli et al[25] found the prevalence of carriers among primary school children was 4.9%.

Bogovac et al[4] reported 6.0% prevalence in all age groups and 11.7% prevalence in 6-13 years old children from Croatia.

Durmaz et al[5] showed the prevalence of *S. pyogenes* nasopharyngeal carriage in 14.3% healthy school children and children in an orphanage in Turkey.

The study done in Pittisburgh, Pennsylvania et al[8] reported the prevalence of carriage of group A streptococci in school children to be 27.0- 32.0%. The isolation rate of GAS was 25.9% according to Ozturk et al[13] in asymptomatic school children in the study done in Turkey.

Kim et al[9] recently reported a high frequency of resistance to erythromycin in GAS, particularly in countries where antibiotics are overused. Of all throat isolates, 95.0% were predominantly resistant to erythromycin, 70.0% to clindamycin, 56.0% to azithromycin and 24.0% to clarithromycin according to the study done in Sindh.

Tamayo et al[12] reported the erythromycin resistance rate to be 21.7% in the study done in Spain in 2004. Ciftci et al[23] reported resistance to erythromycin, clarithromycin, azithromycin and clindamycin as 3.8%, 5.2%, 4.2% and 3.0% respectively.

Alberti et al[13] reported increased resistance of *S. pyogenes* to ciprofloxacin in Spain at the highest rate ever published and it is 63.3%. Of all the isolates analyzed in our study, 15.6% were resistant to erythromycin, 6.6% to tetracycline and 2.2% to azithromycin. All the isolates were sensitive to beta lactam antibiotics (penicillin and amoxycillin). In a large survey done in Iran by Jasir et al[20] found no penicillin resistance strains of *S. pyogenes* and only a few erythromycin resistance strains.

Another study done in France by Binjen et al[11] found all isolates of *S. pyogenes* were susceptible to amoxycillin. Our findings demonstrate that antibiotic resistance of *S. pyogenes*is not clinically significant problem in our country. However, the results of our preliminary study highlights the importance of regular surveillance programs to monitor the rate of GAS carriage and the antibiotic susceptibility of GAS isolates in the community. We, therefore, emphasize the need to carry out this type of study in large sample size with a wide range of geographical and seasonal variations throughout the country.

The major risk factors for infection with *streptococcus pyogenes* infection were cellulitis 32.14%, sepsis 28.57% followed by pharyngitis (10.71%), glomerulonephritis (10.71%) and carditis (10.71%). But the risk factors for infection with quinolone resistant *streptococcus pyogenes* infection were Pharyngitis (25%), Glomerulonephritis (25%) followed by Pneumonia (12.5%), Sepsis (12.5%) and carditis (12.5%).

In study by TadayoshiIk et al.[14] *Streptococcus pyogenes* (group A streptococcus) is one of the most common human pathogens. It causes a wide array of infections, the most frequent of which is acute pharyngitis (streptococcal sore throat). From the late 1980s, streptococcal toxic shock like syndrome caused by *S. pyogenes* became a serious problem in both developed and developing countries. Symptoms such as pharyngitis, fever, and pain may suddenly develop, and the disease may progress very rapidly in some patients to soft tissue necrosis, acute kidney failure, adult respiratory distress syndrome, disseminated intravascular coagulopathy, and multiorgan failure, leading to shock and death.

In study by KR Rijal et al.[16] the frequency of GAS was similar in all age groups of school children, but it was slightly higher in children aged 5-8 years. The study done by Durmaz et al[12] in Turkey, showed that the rates of carriers for boys and girls were similar and the frequency was similar in all age groups of school children, but it was significantly higher in children aged 4-6 years living in the orphanage. Macrolides including erythromycin and clindamyc in have been widely used for treatment of acute pharyngitis and invasive infection of GAS respectively.

Nosocomial Transmission and Challenges in Infection Control

Group A Streptococcus (GAS) consists of a single species, *Streptococcus* pyogenes.[15] It belongs to Lancefield group A and is beta-hemolytic, hence called Group A beta-hemolytic Streptococci (GABHS). Streptococcal carriage bas been defined as the recovery of GAS from the nasopharynx or oropharynx in the absence of any evidence of acute infection.[13] The pathogenesis of GAS is mediated by a variety of factors. One of them is Streptolysin 'O' toxin, which damages cell membranes and accounts for the hemolysis demonstrated on sheep blood agar.[16] Disease spectrum of Group A Streptococcus (GAS) or *S. pyogenes* ranges from mild infectious as pharyngitis, tonsilitis and impetigo to life threatening infections like necrotizing fascitit is and toxic-shock like syndrome. These are often followed by post infective sequelae of rheumatic fever, rheumatic heart disease and post streptococcal acute glomerulonephritis.[29] GAS infection is ordinarily spread by direct person-to-person contact, most likely via drops of saliva, nasal secretions, contaminated fingers, dust or fomites.[12] All beta-hemolytic Group A Streptococcus are sensitive to penicillin G, and most are sensitive to erythromycin. A high frequency of resistance to erythromycin in GAS has been reported, particularly in countries where antibiotics are overused.[18] In Nepal control programs for streptococci does not exist, moreover, such programs are not even in the row. Little information is known about the prevalence of *S.pyogenes* from the throat swab of school children in Nepal.[16]

Current therapeutic options

Azithromycin and erythromycin were very active with susceptibility rates greater than 95% and could be used as first alternative choice. Clindamycin and pristinamycin, less used in therapeutic settings, have shown high degree of efficacy on the β-haemolytic *Streptococcus*. With 97% susceptibility, these two molecules could be used as an alternative or second line antibiotic. Interestingly, chloramphenicol, teicoplanin, vancomycine, and levofloxacin were also very active and could be potential alternative choices of treatment against infections with *S.* pyogens.[15]

One approach to treat severe invasive *S. Pyogenes* infections has been to utilize a combination of penicillin and clindamycin. The rationale is that penicillin provides coverage against 100% of *S. pyogenes* strains and clindamycin has demonstrated greater efficiency in experimental models of necrotizing fasciitis.[16]

Conclusion

Group A β-haemolytic streptococci is one of the major causes of acute respiratory tract infections. Emergence of penicillin-resistant and other antibiotic-resistant clinical isolates of *S. pyogenes* underscores the need for continuous surveillance of antimicrobial resistance patterns.[15,16]

In this study, we investigated antimicrobial activity of 21 widely used antibiotics against *S. pyogenes* isolates. Tetracycline (53.57%), Ampicilin (32.14%), Erythromycin (32.14%) Cotrimoxazole (39.28%), Levofloxacin (28.57%) and Ciprofloxacin (28.57%) were resistant. Cephalosporins like Ceftazidime (21.42%), Cefotaxime (21.42%) and Azithromycin (21.42%) were less resistant. Cefepime (10.71%), Linezolid (7.14%) and Vancomycin (3.57%) were least resistant. All the isolates were sensitive to Meropenem.

Treatment of respiratory streptococcal infections is difficult and there are many factors to consider when choosing an antibiotic regimen. Susceptibility to antibiotics of any isolated strain should be evaluated as this is the only guarantee of prompt and effective treatment. The antibiotic therapy should be associated with adequate preventive methods that must include education of nursing staff in order to avoid as much as possible nosocomial infections, education of the general population for a politic of hygiene and abandonment of the common practice of self-medication, and increased scientific cooperation between clinicians and microbiologists in the interest of improving public health.[15]

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