

## A Preliminary Sentinel Surveillance Report on Antibiotics Resistance Trend of *Streptococcus Pyogenes* in Kolkata Region, India

Dipanwita Ray<sup>1</sup>, Sukanta Sinha<sup>2</sup>, Somnath Saha<sup>3</sup>, Sankar Karmakar<sup>4</sup>, Rathindra Nath Dutta<sup>4</sup>, Sujata Bhattacharya<sup>5</sup>, Nishit Kumar Pal<sup>2</sup> and Basudev Bhattacharya<sup>1</sup>

<sup>1</sup>Biochemistry Research Wing, Department of Biochemistry, <sup>2</sup>Department of Microbiology, Institute of Post-graduate Medical Education & Research (IPGME&R), 244B A J C Bose Road, Kolkata- (India), <sup>3</sup>Department of ENT, B S Medical College, Bankura, (West Bengal), India, <sup>4</sup>Department of Dermatology, Institute of Post-graduate Medical Education & Research (IPGME&R), 244A A J C Bose Road, Kolkata- (India), <sup>5</sup>Department of Microbiology, R G Kar Medical College, 1, Sahid Khudiram Bose Road, Kolkata- (India)

### Abstract

*Streptococcus pyogenes* is an important cause of serious bacterial infection in both adults and school-going children. On the present scenario of emerging changes observed in drug resistant pattern of *S. pyogenes*, this study was undertaken, from October 2008 to July 2009, to evaluate the trend of resistance pattern to various antibiotics by the Kirby-Bauer method of disk diffusion in Kolkata, India. A total of 115 isolates of *S. pyogenes* were identified by standard procedure. All strains were susceptible to penicillin, cephalexin, clindamycin and vancomycin. 60 isolates were resistant to tetracycline. Four isolates have been found to be resistant to erythromycin, which is a very rare occurrence in West Bengal. This finding may be alarming for emergence of resistance against this safe drug, which may complicate the treatment of patients who are allergic to penicillin.

**Keywords:** Antibiotic susceptibility, Modified Kirby-Bauer method, McFarland 0.5, Pyoderma.

### Introduction

*Streptococcus pyogenes* is one of the most frequent human pathogens capable of producing a wide variety of diseases. Such infections range from suppurative sequelae like pharyngitis, impetigo, streptococcal toxic shock-like syndrome (STSS), necrotizing fasciitis to more severe and life-threatening post streptococcal nonsuppurative sequelae like acute rheumatic fever (ARF), acute glomerulonephritis (AGN). In the last decade, there has been an increase in reports of serious streptococcal infections and the sequelae worldwide [1]. In India, prevalence of rheumatic heart disease and pharyngitis varies from 1 to 5.4/1,000 [2] and 4.2% to 13.7% [3-4] school-age children respectively, which is comparable to the rates reported from developed countries [5]. Penicillin is still the drug of choice for the treatment of streptococcal infections, whereas macrolides are used for patients allergic or hypersensitive to penicillin. The first reports of erythromycin-resistant isolates of *S. pyogenes* from human clinical sources appeared in 1959 [6]. Resistance of *S. pyogenes* to tetracycline was initially reported from the United Kingdom in 1954 [7]. In Italy resistance to macrolides has risen markedly in the last decade. Until 1993, erythromycin resistance was between 5 and 8% but from 1995 onwards various reports indicated a sharp rise [8]. Reports on antibiotic resistance by *S.*

*pyogenes* are relatively few in India. No such report is available from Kolkata in last two decades. The present study was undertaken to produce sentinel surveillance data related to *S. pyogenes* antibiotic resistance in Kolkata.

### Materials and Methods

Study was undertaken in a tertiary Health care hospital in Kolkata. Samples were processed in the Department of Biochemistry, I.P.G.M.E&R. A total of 115 *S. pyogenes* isolates were collected. Samples were collected from patients attending skin OPD of SSKM hospital, 10 months from October, 2008 to July, 2009. Consecutive patients of any age group attending the Dermatology OPD, clinically diagnosed as pyoderma [9] and receiving no antibiotic in antecedent 7 days were placed under this study. Prior informed consent and clearance from Institutional ethical committee were obtained. *Collection of samples and Isolation of organism:* Samples were collected using a sterile swab stick for ulcerative lesion. In case of unopened abscess pus was collected by aspiration. Samples were inoculated in 10% sheep blood agar plates. For each sample, inoculation was done in duplicate. One plate was incubated aerobically and the other was incubated anaerobically in a gas packed jar and was allowed to incubate for 24 to 48 hours at 37°C. Gram positive long chain cocci isolates, producing beta-hemolysis on 10% sheep blood agar plate, showing negative result in catalase test and sensitive to Bacitracin disc (0.25 U) were presumptively identified as *S. pyogenes*. Next, the isolates were subjected to Lancefield carbohydrate antigen grouping by sensitized latex agglutination test (Streptex Acid Extraction Kit and Latex suspension Kit, Remel Europe Ltd) for final confirmation as group 'A' *Streptococcus* (GAS). *Determination of antibiotic susceptibility:* The antibiotic susceptibilities of all GAS isolates were tested by the modified Kirby-Bauer method of disk diffusion using turbidity standard equivalent to McFarland 0.5 [10]. The disk diffusion technique was performed on Mueller-Hinton agar with 5% sheep blood using disk of each Penicillin G (10 unit), Erythromycin (15 mcg), Cephalexin (30 mcg), Vancomycin (30 mcg), Tetracycline (30 mcg) and Clindamycin (2 mcg). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. *Streptococcus pyogenes* ATCC19615 strain was used as control.

### Results

Altogether 200 cases, presenting with different types of skin infections, were placed under the study. They belonged to three age groups: upto 16 years (38.5%), between 17-30 years (10%) and above 30 years (51.5%). Out of 200 cases *Streptococcus pyogenes* were isolated from 115 (57.5%) cases. Of these, 55 (47.8%) cases were from ≤ 16 years, 21 (18.3%) cases from 17-30 years and 39 (33.9%) cases were from ≥ 30 years of age group. No isolates showed resistance to vancomycin, Cephalexin and clindamycin. 105 (91.3%) isolates were sensitive to penicillin G and 10 (8.7%) strains had an intermediate level of sensitivity. Of the 115 isolates, 8 (6.9%) and 103 (89.6%) were intermediately sensitive and sensitive to erythromycin respectively. Erythromycin resistance was seen in 4 (3.5%) isolates. Interestingly 60 (52.2%)

isolates show resistance to tetracycline. 12 (10.4%) and 43 (37.4%) were intermediately sensitive and sensitive to tetracycline respectively. *Streptococcus pyogenes* ATCC19615 was sensitive to all six antibiotics. (Table1, Figure1, Figure2)

**Table1:** Antibiotic susceptibility patterns of *S. pyogenes* by disk diffusion method.

Antimicrobial agent(mcg/disk)	Patterns of susceptibility (in percentage) with respect to diameter of zone of inhibition in mm		
	Resistant	Intermediate	Sensitive
PenicillinG (10unit)	Nil	10 (8.7%)	105 (91.3%)
Erythromycin (15mcg)	4 (3.5%)	8 (6.9%)	103 (89.6%)
Cephataxime (30mcg)	Nil	Nil	115 (100%)
Vancomycin (30mcg)	Nil	Nil	115 (100%)
Tetracycline (30mcg)	60 (52.2%)	12 (10.4%)	43 (37.4%)
Clindamycin (2mcg)	Nil	Nil	115 (100%)

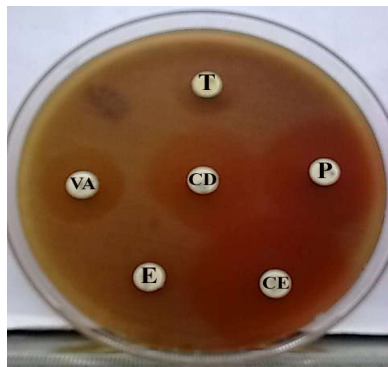


Figure1: Antibiotic sensitivity pattern of a GAS isolate showing erythromycin and tetracycline resistance. Isolate was sensitive to clindamycin, Cephataxime, vancomycin and Penicillin.

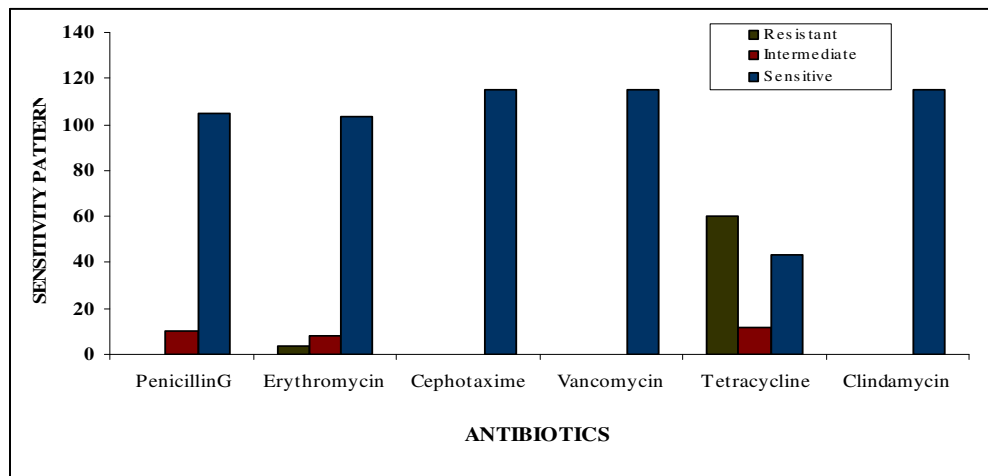


Figure2: Distribution of antibiotic sensitivity pattern of *S. pyogenes* isolated from Kolkata

The number of resistant (and intermediate sensitivity) versus sensitive strains were compared between two individual antibiotics by Fisher's Exact test. The two tail P values are depicted in the Table2.

**Table 2:** Comparison of antibiotics sensitivity patterns between two individual antibiotics

Antibiotics	Level of significance
Penicillin Vs Erythromycin	Not significant (P=0.823 <sup>*</sup> )
Penicillin Vs Vancomycin	Significance (P=0.002 <sup>*</sup> )
Penicillin Vs Tetracycline	Significant (P<0.001 <sup>*</sup> )
Erythromycin Vs Tetracycline	Significant (P<0.001 <sup>*</sup> )
Vancomycin/Cephotaxime/Clindamycin Vs Tetracycline	Significant (P<0.001 <sup>*</sup> )

### Discussion

In this sentinel surveillance we have found 10 (8.7%) strains of *S.pyogenes* showing intermediate sensitivity to PenicillinG. Rising MIC to penicillin (0.25-0.75µg/ml) in 10(5%) *S.pyogenes* isolates was reported from Mexico [12]. In a recent study, 7(20.6%) isolates have been found to show rising MIC to penicillin in India [2]. Our finding of intermediate resistance attributed to these reports (see table1). Lack of judicious prescribing habit of this antibiotic may be responsible for its intermediate sensitivity as has been advocated by Amabile-Ceuevas, 2001 [12]. It appears from this study that all strains were susceptible to vancomycin and Cephotaxime in this region. In our study we found 52.2% of tetracycline resistance, which is noticeably higher in this region (see table1). There was a report of higher rate (42%) of tetracycline resistance from Iranian population and that is in support of our finding. They have also mentioned a global rising in tetracycline resistance [13]. Due to rising allergy to PenicillinG, use of erythromycin, a macrolide compound, has been increased in treatment of *groupA* streptococcal infections [14]. During last one decade several studies have reported high frequencies of erythromycin resistance in many parts of the world [15-17]. Few studies have reported erythromycin resistance in *S.pyogenes* isolates collected from different parts of India [2]. In this present study, we have found intermediate susceptibility in 8 (6.9%) isolates and 4 (3.5%) isolate was found resistant (see fig.1). Our observation correlates with these previous findings. This increasing resistance, though insignificant, is again accredited to the increasing use of erythromycin and its derivatives by the physicians. The molecular mechanism of erythromycin resistance in *S.pyogenes* is frequently associated with M phenotype harboring *mefA* gene [14]. In this part of our study we did not analyze the molecular aspect of our observations. Though, there were some reports of clindamycin resistance to *s.pyogenes* from different parts of world [18-19], but we did not find any clindamycin resistant isolate in this region. Comparison of antibiotic sensitivity patterns between individual antibiotics is required for the selection of appropriate drug application (see fig.2). It has been found that 91.3% strains were sensitive to penicillin compare to 89.6% sensitive to erythromycin, however the result was not significant (P=0.823<sup>\*</sup>). It was seen that erythromycin and

penicillin will be better drug of choice than tetracycline as a high percentage of tetracycline resistance has been observed in this region (see table2). In comparison with tetracycline it was found that vancomycin/clindamycin/Cephotaxime was more sensitive to *s.pyogenes* isolates ( $P<0.001^*$ ). From these observations it can be said that vancomycin, clindamycin, Cephotaxime will be more effective drugs to treat GAS infections as because of the rising resistance of erythromycin, penicillin and tetracycline to *s.pyogenes* isolates.

From this study, it can be concluded that emergence of erythromycin resistance even in four cases should be considered very seriously as this is the drug of choice for patients allergic to PenicillinG. Surveillance study with tetracycline and erythromycin should be continued in large scale. This finding may further be analyzed by determining the genotypic variation in resistance determinants.

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All correspondences to Prof. Basudev Bhattacharya, Biochemistry Research Wing, Department of Biochemistry, Dr.B C Roy PG Institute of Basic Medical Sciences (IPGME&R), 244B A J C Bose Road, Kolkata-700020 (India) Tel:+91-9830343484, Fax: 091-33-2223-5076 Attn. Basudev Bhattacharya Email: bbasudev@rediffmail.com