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Original Article

Microbiology Section

Molecular Basis for Erythromycin Resistance in *Group A Streptococcus* Isolated From Skin and Soft Tissue Infections

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ABSTRACT

Background: In recent years there has been an increase in the use of erythromycin in the treatment of infections caused by bacteria other than Group A Streptococcus (GAS), which has resulted in increased resistance to this antibiotic. Erythromycin and other macrolides are alternative agents for treating GAS infections in patients, who are allergic to penicillin and its derivatives.

Aim: The main aim of this study was to identify frequency, pattern and genetic determinant of erythromycin resistance among the GAS isolated from skin and soft tissue infections.

Materials and Methods: A total 100 isolates of GAS were screened for erythromycin resistance by phenotypic and genotypic method.

Results: The results of the present study showed that 38% isolates were resistant to erythromycin. The iMLS (inducible macrolide-lincosamide-streptogramin) phenotype was predominant (55.26%) followed by M phenotype (26.32%) and cMLS (constitutive macrolide-lincosamide-streptogramin) (18.42%).

Conclusion: Phenotypic and genotypic analysis showed that the MLS_B phenotype with *ermB* mediated mechanism of resistance was found the most common (76.31%) followed by *mefA* (20.51%). The *ermTR* genes was absent in all the isolates.

Keywords: *Erythromycin resistance, Genotypes, Phenotypes*

INTRODUCTION

Group A Streptococcus (GAS) causes a variety of infections which are generally treated with penicillin and related β lactam antibiotics [1]. Erythromycin and macrolide are used as an alternative for patients, who are allergic to penicillin [2,3]. In recent years resistance to erythromycin has been reported in many parts of the world including India. There is a wide variation in erythromycin resistance ranging from 5%-40% has been observed in previous studies. The limitations of these studies are that they have used only phenotypic method like disc diffusion method and have not detected genetic determinants of resistance [4-8]. There are two major resistance phenotypes ie MLS B and M phenotypes and genes coding for them are erm and mef [9-11].

The main aim of this study was to assess the frequency, pattern and genetic determinant of erythromycin resistance of GAS strains isolated from skin and soft tissue infections.

MATERIALS AND METHODS

Patients with skin and soft tissue infections attending the surgical OPD of a rural teaching hospital at Puducherry between January 2009 to December 2010 were included in the study. The study was approved by Institutional research and ethic committee. Patients were divided in two groups: Group I with deep cellulitis (80) cases and Group II with other superficial skin infections i.e. pyoderma, folliculitis and wound infections (20) cases. All the patients were in the age group between 31–60 year

Specimen Collection

Specimens were collected from the patients with sterile cotton swab or by fine needle aspiration inoculated on a 5% sheep blood agar and MacConkey agar plates plates. All the plates were incubated in 5% CO_2 at 37°C and bacterial growth was observed at 24 hour and 48 hour. All suspected β-hemolytic streptococcal isolates were tested for sensitivity to bacitracin disc (50IU) (Hi Media Lab, India) and the latex agglutination test (Span diagnostic, India Ltd) was done to serogroup the isolates. The isolates identified as GAS were stored at -70°C [12].

Erythromycin Resistant Phenotypes

All GAS strains were tested for their susceptibility to antibiotics by disc diffusion method as per CLSI guidelines. Erythromycin resistant GAS strains were tested for their resistance phenotype by double disc method. An erythromycin disc (15mg) and a clindamycin disc (2mg) (Hi Media Lab, India) were placed 16 mm apart on plates inoculated with the test strains. The plates were incubated overnight at 37°C in 5% CO_2. After overnight incubation the presence of resistance to both discs was indicative of constitutive macrolide-lincosamide-streptogramin (cMLS) phenotype; susceptibility to clindamycin with no blunting of the inhibition zone around clindamycin disc was indicative of the M phenotype. Inducible macrolide-lincosamide-streptogramin phenotype (iMLS) showed resistance to erythromycin with blunting of the clindamycin zone proximal to the erythromycin disc [13].

Erythromycin Resistant Genotype

All the erythromycin resistant isolates by phenotypic method were further screened for erythromycin resistance genes *mefA*, *ermB* and *ermTR* by a multiplex PCR. The methods and primers used were adapted from the previous study [10]. The template DNA was extracted by alkali hydrolysis method as described earlier [14]. Each 25µl reaction mixture contained 5ul template DNA, 1ul of each *ermB* and *mefA* specific primer sets,1.5ul *ermTR* specific primer set, 1ul dNTP mix and 2ul Taq DNA polymerase in 5ul of 10X PCR buffer. The reactions were carried out in thermocycler (Eppendoff Germany) under the following conditions. Initial denaturation 95°C -2 minute and 30 cycles of denaturation 95°C –1 minute, annealing 55°C- 2 minute and extension 72°C –10 minute. The PCR amplicons were resolved in 1.2% agarose gel by electrophoresis. The amplicon size of *ermB* gene was 616bp, *mefA* gene was 348bp and *ermTR* gene was 206 bp [15].

emm Genotyping

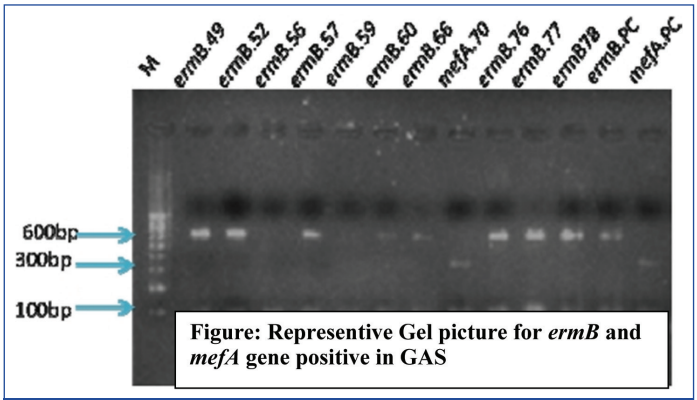
All the isolates were subjected to *emm* gene PCR by using specific set of primers and product sizes ranged between 800bp to 1400bp. The *emm* gene PCR product was sequenced (*Macrogen korea*) and the first 180 bp of sequence of every strain was compared with the sequences in the CDC *emm* database http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm) to determine *emm* type [16].

RESULTS

A total of 100 (22.83%) GAS strains were collected from a pus sample collected from 438 patients suspected of skin and soft tissue infections over a period of two years.

Erythromycin – Resistance

The result of present study showed that 38 (38%) isolates were resistant to erythromycin. The iMLS phenotype was observed to be predominant with (21/38) followed by M phenotype (10/38) and cMLS with (7/38). The iMLS and cMLS were present in 28(73.68%) of isolates. Of which 27 isolates carried ermB gene [Table/Fig-1]. None of the isolate was positive for *mefA* or *ermTR* gene. One isolate was positive for both *ermB* and *mefA*. Of the 10 M phenotypes, 7 were positive for *mefA* gene and *ermB* was present in 3 cases [Table/Fig-2].



[Table/Fig-1]: erm gene PCR

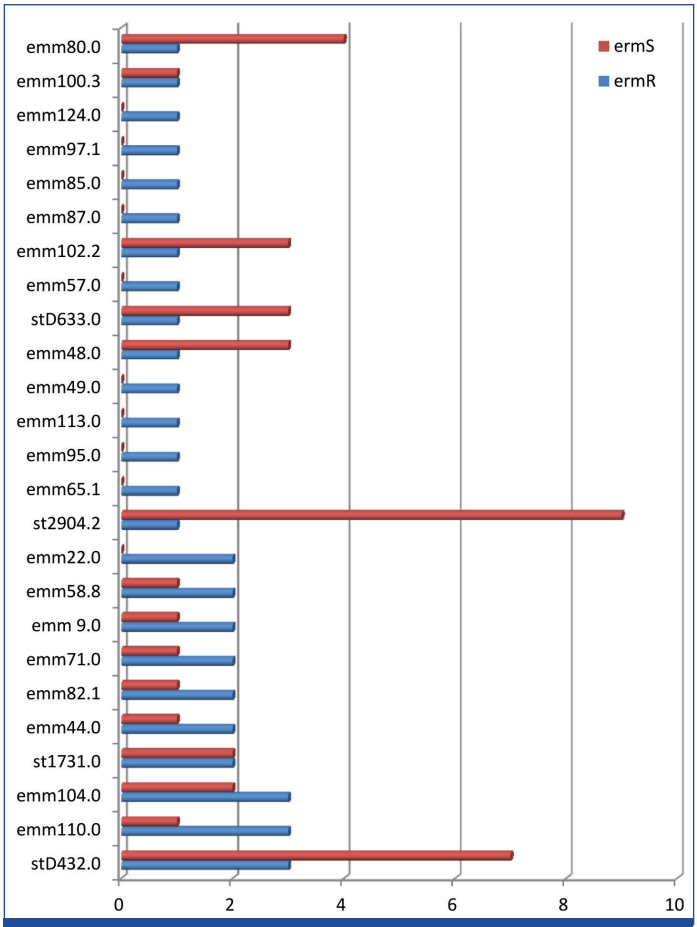
	Phenotype (38)		Genotype (38)	
	mefA	ermB	mefA+ ermB	ermTR
M type (10)	07	03	0	0
iMLS (21)	0	21	0	0
cMLS (07)	0	06	01	0
Total	07	30	01	0

[Table/Fig-2]: Erythromycin resistant phenotype and genotype.

The present study showed a higher rate of *ermB* (30/38, 76.31%) mediated genetic determinant of resistance in Indian isolates followed by *mefA* mediated resistance (7/30, 18.4%). The *ermTR* mediated mechanism was not detected in any of the isolates [Table/Fig-2].

Erythromycin Resistance and emm Types

The highly heterogeneous pattern of emm types was observed with 26 different *emm* types. The *emm 110.0, emm104.0 and st D432.0* were the most frequent and contributed for 36% of GAS showing erythromycin resistance. There was no evidence of dominance of a particular clone of *emm* type. Among the 26 different *emm* types only 6 *emm* types showed more than one erythromycin resistance phenotype and 20 *emm* types showed single erythromycin resistance phenotype indicating minimal overlapping of *erm* phenotypic pattern among the *emm* types [Table/Fig-3].



[Table/Fig-3]: Distribution of *emm* types in erythromycin resistant and sensitive strains of GAS

DISCUSSION

Erythromycin and other macrolide antibiotic are the alternative therapeutic option to those who are allergic to penicillin. In India the use of macrolide group of antibiotics to treat pharyngeal infections has increased since last two decades [17]. The earlier reports in India on erythromycin resistance in GAS indicated a sharp increase in resistance to erythromycin from 2%-38% between 1989-2010. IIn this study we report 38% resistance of GAS to erythromycin. Similar findings were also reported in studies from Manipal (38.13%) [4] and Delhi (29.4%) [5]. The studies carried out earlier in Chennai (9.04%) [6], Vellore (13.8%) [7] and Lucknow (10.2%) [8] showed lower resistance to erythromycin compared to the present study. This is probably because all these studies were carried out on pharyngeal isolates. The present study includes isolates from skin and soft tissue infections, which are more invasive and known to have higher resistance to erythromycin. A similar resistance rate was also observed in Italy (50%), Poland (42%), Spain (27-34%) and UK (> 50%) [18,19]. The earlier studies from Chennai and Delhi has reported the predominance of M type where as present study showed the predominance of iMLS phenotype.

The previous Indian studies have not determined the genetic determinant for erythromycin resistance. This is the first study on genotypic analysis of erythromycin resistance among the GAS strains isolated from skin and soft tissue infections showing predominance of *ermB* gene. The similar *ermB* predominance was also reported earlier from Belgium, France and Italy [20-22].

The high diversity of emm types was also observed among both the erythromycin sensitive and resistance isolates. The present study also showed higher resistance rates compared to previous studies.

This may have resulted from the regular use of erythromycin in India for treating the infections other than GAS which may have caused the organisms to acquire resistance to erythromycin.

CONCLUSION

The result of present study indicates the high resistance of GAS against the 14- membered macrolide group of antibiotics. The infections caused by these strains can be treated with other group of macrolide such as spiramycin, josamycin and midecamycin (16-membered macrolides) with the regular surveillance for monitoring emergence of resistance.

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