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Prevalence and Molecular Characterization of Macrolide Resistance in Clinical Isolates of Beta Hemolytic Streptococci from a Tertiary Care Teaching Hospital in Kerala, India

*Ann George, Rosmi Jose *, Chithra Valsan*

Department of Microbiology, Jubilee Mission Medical College & Research Institute, Thrissur, Kerala, India.

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ABSTRACT

Background: Beta-hemolytic streptococci (BHS) are responsible for both invasive and noninvasive infections, and the preferred treatment for these infections is penicillin due to the distinctive characteristics of these bacteria. However, in patients who cannot tolerate β-lactam antibiotics, macrolides and clindamycin are important alternative options for treating BHS infections. This study aimed to analyse the pattern of macrolide resistance among clinical strains of beta-hemolytic streptococci (BHS).

Methods*:* Beta hemolytic streptococci isolated from clinical specimens during December 2018 to May 2020 were included in this study. Identification of the isolates were done by conventional and Vitek 2 method. All isolates were subjected to serogrouping. Antibiotic susceptibility testing done by disc diffusion method. Genes encoding macrolide resistance were detected by conventional multiplex polymerase chain reaction.

Results: A total of 129 beta hemolytic streptococcal isolates were obtained which included 27 *S. pyogenes* (20.9%), 77 *S.agalactiae* (59.7%), 23 *S.dysgalactiae* spp equisimilis (17.8%) and one isolate of *S.anginosus* and *S. porcinus* each (0.8%). Erythromycin, clindamycin, quinuprisitn and tetracycline resistance were found to be 20.2%, 12.1%, 16.3% and 51.2% respectively. Among the 26 erythromycin resistant isolates, 12(46.2%) were inducible clindamycin resistant phenotype. Out of 26 erythromycin resistant isolates, 7(26.9%) isolates were harbouring *erm(A)* gene, 10(38.5%) *erm(B)* and 9(34.6%) *mef(A)* gene.

Conclusion: Our study highlights the importance of routine antibiotic susceptibility testing for beta-haemolytic streptococci, as well as the detection of inducible resistance to prevent therapeutic failure.

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*Corresponding Authors: Rosmi Jose, Department of Microbiology, Jubilee Mission Medical College & Research Institute, Thrissur, Kerala, India.

Tel: +91-94-95953733, *E-mail:* rosdinmi@gmail.com

Introduction

Beta-hemolytic streptococci (BHS) are known to cause a diverse array of clinical presentations ranging from mild infections to potentially fatal ones. Group A streptococci (GAS) and Group B streptococci (GBS) constitute the most important and prevalent members of this group. GAS can cause conditions like self-limiting pharyngitis or even life-threatening toxic shock syndrome. Group B streptococci (GBS) remains as a leading cause of infections in neonates and pregnant women and also cause invasive diseases in children and non-pregnant adults. Group C (GCS) and group G (GGS) streptococci are commonly found in the throat, skin, gastrointestinal tract, and female genital tract as harmless inhabitants. Recent studies indicate that GCS and GGS, previously considered weak pathogens, can cause infections similar to those caused by GAS (1, 2).

Penicillin remains the drug of choice of BHS infections but there are reports of rising minimum inhibitory concentration (MIC) or diminished susceptibility to penicillin. Macrolides and clindamycin are important alternatives in the treatment of BHS infections in β-lactam-intolerant patients. But high variable resistance to macrolide and therapeutic failure are increasing which substantiate the need for antimicrobial susceptibility testing of BHS isolates (3, 4).

This study was conducted to analyse the pattern of macrolide resistance among clinical isolates of beta hemolytic streptococci.

Materials and Methods

Study design

The study was conducted in the department of Microbiology, Jubilee Mission Medical College & Research Institute, Thrissur, for a period of 18 months (December 2018 to May 2020) on clinical isolates of beta hemolytic streptococci obtained from various specimens received for routine culture. All consecutive non repetitive beta haemolytic streptococci obtained during the study period were included in this study.

Identification of the isolates

All isolates were subjected to Gram stain, catalase test, bacitracin susceptibility, CAMP test and bile esculin hydrolysis. Species were identified based on the biochemical characteristics (5) and confirmed by Vitek 2 GP cards (bioMeriux India Pvt Ltd). Serogrouping of the isolates were done using *Streptococus* grouping kit (Lab21Healthcare, Cameberley).

Antibiotic susceptibility testing

All isolates were subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method on Mueller Hinton agar with 0.5% sheep blood. Susceptibility to penicillin, erythromycin, clindamycin, tetracycline, quinupristin, linezolid and vancomycin were tested and the results interpreted based on CLSI guidelines (6). All isolates were subjected to D test by placing erythromycin and clindamycin discs at 12mm apart (edge to edge). Erythromycin resistant isolates were classified into inducible clindamycin resistance(iMLSB), constitutive clindamycin resistance (cMLSB) and M phenotype based on the D test phenotype (7).

Genotyping of Macrolide resistance

All macrolide resistant strains were subjected to conventional multiplex polymerase chain reaction to detect the genes responsible for the macrolide resistance. The genes studied were ermA, ermB, and mefA. Forward and reverse primers for the corresponding genes were obtained from a previous published study (8).

The PCR products were analyzed by electrophoresis with 2% agarose gels in TBE buffer. The gels were stained with ethidium bromide (75 µl in 500ml distilled water) and PCR products were visualized with UV light using gel documentation system.

Result

A total of 129 beta hemolytic streptococcal isolates were obtained from various clinical samples during the study period. BHS isolates included 27 *S. pyogenes* (20.9%), 77 *S. agalactiae* (59.7%), 23 *S. dysgalactiae* spp equisimilis (17.8%) and one isolate of *S. anginosus* and *S. porcinus* each (0.8%). Majority of the isolates were obtained from pus swabs or exudates (n=71, 55 %), 32 were from urine (24.8%), 13 from cervical swabs (10.1%), 8 isolates from blood (6.2%), 4 from throat swab (3.1%) and one isolate from cerebrospinal fluid (0.8%) (Table 1).

All isolates were susceptible to penicillin, vancomycin and linezolid. Erythromycin, clindamycin, quinupristin and tetracycline resistance were found to be 20.2%, 12.1%, 16.3% and 51.2% respectively (Table 2). Among the 26 erythromycin resistant isolates, majority were inducible resistant phenotype (46.2%), followed by M phenotype (34.6%) and constitutive resistance (19.2%) (Table 3). Out of the 26 erythromycin resistant isolates, 7(26.9%) isolates harboured *erm(A)* gene, 10(38.5%) *erm(B)* and 9(34.6%) *mef(A)* gene (Table 4). Among the 12 isolates with inducible clindamycin resistance six isolates harboured *erm(A)* and six had *erm(B)* gene. Among the isolates with constitutive resistance four had *erm(B)* and one had erm(A). All nine isolates with M phenotype harboured *mef(A)* gene (Table 5).

Discussion

Beta hemolytic streptococci are important causative agent of various invasive and noninvasive infections. Even though these bacteria remain susceptible to penicillin, variable resistance to other antibiotics have been reported from India and other parts of the world (9-11).

The present study was conducted in an 1800 bedded tertiary care teaching hospital, catering to patients from three neighboring districts of central Kerala. All clinically significant isolates of beta hemolytic streptococci obtained from different clinical specimens during the study period were included in this study. Although our study was conducted at a single centre, we were able to collect a substantial number of BHS isolates over an 18-month period. In contrast to previous studies where GAS was more common (8), our study found that GBS isolates made up nearly 60% of all isolates. Low isolation rate of GAS may be attributed to variations in the way cultures are sent for treatment of streptococcal pharyngitis.

The susceptibility of all isolates to penicillin was similar to previous studies, but resistance to other antibiotics varied, with the highest resistance observed for tetracyclines. Over half of the isolates were found to be resistant to tetracycline. In comparison to a prior multicentric study in India where 79% of BHS isolates were found to be resistant to erythromycin, the macrolide resistance in our study was low (20.2%) (8). This might be due to the prevalence of non-Group A BHS isolates, particularly GBS, in our study. Among the BHS isolates, GAS isolates exhibited higher macrolide resistance than the others. A study from south India conducted by Tintu et al. reported resistance rates of 53%, 33%, and 58% to erythromycin, clindamycin, and tetracycline, respectively (12). Clindamycin and quinupristin resistance in our isolates were slightly lower than macrolide resistance, which is in accordance with the phenotypes demonstrated in the D test, indicating that 34.6% of macrolide-resistant isolates were of the M phenotype.

Out of the 26 macrolide-resistant isolates in this study, 12 (46.2%) isolates demonstrated an inducible resistance phenotype. A higher percentage of GAS isolates exhibited inducible resistance in comparison to the other BHS isolates.

Table 1. Sample wise distribution of BHS isolates.

BHS(n)	Pus	Cervical Swab	Urine	Throat Swab	Blood	CSF
GAS (27)	23 (85%)	0	0	1(3.7%)	3(11%)	0
GBS (77)	28 (36.4%)	13(16.8%)	31(40%)	1(1.3%)	3(3.9%)	1(1.3%)
GGS (23)	20 (86.9%)	0	0	2(8.7%)	1(4.3%)	0
GFS (1)	0	0	0	0	1(100%)	0
S.porcinus(1)	0	0	1(100%)	0	0	0
Total (129)	71(55.03%)	13(10.07%)	32(24.8%)	4(3.1%)	8(6.2%)	1(0.8%)

Table 2. Antibiotic resistance profile of BHS isolates.

BHS (n)	Erythromycin	Clindamycin	Quinupristin	Tetracycline
GAS (27)	9 (33.3%)	6(22%)	6(22%)	11(40%)
GBS (77)	13(16.8%)	9(12%)	11(14%)	43(56%)
GGS (23)	3(13%)	1(4%)	3(13%)	11(48%)
GFS (1)	0	0	0	0
S.porcinus(1)	1(100%)	1(100%)	1(100%)	1(!00%)
Total (129)	26(20.15%)	17(12.14%)	21(16.3%)	66(51.2%)

Table 3. Macrolide resistant phenotypes in BHS isolates.

Table 3 Macrolide resistant phenotypes in BHS isolates

Macrolide resistant BHS (n=26)	Inducible (n=12)	Constitutive (n=5)	M phenotype(n=9)
GAS (9)	6 (66.6%)	0	3 (33.3%)
GBS (13)	5 (38.5%)	4 (30.7%)	4 (30.7%)
GGS (3)	1 (33.3%)	0	2 (66.6%)
S. porcinus (1)	0	1 (100%)	0

Table 4. Distribution of macrolide resistance genes among BHS isolates.

Macrolide Resistant BHS (n)	*erm(A) (n=7)*	*erm(B) (n=10)*	*mef(A) (n=9)*
GAS (9)	0	6 (66.6%)	3 (33.3%)
GBS (13)	6 (46.2%)	3 (23%)	4 (30.8%)
GGS (3)	1 (33.3%)	0	2 (66.6%)
S.porcinus (1)	0	1 (100%)	0

Table 5. Genotype distribution among different phenotypes of macrolide resistant BHS.

Macrolide resistant BHS (n=26)	iMLSB (n=12)		cMLSB (n=5)		Mphenotype (n=9)
	erm(A)	*erm(B)*	*erm(A)*	*erm(B)*	*Mef (A)*
GAS (9)	0	6 (66.6%)	0	0	3 (33.3%)
GBS (13)	5 (38.46%)	0	1 (7.7%)	3 (23%)	4 (30.8%)
GGS (3)	1 (33.3%)	0	0	0	2 (66.6%)
S.porcinus (1)	0	0	0	1 (100%)	0

Similar to previous studies conducted in India, there was a higher prevalence of inducible resistance (8, 12). The iMLSB phenotype was also found to be predominant in studies conducted in Norway and Bulgaria (13). These findings highlight the importance of detecting inducible resistance in routine antimicrobial susceptibility testing to prevent treatment failure due to false susceptibility reporting.

Resistance to macrolides in the *Streptococcus* genus is mainly caused by modification of the drug target site through methyltransferases encoded by erm genes or active efflux via an efflux pump encoded by the mef gene. Among these genes, *erm(A)* is expressed in an inducible manner, while *erm(B)* can be expressed constitutively (cMLSB) or inducibly (iMLSB) and leads to high-level resistance (14). This information suggests that different genes and modes of resistance can lead to macrolide resistance in Streptococcus, which is important to consider when testing for antibiotic susceptibility.

The macrolide-resistant isolates exhibited a diverse distribution of resistance genes, as revealed by molecular characterization. In GAS isolates, the *erm(B)* gene was the most common genotype detected, followed by *mef(A)*, whereas in GBS isolates, the *erm(A)* gene was the most frequently detected genotype. In our study six GBS isolates and one GGS isolate were found to carry the erm(A) gene. This contrasts with previous studies conducted in India where none of the BHS isolates were found to carry the *erm(A)* gene [8,12]. However, Balaji et al. from India and Michos et al. from Greece have reported the predominance of the *erm(A)* gene in GAS isolates [15,16]. Inducible resistance in GAS isolates was exclusively associated with the *erm(B)* gene, while it was linked to the *erm(A)* gene in GBS and GGS isolates. The *erm(B)* gene was the predominant genotype detected among isolates with a constitutive resistance phenotype. In line with previous studies conducted in India and other parts of the world, all isolates with the M phenotype harboured the *mef(A)* gene. These findings suggest a diverse distribution of macrolide-resistant genes across different *Streptococcus* species.

Conclusion

Our study was one of the first study from Kerala, reporting the antimicrobial resistance in BHS isolates. This study was done just before the beginning of the COVID 19 pandemic and macrolide resistance was found to be relatively low at the time of the study. However, macrolides are commonly misused antibiotics, particularly during the COVID-19 pandemic, and have been identified as a major driver of macrolide resistance in streptococci, both at the population and individual levels (17, 18). In this study we did not investigate the impact of macrolide consumption on resistance rates. Therefore, further research on macrolide resistance and its consumption is necessary to develop policies to regulate the misuse of this agent. Our study highlights the importance of routine antibiotic susceptibility testing for beta-haemolytic streptococci, as well as the detection of inducible resistance to prevent therapeutic failure. Additionally, genotypic analysis will provide insights into the variability of strains in different regions of the country and the world.

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Ethics approval and consent to participate

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Conflict of interest

The authors notified that there are no conflicts of interest.

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