Original Article

Antimicrobial Resistance Pattern Of Invasive Group A Streptococci From Various Clinical Samples In A Tertiary Care Hospital

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Abstract

Background: Group A streptococcus[GAS] causes illness ranging from self- limiting pharyngitis to life threating systemic and invasive infection. In India, there is a sharp rise of 2%-38% macrolide resistance among GAS isolates. Hence this study is done to know the prevalence of antibiotic resistance among GAS isolates.

Materials and Methods: Samples (pus, sputum, blood) were collected & processed as per standardprotocol; Group A Streptococcus were isolated. Antibiogram done as per CLSI guidelines, MIC done by E-test.

Results: 52 Beta haemolytic streptococci(BHS) were isolates of which 36(69.23%) were Group AStreptococci(GAS), 28(53.84%) were in the age group above 60 years. Erythromycin resistance is seen in 19 (52.77%) isolates of GAS, followed by Tetracycline resistance seen in 17(47.22%) isolates. MIC of Erythromycin were in the range of (0.5 to 256) for 17 isolates, of which 12 isolates exhibited M phenotype.

Conclusion:
In our study GAS isolates showed higher resistance towards erythromycin, this prevailing rise in macrolide resistance is due to the misuse, over use, and easy availability of drugs over the counters. Hence there must be a regular surveillance of antibiotic resistance phenotypically to accurately identify the resistant isolates.

Keywords: Beta hemolytic streptococci, Group A streptococci, Erythromycin resistance, Macrolide resistance.

Introduction

Streptococcus pyogenes (Lancefield Group A, GAS) is a significant pathogen in humans which causes wide range of infection ranging from pharyngitis to life threatening invasive infection(1).GAS colonizes the nasopharynx or skin and is responsible for causing many of the suppurative and non- suppurative sequalae (2)
In 1980's there is a increase in streptococcal disease in causing serious life threatening infection(3). If untreated, the mild streptococcal infection can lead to glomerulonephritis and acute rheumatic fever, which is a major problem in developing countries(1)

GAS isolates are susceptible to penicillin which is reported globally and is the main stay of therapy with β lactam antibiotics. Macrolides, lincosamides and fluoroquinolones are the alternative treatment options in penicillin allergic patients(1)

Macrolides are widely used for treating streptococcal pharyngitis and respiratory infection, Clindamycin is widely used for serious soft tissue(4)
In India have reported that there is a sharp increase of 2%-38% Macrolide resistance among GAS isolates(1)

Various studies reported the resistance of GAS isolates varied from 1-2%, but nil resistance has been reported in six intervening years and the currently the resistance is increasing due to the fact that they are freely available as " over the counter drugs" in pharmacy.(5)

Studies on GAS and its antimicrobial resistance is done worldwide, but there is paucity of the information from India. Where the epidemiology of GAS differs, the streptococcal isolates are common in our hospital samples, and the resistance pattern also is sometimes alarming. Hence this study was done to study the phenotypic characterization of the strains prevalent in our hospital. This will help the clinician to start the empirical therapy early.

Material and Methods

This study was carried out in Microbiology department in Meenakshi Medical College Hospital & Research Institute (MMCH & RI) at Kanchipuram. Ethical committee clearance was obtained from the Institute, Study period: April 2021 to March 2022, Sample Size- 188

Samples: Sputum, pus, urine, blood, throat swab, body Fluids.

Inclusion criteria: is Outpatient and Inpatients with suspected cases of acute pharyngitis, rheumatic fever, cellulitis, impetigo, erysipelas, necrotizing fasciitis, mastoiditis, tonsillitis, otitis, pneumonia and bone and joint infections with age above 21 years, both sexes were included.

Exclusion criteria: is Those not willing for the study and those who were on antibiotics for past 3 months.
After collecting the sample pus, wound swab, and sputum were subjected from Gram staining and inoculation into various medias. All the samples except blood were streaked onto the surface of various media such as 5% sheep blood agar (incubated in a candle jar at atmosphere of 5% CO2) and MacConkey agar, Crystal violet blood agar, Polymyxin neomycin fusidic acid agar plates and incubated at 37°C for 24-48 hours, and observed for signs of growth and colony appearance for Streptococcus pyogenes.
Blood sample, after inoculation into Brain Heart Infusion (BHI broth) were sub cultured on various media and observed for growth.
Identification of Group A Streptococci is done by routine laboratory techniques such as Gram staining, catalase test, Bacitracin sensitivity, PYR test.
Antibiotic susceptibility test for bacterial isolates was done by kirby- Bauer disc diffusion method with commercially available discs (Hi- Media) on Muller Hinton agar supplemented with 5% sheep blood. Colonies were picked up in Todd Hewitt broth until a turbidity of 0.5 McFarland (1x 10 6 CFU/ml) following antimicrobial discs (Penicillin (10 units), Cefotaxime(30µg), Erythromycin(15µg), Clindamycin(2µg), Ciprofloxacin(5µg), Tetracycline(30µg), Vancomycin(30µg), Linezolid(30µg)) were placed on the inoculated plate. Zone of inhibition was measured around the disc as recommended by CLSI guidelines
MIC of Erythromycin was determined by E-test according to manufacturer's instructions (Hi-media). E- test strips were placed on the plate using sterile forceps, and the plates were incubated overnight at 37°C. MIC reading was taken as the point of intersection of the zone of inhibition of growth and the E-Strip. The results were interpreted according to CLSI guidelines.

D test (Determination of macrolide resistant phenotype)

This test is done for isolates which was erythromycin resistant and clindamycin susceptible. Standard disk diffusion D zone testing was performed using erythromycin and clindamycin disk placed 15-20mm apart on Mueller-Hinton 5% sheep blood agar plates incubated at 37°C in 5% carbon dioxide for 48hours, Blunted D-shaped clear zone around clindamycin disk proximal to the erythromycin disk indicated a positive D-test result.

Results

Out of the 188 samples, 52(27.56%) Beta hemolytic streptococci (BHS) were isolated.
Of the 52 BHS, 36 (69.23%) were Group A Streptococci. BHS were isolated more frequently from males 34(65.38%) than females (34.61%).

Table 1: showing the age wise distribution of BHS

Age	No of BHS (n=52)	Percentage (%)
21-40 years	9	17.31
41-60	15	28.85
Above 60 years	28	53.84

Majority of the patients belong to the age group of above 60 years.

Table 2: shows antibiotic resistant pattern of GAS.

Antibiotic	Resistant	Percentage
Erythromycin	19	52.77
Tetracycline	17	47.22
Ciprofloxacin	12	33.33
Vancomycin	8	22.22
Clindamycin	12	33.33
Ceftriaxone	5	13.88
Penicillin	2	5.5
Linezolid	2	5.5

Highest resistance is seen in erythromycin (52.77%) followed by Tetracycline (47.22%), ciprofloxacin (33.33%) and least resistance was seen in penicillin(5.5%) and linezolid(5.5%).

Out of the 36 GAS isolates, 12 (33.33%) isolates exhibited co- resistance to Erythromycin and tetracycline. 2 (5.5%) isolates were multidrug resistance.

Of 36 GAS isolates, 12 isolates showed M phenotype that is erythromycin resistant and clindamycin sensitive without blunting.

19 Erythromycin resistant strains were subjected to MIC of E-test, of which 17 resistant isolates (>1-256) were confirmed by E test (89.47%), 2 (10.52%) isolates were moderately sensitive(0.5-256).

Discussion

Due to selection pressure exerted by usage of antibiotics is a major cause for development of antibiotic resistant organisms. This is even worsened by the availability of ‘over the counter drugs’, which is practiced in India without any surveillance of the resistant organism(1).

In our study 52 (27.56%) isolates of beta hemolytic streptococci were collected from various clinical samples. Which is concordant with the study done by Nirmal Kushwaha et al who reported 30.7%of beta hemolytic streptococci(6)and also study done by Vanita Dhanda et al who reported a much higher incidence of 37.4% of BHS(7)

In our study 69.23% of GAS were isolated, which is concordant with study done by Vanitha et al who also reported 65.8% of GAS, and also study done by Nidhi Bhardwaj et al who reported a much higher incidence of 74% of GAS(8,9)

In our study among the 52 BHS, Male predominance were seen as 61.53% and female were 38.64%, which is concordant with study done by Abraham and Sistla et al who reported 62% male and 38% female(10)In this study our finding are much lower than Nirmal Kushwaha et al who reported 47% male and 64% female(6)

Male predominace is due to males being involved more in outdoor activities hence being exposed to trauma and infection(11)

In our study BHS isolate is more in age group above 60 years, this findings were concordant with the study done by Abraham and Sistla et al who also reported age above 60 years were predominant (10)

Erythromycin resistance is progressively increasing since 1955 worldwide. Erythromycin resistance varies between 5% to 40%, highest being reported in Asia and lowest prevalence being reported in Europe and USA. This is of great concern since macrolides are the drug of choice for penicillin allergic patients and for treating severe streptococcal disease. This increase in resistance is due to injudicious overuse of antibiotics.(1)

In our study erythromycin resistant is seen in 19(52.77%) isolates which is concordant with the study done by Abraham and sistla et al who have reported 53% of erythromycin resistance .(1) Much lower incidence reported in a study from Puducherry by Khyriem AB et al who reported 1% of erythromycin resistance. (12)Over the years of 1986-1993 erythromycin resistance was 2.7%, increased to 5.8% in 1999 and reached 13.8% in 2002(5)Past Indian studies by Jacob et al, Jain et al and Lloyd et al. go in line withBrahmadathan et al with a report prevalence of 9.04%, 10.2% and 16.2% respectively.(13–15)Shivekar and Menon in 2015 reported a slighter higher rate 38% of macrolide resistance among south Indian GAS isolates.(16)In the year of 2012-2014 antibiotic surveillance done showed 78 GAS isolates collected from four sites across India, reported erythromycin resistance as 76.3%(17) in the contrary to the above finding of Ray et al from eastern india, reported that the erythromycin resistance in GAS was very low(2.85%).(18)This massive increase in the macrolide resistance can be due to increase usage of azithromycin for all common respiratory infection.(1)

In our study erythromycin and tetracycline co-resistant were seen in 12(33.3%) isolates which is concordant with the study done by Abraham and sistla et al who reported erythromycin and tetracycline co-resistant as 39%.(1) and study done by Eleonora Giovanetti et al who reported a higher co-resistant of 73%(19) and study done by Bhardwaj et al reported co-resistance of 30.6% which is lesser compared to our study.(9)

In our study M phenotype is seen in 12(33.33%) isolates were erythromycin resistant and clindamycin sensitive which is concordant with the study done by Abraham and sistla et al who reported 37% of erythromycin resistant but clindamycin sensitive.(1) Bhardwaj et al reported a lesser of 28% of erythromycin resistant and clindamycin sensitive phenotype(M phenotype)(9)
In our study 100% concordance was found in D-test for inducible clindamycin resistance .
Hence D-test must be made as a routine method for detection of inducible clindamycin resistance, which is helpful in the empirical treatment of the patients.
In our study Multidrug resistance is 5.5%, which is concordant with the study done by Bhardwaj et al who reported multidrug resistance as 4%(9) study done by Destawkebede et al reported MDR of 21.3%(20) is higher than our study.

Conclusion

In our study co-resistance of erythromycin and tetracycline is seen in 33.33% GAS isolates, multidrug resistance is seen in 5.5% of GAS isolates, M phenotype was observed in 33.33% of GAS isolates, which is erythromycin resistant and clindamycin sensitive.
Hence there must be a regular surveillance of antibiotic resistance both phenotypically and genotypically to accurately identify the resistant isolates.Due to the emerging antibiotic resistance developing all over the world, there must be stringent measures taken to avoid the easy availability of antibiotics over the counters and educating

pharmacist about the development of antibiotic resistance caused due to issue of drugs over the counters without proper prescription, thereby preventing the rise of antimicrobial resistance in developing country like India.

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