

Original Research Article

Characterization of Beta-Hemolytic Streptococci from Clinical Isolates in a Tertiary Care Centre in North-East India

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ABSTRACT

Introduction: Hemolytic streptococci are one of the most frequent human pathogens capable of producing a variety of diseases ranging from pharyngitis, impetigo, to more severe and life threatening diseases like Toxic Shock Syndrome, neonatal sepsis, pneumonia and meningitis. Though they are still susceptible to commonly used antibiotics, resistance is increasingly reported.

Materials and Methods: This was a hospital based prospective study conducted to characterize the beta hemolytic streptococci prevalent in a tertiary care centre in North-east India. All the catalase negative, beta hemolytic gram positive cocci were subjected to phenotypic tests for presumptive identification and Lancefield antigen grouping was performed using Latex agglutination kit test. Antimicrobial sensitivity testing by Kirby-Bauer disk diffusion method and broth macrodilution method were performed.

Results: A total of 91 non-duplicate, consecutive patient-specific clinical isolates of β -hemolytic streptococci were obtained. No gender predilection was noted except for Group B Streptococcus. More than half the isolates were obtained during winter months. GAS was the most common group isolated in our hospital followed by GBS, GGS, GCS and GFS. All the isolates were sensitive to beta lactams. Though disk diffusion method showed 100% sensitivity for penicillin, 3 GBS isolates showed increased MIC for penicillin. Of the isolates, 94.5% and 84.61% were sensitive to erythromycin and tetracycline respectively.

Conclusion: Invasive streptococcal infections have increased worldwide in the recent past, despite the organism's susceptibility to commonly used antibiotics. Large scale surveillance programmes like LEADER, SENTRY surveillance etc., had reported increasing resistance among beta hemolytic streptococci. Further evaluation of the strains and continued monitoring of the antimicrobial sensitivity pattern will help in better understanding of these bacterial infections.

Keywords: beta-hemolytic, streptococci, resistance, latex agglutination, seasonal variation, MIC.

INTRODUCTION

Streptococcus is a genus that groups catalase negative, gram-positive chain forming cocci which are widely distributed in nature. [1] Most species are facultative anaerobes, and some grow only in an atmosphere enhanced with carbon-

dioxide. Streptococci are divided into more than 40 species and multiple groups. The taxonomy has changed several times over the years. [2] The differentiation of species within the genus is complicated because several different schemes are used to classify the organisms. [3]

Over the years, the classification of streptococci into major categories has been based on a series of observations. (i) colony morphology and hemolytic reactions on blood agar; (ii) serological specificity of the cell wall group specific substances (Lancefield classification) and other cell wall or capsular antigens; (iii) biochemical factors and (4) ecological features. [1] The most common method of classification is based on the appearance of colonies grown on blood agar plates. Hemolysis is used as a guide for managing patients as well as an aid in classification of bacterium to the species level. [4] Beta hemolytic streptococci can be subdivided into large colony and small colony (<0.5mm in diameter) formers. *Streptococcus pyogenes* (Lancefield Group A), *Streptococcus agalactiae* (Group B) and *Streptococcus dysgalactiae* subsp. *equisimilis* (Group C, G) belong to large colony formers. [4] *Streptococcus anginosus* belong to small-colony formers. [5]

Hemolytic streptococci can be divided into serologic groups, and some of these groups can be subdivided into types. Several antigenic substances are found and group specific cell wall antigen is one of them. This carbohydrate is in the cell wall of many streptococci and forms the basis of serologic grouping (Lancefield groups). [1]

Based on the presence of these antigens, twenty one groups (Lancefield groups) have been identified so far and named from A to W (without I and J). Among these, only four (A, B, C and G) are commonly isolated from human infections and other groups are very rarely encountered.

Group A streptococci are one of the most frequent human pathogens capable of producing a variety of diseases. Such infections range from suppurative sequelae like pharyngitis, impetigo, necrotizing fasciitis, to more severe and

life threatening disease like Streptococcal Toxic Shock Syndrome (STSS).

Group B beta-hemolytic streptococci have gained increasing significance as a human pathogen, causing sepsis, meningitis, pneumonia, and severe genitourinary tract infections. [6] Group B streptococci are the most frequent cause of sepsis and meningitis in newborn infants. [7]

Group C and G streptococci resemble group A streptococci but cause a number of different diseases in animals like horses, cows, cats, dogs and pigs. [7] Though group C and G streptococci had been regarded as weak pathogenic bacteria, it has been reported in recent years that these streptococci can also cause STSS similar to Group A streptococci. [8,9]

Macrolide resistance rates among isolates of *S. pyogenes* and *S. agalactiae* have been increasing in North America as well as in Europe. [10] Resistance rates correlate well with the use of macrolides in clinical practice and geographic differences in resistance rates are often due to differences in macrolide use.

Beta hemolytic streptococcal isolates with a reduced susceptibility to vancomycin have not been reported. [11]

There is a dearth of published data about the prevalence of various serogroups of β -hemolytic streptococci and their antimicrobial sensitivity pattern in North East India. This study was conducted in view of the relevance and impact created by the above with the following objectives: i) To identify the β -hemolytic streptococci from clinical isolates. ii) To study the prevalence of different serogroups of β -hemolytic streptococci and iii) To determine the trends in antimicrobial susceptibility of β -hemolytic streptococci.

MATERIALS AND METHODS

This was a hospital based prospective study conducted in Department of Microbiology,

NEIGRIHMS, Shillong over a period of one and half years (December' 2012 – May' 2014). All isolates of β -hemolytic streptococci from the following clinical samples were included: throat swab, respiratory secretions (sputum, endotracheal secretions and broncho alveolar lavage), pus and wound swabs, vaginal swabs, sterile body fluids (pleural fluid, cerebrospinal fluid, peritoneal fluid, synovial fluid) and blood.

These isolates were initially identified by observing the morphology of colonies with reference to size, shape, moistness and the relative size of zone of hemolysis. All the catalase negative, beta hemolytic gram positive cocci were subjected to phenotypic/biochemical tests namely PYR test, bacitracin sensitivity test, CAMP test, hippurate hydrolysis test and VP test. The isolates showing positive result with PYR test and sensitive to bacitracin (0.04U) were presumptively considered as Group A streptococci. The isolates which showed positive CAMP test and hydrolyzed hippurate were presumptively considered as Group B Streptococci. Lancefield antigen grouping was performed using Latex agglutination test (PASTOREXTM STREP (BIO-RAD)). The individual biochemical tests were compared with the latex agglutination test for the assessment of their diagnostic efficacy. Antimicrobial sensitivity testing by Kirby-Bauer disk diffusion method was performed for penicillin (10 μ g), erythromycin (15 μ g), ceftriaxone (30 μ g) and tetracycline (30 μ g). Broth macrodilution method was performed to detect minimum inhibitory concentration of penicillin. All the results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) 2014 guidelines. [12]

RESULTS

A total of 91 non-duplicate, consecutive patient-specific clinical isolates of β -hemolytic streptococci were

obtained during the study period (December 2012 to May 2014). The 91 isolates of beta hemolytic streptococci from the clinical samples were obtained from 46 (50.55%) males and 45 (49.45%) females. Sixty six isolates (n=91, 72.53%) were obtained from age group of 20-50. Prevalence of β -hemolytic streptococci was found to be less in the extremes of age groups. One (1.1%) isolate each was obtained from an infant and a person above 70 years respectively. Most of the samples were obtained from General medicine and General surgery departments (29.6% each). The distribution among other departments in decreasing order of frequency was Otorhinolaryngology (14.3%), Obstetrics & Gynaecology (10.99%), Intensive care units (7.7%), Orthopaedics (4.4%) and Dermatology (3.3%).

The prevalence of β -hemolytic streptococci showed considerable variation among four different seasons. Statistically significant increase in the number of isolates was seen in winter season comprising November, December and January months (More than half the number of total isolates were obtained in these 3 months). Spring (14.91%, p=0.0262), summer (13.16%, p=0.0091), autumn (15.79%, p=0.0425) and winter (56.14%, p<0.0001).

The β -hemolytic streptococci isolates were obtained from the following clinical samples in decreasing order of frequency: pus (32), sputum (23), high vaginal swabs (10), throat swabs (10), wound swabs (9), blood (4), tracheal secretions (1), peritoneal fluid (1) and uterine endometrial swab (1).

All the β -hemolytic streptococci isolates were initially subjected to the phenotypic tests for presumptive grouping. The isolates which showed Bacitracin sensitivity and positive result in PYR test were presumptively grouped under Group A Streptococci. The isolates which showed positive result in CAMP test, hydrolyzed

hippurate and showed positive Voges-Proskauer reaction were presumptively grouped under Group B Streptococci. Rest were grouped under non-A non-B (NANB) Streptococci. The Latex agglutination test for identification of group specific

carbohydrate antigen was taken as a standard to evaluate these presumptive tests. The diagnostic characteristics of Bacitracin test and CAMP test are summarized in Table 1.

Table 1 : Evaluation of Bacitracin disk test and CAMP test as standalone tools for presumptive identification of Group A Streptococci and Group B Streptococci respectively

	Bacitracin disk test	CAMP test
Sensitivity	82.26% (95% CI: 70.46% to 90.78%)	84.21% (95% CI: 60.4% to 96.43%)
Specificity	72.41% (95% CI: 52.76% to 87.23%)	100% (95% CI: 94.96% to 100%)
Positive likelihood ratio	2.98 (95% CI: 1.63 to 5.44)	NA
Negative likelihood ratio	0.25 (95% CI: 0.14 to 0.44)	0.16 (95% CI: 0.06 to 0.45)

The latex agglutination tests showed the following results: 62 Group A Streptococci (68.13%), 19 Group B Streptococci (20.87%), 8 Group G Streptococci (8.79%), Group C and F Streptococci 1 (1.09%) each. Among 62 Group A Streptococci 35(56.45%) and 27(43.54%) were obtained from males and females respectively. Among 19 Group B Streptococci 13 (68.42%) were from females and 6(31.58%) were from male patients. The only Group C streptococcus isolated was from a female patient and the only Group F Streptococcus isolated was from a male patient. The Group G Streptococci were isolated equally from

male (4) and female (4) patients. Distribution of different groups of β -hemolytic streptococci among different clinical samples is presented in

Table 2. The antimicrobial sensitivity pattern is shown in Table 3.

Table 2 : Distribution of different groups of β -hemolytic streptococci among different clinical samples

Clinical Sample	A	B	C	F	G
Blood	1	3	-	-	-
High Vaginal Swab	1	8	1	-	-
Peritoneal fluid	1	-	-	-	-
Pus	27	3	-	1	1
Sputum	17	1	-	-	5
Throat swab	8	1	-	-	1
Tracheal secretion	-	-	-	-	1
Uterine endometrial swab	-	1	-	-	-
Wound swab	7	2	-	-	-
Total	62	19	1	1	8

Table 3 : Antimicrobial sensitivity pattern of different groups of beta hemolytic streptococci (S – Sensitive, I – Intermediate, R – Resistant)

Group	Penicillin			Erythromycin			Tetracycline			Ceftriaxone		
	S	I	R	S	I	R	S	I	R	S	I	R
A	62	0	0	60	0	2	51	5	6	62	0	0
B	19	0	0	17	0	2	17	0	2	19	0	0
C	1	0	0	1	0	0	1	0	0	1	0	0
F	1	0	0	0	0	1	1	0	0	1	0	0
G	8	0	0	8	0	0	7	1	0	8	0	0
Total	91	0	0	86	0	5	77	6	8	91	0	0

DISCUSSION

Beta hemolytic streptococci are clinically an important group of virulent bacteria and the incidence of invasive infections is found to be increasing. [11] Though resistance to antimicrobial agents is found to be less in hemolytic streptococci, the resistance to certain group of antibiotics has been increasing over the years and this warrants continued and close monitoring of the susceptibility

patterns of these bacteria. This study was an endeavour to determine the prevalence of beta hemolytic streptococci in various clinical samples obtained from a tertiary care hospital and to characterize them.

In our study, Group A and Group B were the predominant hemolytic streptococci which was similar to findings of studies by Megged *et al* (n=245) and Kocuglu T *et al* (n=260) in which 51.84% GAS, 35.90% GBS, 2.86% GCS, 2.04%

GFS, 7.34% GGS and 83.80% GAS, 1.2% GBS, 6.5% GCS & 3.5% GGS were isolated respectively. [13,14] In a similar study by Srifuengfung *et al* (n=304) equal number of GAS (24.01%) and GBS (23.03%) were isolated from clinical samples. [15] In contrast, few studies showed predominant GBS isolation from clinical samples. A study by Traub *et al* (n=312) showed that 46.40% of the isolates of beta hemolytic streptococci belonged to Group B, followed by GAS (20.19%), GCS (16.02%), GFS (8.65%) and GGS (8.65%). [16] In a study by Young Uh *et al* (n=540), 57.03% GBS, 26.48% GAS, 12.40% GGS and 4.07% GCS were isolated. [17] In a recent study conducted by M Ichikawa *et al* (n=918) seventy seven percent (77.88%) of the isolates belonged to Group B, followed by Group A (9.14%), Group G (9.04%) and Group C (3.92%) streptococci. [18] However a study conducted in South India by Thangarajan *et al* (n=124) showed predominantly GCS (59.70%) followed by GGS (25.00%) and GAS (15.30%). [18] These findings establish the fact that prevalence of different groups of beta hemolytic streptococci differs with time and geographical location and warrants characterization of beta hemolytic streptococci in different locations from time to time.

The β -hemolytic isolates obtained in the present study were distributed equally among males (50.55%) and females (49.45%) ($p=0.9164$). However, among different groups, Group B streptococci were isolated more (68.42%) from female patients and the difference is statistically significant ($p=0.0004$). These findings correlated well with results of a study by Douglas JB *et al* (2003), in which the distribution of male and female patients was nearly even for all serogroups except for patients infected by serogroup B (females represented 69.8%). [19] Similar findings were seen in a study conducted by M Ichikawa *et al* where no significant

gender predilection among other groups and significantly ($p<0.05$) higher number of Group B isolates were obtained from female patients. [20]

In our study, 72.53% (66/91) of the isolates were obtained in the age group of 20-50 years. Prevalence of β -hemolytic streptococci was found to be less in the extremes of age groups. One (1.1%) isolate was obtained from an infant and one (1.1%) from a person above 70 years. These findings correlated with the surveillance study conducted by Douglas J B *et al*. [19] Similar age distribution was seen even with individuals groups A, B, C and G isolated. The only group F streptococcus isolated, was from a 13 year old patient. In a study by M Ichikawa *et al*, Group A Streptococci were predominantly seen in those less than 30 years. Twenty-five percent of GBS were isolated from patients in their 30s and twenty-four percent of GBS from patients above 70 years. GGS was isolated more from patients above 60 years than younger patients. [20] In contrast to this study, no significant difference was observed in our study except that less number of Group B and Group G streptococci were isolated from older patients.

In the present study, the prevalence of β -hemolytic streptococci in different seasons of the year showed significant variation. There was an increased incidence in winter months (56.14%) ($p<0.0001$) which was consistent with the findings of a study conducted by Madhur *et al* in which 50% (n=529) of the beta hemolytic streptococci were isolated in the winter months. [21] Though the total numbers of samples tested were less in winter months, the numbers of study isolates were significantly higher during this period. In our study 13.16% of the isolates were obtained during summer months. Among different groups in our study, Group A (60.52%) and Group B (48.14%) streptococci showed a significant increase in winter months whereas Group

G streptococci did not show any seasonal variation. The single Group C streptococcus was isolated in the month of December and the single Group F streptococcus was isolated in the month of May. A study by I Sevinc *et al* reported similar findings of Group A streptococci isolated at 47.82% (n=230) during the winter months. [22] The least number of isolates (9.13%) were obtained during summer months. In contrast to the above findings F Baquero *et al* reported an isolation rate of 11.6% (n=786) of GAS during summer months. Even the frequency of macrolide-resistance exhibited a seasonal pattern, being 13.2, 25.0, 31.7, and 31.3% in summer, autumn, winter, and spring, respectively (P<0.001). Antibiotic consumption patterns and existence of a seasonal clone variation of new phenotypes with respect to virulence and antibiotic susceptibility were thought to be reasons for this seasonal variation. [23]

In our study, pus and wound swabs together yielded 45.05% (41/91) of the total beta hemolytic streptococcal isolates followed by sputum (23/91), throat swab (10/91), high vaginal swabs (10/91) and blood (4/91). One isolate each was obtained from a peritoneal fluid, tracheal secretion and uterine endometrial swab. A study by Megged *et al* however showed more number of beta hemolytic streptococci from throat swabs, followed by vaginal swabs and wound swabs/pus. [13] In a study by Young Uh *et al* 35.3% of isolates were from genitourinary specimens followed by wounds (33.3%), blood (15.7%), lower respiratory tract specimens (8.8%), throat (5.3%) and other body fluids (1.5%). [5] Among the different clinical samples pus (27) followed by sputum (17), throat swab (8) and wound swabs (7) yielded high number of Group A Streptococci. Group B Streptococci were isolated more from high vaginal swabs (8) and pus (3). The only group C streptococci isolated was from high vaginal swab and

the only group F streptococci isolated was from pus. Group G streptococci were isolated mostly from sputum samples (5). Blood culture from a case of neonatal septicaemia yielded Group B Streptococci and this is the only group isolated from a neonate in our study. In a study by M Ichikawa *et al* Group A streptococci were predominantly (21%) obtained from pharyngeal mucus, Group B streptococci from urine, Group C streptococci from sputum and Group G streptococci from pus. [20]

The biochemical tests evaluated for the characterization and presumptive identification of beta hemolytic streptococci yielded results similar to that of David *et al*, D Nsanga *et al* and H B Ratner *et al*. [24-26] Bacitracin susceptibility testing alone gave a sensitivity of 82.26% and specificity of 72.41% in the identification of Group A beta hemolytic streptococci, but when combined with PYR test the sensitivity increased to 93.55% and specificity increased to 86.21%. Combination of two or more tests in the identification of Group A streptococci yielded better results. Similarly CAMP test done for identification of Group B Streptococci showed a sensitivity of 84.21% and specificity of 100%. The combination of CAMP test with hippurate hydrolysis test for identification of Group B streptococci increased the sensitivity above 90% (94.74%).

Among different clinical syndromes beta hemolytic streptococci were isolated mostly from skin and soft tissue infections in our study (45.05%). Isolates from lower respiratory tract infections were 26.37%, 12.08% from genitourinary infections and 10.98% from upper respiratory tract infections comprising of pharyngitis and tonsillitis. Among the beta hemolytic streptococci, Group B streptococci were the leading cause of genitourinary infections (81.81%) and septicemia (75%). Group A

streptococci predominated in respiratory tract infections (73.52%) followed by Group G streptococci (25%). Majority of skin and soft tissue infections were caused by Group A streptococci (82.92%) followed by Group B streptococci (12.2%).

Our study revealed findings similar to that of M Ichikawa *et al* in which GBS predominated in genitourinary system disorders and GAS in skin and soft tissue infections. [20] Large numbers of GGS obtained were from isolated from sites similar to GAS which suggested similar virulence factors among these two groups which were reinforced in our study.

Antimicrobial sensitivity pattern

Group A Streptococcus : In our study all the sixty two isolates (100%) were susceptible to penicillin when tested using disk diffusion method and the minimum inhibitory concentration determined using broth macrodilution was ≤ 0.06 $\mu\text{g/ml}$. Sixty isolates (96.77%) were susceptible and two (3.23%) were resistant to erythromycin. Fifty one isolates (82.25%) were susceptible to tetracycline. Five isolates (8.06%) showed intermediate susceptibility and six isolates (9.67) were resistant to tetracycline. All the sixty two isolates (100%) were susceptible to ceftriaxone.

In many studies conducted in the past, there has been no documented case of penicillin resistance among beta hemolytic streptococci. [5,17,19,23,27-31] There are no Clinical Laboratory Standards Institute (CLSI) guidelines to interpret penicillin non-susceptible strains. [12] Recent studies however showed that among different groups of beta hemolytic streptococci only Group A streptococci has remained susceptible to penicillin with few reported penicillin non-susceptible strains among other serogroups. [20,22 23]

In a study by Douglas JB *et al* (n=397) 100% of the GAS isolates were sensitive to penicillin and cephalosporins. The isolates however, showed 8.6% and

14.4% resistance to erythromycin and tetracycline respectively. [19] A study by Young Uh *et al* (n=143) showed that 16.1% and 55.2% of the GAS isolates were resistant to erythromycin and tetracycline respectively. [17] In a study by Kim H Y *et al* (n=70) 25.7% and 47.1% of the GAS were resistant to erythromycin and tetracycline respectively. [30] In a study conducted by Devi U *et al* (n=18) all the GAS isolates were sensitive to penicillin. However, 27.80% of the GAS isolates were resistant to cefotaxime and 5.6% of the GAS isolates were resistant to erythromycin. [32] A study conducted by Megged *et al* showed 4.7% of the GAS isolates exhibited macrolide resistance. [13] In a study conducted by Dhanda V *et al* (n=102), 100% of the GAS isolates showed sensitivity to penicillin and 6.9%, 37.3% isolates showed resistance to erythromycin and tetracycline respectively. [33]

Group B streptococcus: In our study, all the nineteen GBS isolates (100%) were susceptible to penicillin when tested using disk diffusion method, but four isolates (21.05%) showed raised MIC when tested using broth macrodilution method. Three isolates (15.78%) exhibited MIC > 0.24 $\mu\text{g/ml}$. One isolate (5.26%) showed MIC > 0.12 but ≤ 0.24 $\mu\text{g/ml}$. Seventeen isolates (89.47%) were susceptible and two isolates (10.53%) were resistant to both erythromycin and tetracycline. All the nineteen isolates (100%) were susceptible to ceftriaxone.

In a study by Douglas JB *et al* (n=318) 100% of the GBS isolates were sensitive to penicillin and cephalosporins, but 30.2% and 85.2% of the GBS isolates showed resistance to erythromycin and tetracycline. [19] A study by Young Uh *et al* (n=308) showed that 25.3% and 95.8% of the GBS isolates were resistant to erythromycin and tetracycline respectively. [17] In a study by Kim H Y *et al* (n=138) 41.3% and 95.7% of the GBS were resistant to erythromycin and

tetracycline respectively. [30] In a study conducted by Devi U *et al* (n=18) all the GBS isolates were sensitive to penicillin and 25% of the GBS isolates were resistant to erythromycin. [32] A study conducted by Megged *et al* (N=88) showed 17% of the GBS isolates exhibited macrolide resistance. [13]

Group C Streptococcus: The single GCS isolate obtained in the present study was sensitive to all the four drugs tested and the MIC of penicillin was <0.06µg/ml.

In a study by Douglas JB *et al* (n=19) 100% of the GCS isolates were sensitive to penicillin and cephalosporins, but 5.3% and 42.1% of the GCS isolates showed resistance to erythromycin and tetracycline respectively. [19] A study by Young Uh *et al* (n=22) showed that 9.1% and 50% of the GCS isolates were resistant to erythromycin and tetracycline respectively. [17] In a study by Kim H Y *et al* (n=9) 33.3% and 77.8% of the GCS were resistant to erythromycin and tetracycline respectively. [30] Devi U *et al* (n=6) reported that 50%, 66.7% and 100% of the GCS isolates were sensitive to penicillin, cefotaxime and erythromycin respectively. [32] A study conducted by Megged *et al* (n=7) showed 100% of the GCS isolates were susceptible to macrolides. [13] In a study conducted by Dhanda V *et al*(n=25), 24% of the GCS isolates showed non-susceptibility to penicillin and 12% of the isolates showed resistance to tetracycline. All isolates were susceptible to erythromycin. [33]

Group F Streptococcus: In our study, the single GFS isolate obtained was sensitive to penicillin, tetracycline and ceftriaxone, but was resistant to erythromycin. The MIC for penicillin was <0.06µg/ml.

A study by Douglas JB *et al* (n=8) reported, all the GFS isolates (100%) were sensitive to penicillin, cefotaxime and erythromycin and 25% were resistant to tetracycline. [19] In contrast a study conducted by Devi U *et al* (n=57) showed that 45.6% of GFS isolates were not

susceptible to penicillin and 56.15% were non-susceptible to cefotaxime. [32]

Relatively fewer isolates (12.3%) exhibited macrolide resistance, while a study by Megged *et al* (n=5) showed that all the GFS isolates (100%) were susceptible to macrolides. [13]

Group G Streptococcus: The eight isolates (100%) obtained in the present study were sensitive to penicillin with MIC ≤0.06 µg/ml. All the eight isolates (100%) were also sensitive to erythromycin and ceftriaxone, but only seven isolates (87.5%) were sensitive to tetracycline.

In a study by Douglas JB *et al* (n=45) 100% of the GGS isolates were sensitive to penicillin and cephalosporins, but 15.6% and 51.1% of the GGS isolates showed resistance to erythromycin and tetracycline respectively. [19] A study by Young Uh *et al* (n=67) showed that 9% and 70.1% of the GGS isolates were resistant to erythromycin and tetracycline respectively. [17] In a study by Kim H Y *et al* (n=32) 12.5% and 50.1% of the GGS were resistant to erythromycin and tetracycline respectively. [30] In a study conducted by Devi U *et al* (n=21) 19.05% the GGS isolates were not susceptible to penicillin and erythromycin whereas 57.15% of the GGS isolates were non-susceptible to cefotaxime. [32] A study conducted by Megged *et al* (n=18) showed that 38.9% of the GGS isolates exhibited macrolide resistance. [13] In a study conducted by Dhanda V *et al* (n=28), 100% of the GGS isolates showed sensitivity to penicillin and cefotaxime. However, 3.6% and 14.3% isolates showed resistance to erythromycin and tetracycline respectively. [33]

CONCLUSION

Beta hemolytic streptococci remains an important global health problem even though the resistance rate is less compared to other pathogens. Invasive GAS infections have increased worldwide

in the recent past, despite the organism's susceptibility to penicillin and other commonly used beta-lactam antibiotics. Along with an array of high virulence factors, findings from recent studies suggesting clones of resistant beta hemolytic streptococci warrants structured surveillance programmes to detect and monitor the patterns of beta hemolytic streptococci continuously. Large scale surveillance programmes like LEADER, SENTRY surveillance etc., had reported increasing resistance among beta hemolytic streptococci. This study conducted in NEIGRIHMS was an initiative to study the prevalence of beta hemolytic streptococci and to characterize the isolates phenotypically with special reference to the antibiotic susceptibility profile. Further evaluation of the strains genotypically and continued monitoring of the antimicrobial sensitivity pattern will help in better treatment and prevention of the infections associated with beta hemolytic streptococci.

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