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PREVALENCE OF STREPTOCOCCAL PHARYNGITIS IN PEDIATRICS.

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ABSTRACT

A microbiological diagnosis of pharyngitis is essential for confirmation of Group A Streptococci. Macrolide is given to those patients who are allergic to Penicillin. Various rates of Macrolide resistance of *Streptococcus pyogenes* are reported worldwide. A total of 400 children was included in the study. All samples were processed by standard Microbiological protocol. Antibiotic susceptibility testing was done for all the significant isolate. Minimum inhibitory concentration test and double disc diffusion test were done for Macrolide resistant Streptococci. All isolates were screened for the detection of biofilm formation. In this study 139(34.75%) children were positive for Beta haemolytic streptococci: Group A Streptococci 71(51.1%), Group C Streptococci 29(20.8%) and Group G Streptococci 39(28.05%). Macrolide resistance in Group A Streptococci was found to be 28.1% and Biofilm formation was 35.2%. In Conclusion this study highlights high rate of Streptococcal infection in this population and also alarmingly high rates of Macrolide resistance.

KEY WORDS: **Group A streptococci, Pharyngitis, Macrolide resistance**

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INTRODUCTION

Pharyngitis is one of the most common infections found in children and Group A Streptococci (GAS) is one of the predominant causative agent. A microbiological diagnosis of pharyngitis is essential for confirmation of GAS infection as two third of pharyngitis is caused by viruses. Adequate treatment of Streptococcal pharyngitis is necessary for the prevention of life threatening post streptococcal sequelae like Rheumatic carditis. Patients with GAS pharyngitis complain of pain while swallowing, fever, enlarged cervical lymph nodes and fatigue. Tonsil is red and swollen, headache, nausea, vomiting etc. are seen commonly in Children.[1] GAS strain colonized in upper respiratory tract of children, play an important role in the spread of their infection, especially in the overcrowded areas. Rate of carriage of GAS was found to be 15.4% in Chennai[2]. Although Penicillin is the drug of choice for GAS, Macrolide is given to those patients who are allergic to Penicillin. Various rates of Macrolide resistance of *Streptococcus pyogenes* are reported worldwide, like 37% in Pennsylvania, 6.2%, Poland 12%, etc. [3,4,5] . According to Indian studies, from Chennai 16.2 % was reported, 38.23% from Mangalore and 29.4% from North India by MR.Capoor. [6,7,8,] The aim of the study is to detect the prevalence of *Streptococcus pyogenes* in causing Pharyngitis in this population and also to detect the rate of Macrolide resistance among the isolates. All the isolates were also screened for biofilm formation.

MATERIALS AND METHODS

This is a hospital based study done in the Paediatric Department of SRM medical college**.** A total of 400 children were included in the study in the age group of 6 months to 14 years presenting with symptoms of pharyngitis. Approval from institutional ethical clearance board was attained. Informed Consent form was signed by the parent/guardian of the patient. A detailed case history which included the demographic, anthropometric, vaccination and clinical details about each patient was recorded.

i) Collection of specimen

For patients with tonsillitis, pharyngitis and laryngitis, throat swab was collected by rubbing tonsillar and peri-tonsillar area. All the samples were subjected to Gram's stain and checked for presence of Gram positive cocci in chain. The samples were placed on Blood agar for isolation of beta haemolytic Streptococci and incubated at 37°C over night. Significant colonies were identified by standard Microbiological protocol. Antibiotic susceptibility testing was carried out for significant isolates by Kirby Bauer's disc diffusion method, according to CLSI guidelines[9]. Antibiotics used were Penicillin, Ampicillin, Erythromycin, Clindamycin, Cephalothin, Cefuroxime, Ofloxacin and Co-trimoxazole.

ii)Beta haemolytic streptococci

All Beta haemolytic Streptococci were included for further study. Bacitracin sensitivity and PYRase test was done for all isolates for presumptive identification. All the positive strains are further grouped by antisera grouping kit, Group A, B, C, and G (Hi media).The antigen extraction of group specific carbohydrate was done by enzymatic digestion method (Hi strep latex test kit from Hi Media).

iii) Minimum inhibitory concentration (MIC).

MIC was done by agar dilution method for Erythromycin and Clindamycin on Muller Hinton agar with 5% sheep blood. Dilution ranging from drug concentration of 0.25µg/ml to 128µg/ml was tested. (CLSI guidelines)[9].

iv) Double disc diffusion test (D test)

D test was performed with Erythromycin and Clindamycin disc to detect the MLS type of resistance. Muller Hinton agar plate containing 5% sheep blood was inoculated with overnight culture of Beta haemolytic Streptococci, a Clindamycin (2µg) disk and an Erythromycin (15µg) disk was placed 12mm apart. (CLSI guidelines, 2013).Inoculated agar plates were incubated at 35˚C in 5% CO_2 overnight. Three types of zone were seen. They are i) inducible MLS_B: iMLS$_B$- The Clindamycin zone is blunted towards the Erythromycin because the

Erythromycin induces Clindamycin resistance. ii) Constitutive MLS_B: cMLSB- No zone of inhibition is seen around either Erythromycin or Clindamycin because erm gene is fully expressed. iii) M type: It is sensitive to clindamycin, so no change in the Clindamycin zone induced by erythromycin. Resistance is because of mef gene by efflux mechanism.

v) Detection of Biofilm formation

This was done by Micro titre culture plate method (TCP).All the isolates were done in triplets in Trypticase soya broth with 1% glucose. Each isolate was inoculated into 5 ml of broth and incubated over night. 230 µl of broth was dispensed along with 20µl of test sample into each well and incubated at 37° C over night and washed with Phosphate buffer saline 3 times. 250µl of methanol was dispensed in each well and incubated for 20 min. Methanol was discarded, 300 µl of Saffranin was added and incubated for 10 min. 33 % acetic acid was added to each well, rocked for 10 min and read with ELISA reader at 490nm.[10,11,12]

RESULTS

A total of 400 samples were collected and out of which 139 were positive for growth of Beta haemolytic Streptococci. Age group of the patients included in the study is given in table 1, which shows maximum number of patients in age group of 7-10 years. Antibiogram of the isolates are given in table 2.Minimum inhibitory concentration values and D test results are given table 3 and 4 respectively

Table 1

Age group distribution of Patients positive for Streptococci.(n=139)

Age group of patient	Positive for streptococci	Total
6 months – 3years	10(28.6%)	35
4-6 years	38(37.3%)	102
7-10 years	55(37.9%)	145
11-14 years	36(30.5%)	118
Total	139(34.75%)	400

Table 2

Antibiotic resistance pattern of beta haemolytic Streptococci(n=139)

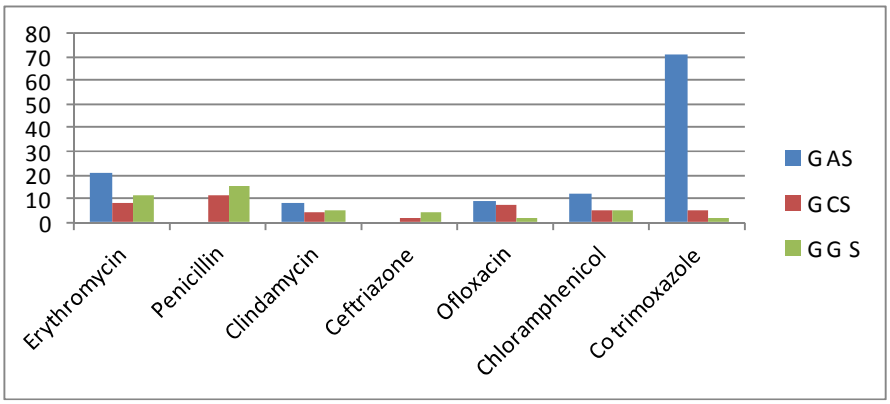
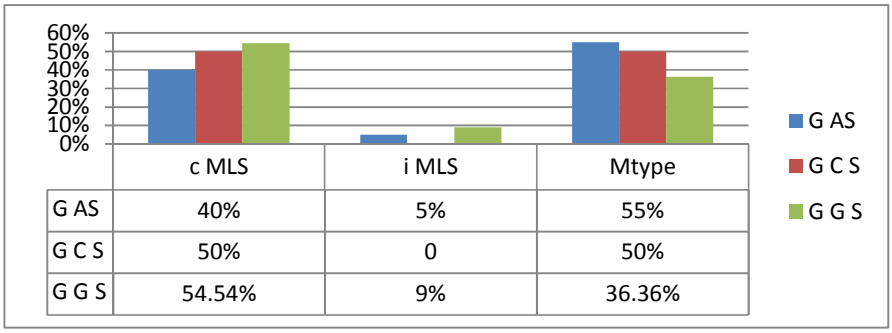


Table 3
MIC values Erythromycin and clindamycin of resistant Streptococci

Drug conc µg/ml	Erythromycin			Clindamycin		
	G AS	G CS	G GS	GAS	GCS	G G S
0.25	0	0	0	0	0	0
0.5	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	2(25%)	1(25%)	0
4	3(15%)	1(12.5%)	1(9%)	1(12.5%)	1(25%)	2(40%)
8	4(20%)	1(12.5%)	2(18.2%)	2(25%)	1(25%)	2(40%)
16	3(15%)	2(25%)	1(9%)	1(12.5%)	0	0
32	2(10%)	0	1(9%)	2(25%)	1(25%)	1(20%)
64	2(10%)	2(25%)	2(18.2%)	0	0	0
126	6(30%)	2(25%)	4(36.6%)	0	0	0
Total	20	8	11	8	4	5

Table 4
D test results of Macrolide resistant beta haemolytic Streptococci



	c MLS	i MLS	Mtype
G AS	40%	5%	55%
G C S	50%	0	50%
G G S	54.54%	9%	36.36%

DISCUSSION

In this study 400 samples were taken and 139(34.75%) were positive for Beta haemolytic Streptococci. Among the Positives, Group A Streptococci GAS) was 71(51.1%), Group C Streptococci GCS was 29(20.8%) and Group G Streptococci GGS was 39(28.05%). GAS isolated were completely sensitive to Penicillin and amoxicillin, Cephalothin and cefuroxime showed only 28.1% resistance to Macrolides, 11.26% to Clindamycin. 12.7% to Ofloxacin and 17% to Chloramphenicol. Macrolide resistance in GAS is 28.1% which is in par with 29.4% in New Delhi as reported by Capoor.MR .It was higher than 16.2% in Chennai as reported by SE Jacob. Macrolide resistance in GCS and GG S was 27.6% and 28.2% respectively. In D test, For GAS M type 55% of resistance is the most common type followed by cMLS 25% and iMLS 10%, similar results were seen in GGS, M type 56%, cMLS 36%, iMLS 8%. GCS showed only M type 8% and cMLS 10%, and no iMLS.MIC values for Erythromycin ranged from 4 µg/ml to 128µg/ml and Clindamycin ranged for 2 µg/ml to 128 µg/ml. 35.2% Biofilm formation was detected among the Streptococcal isolates. Among the biofilm formers, 56% were macrolide sensitive GAS .Similar results were reported by various other studies.[13,14]

CONCLUSION

This study highlights high rate of Streptococcal infection in our population, and also alarmingly high rates of Macrolide resistance among the isolates. High level of antibiotic resistance and biofilm formation may be the reason for chronic carriage of Streptococcal infection and difficulty in eradication. Therefore attention has to be given in treating this infection, with suitable antibiotics as per the antibiotic sensitivity testing results to prevent further post streptococcal sequelae like Rheumatic fever.

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