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# DETECTION OF ERYTHROMYCIN RESISTANCE AND SERUM OPACITY FACTOR IN GROUP A *STREPTOCOCCI* CAUSING PHARYNGITIS

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**ABSTRACT**

Group A Streptococcus (GAS) accounts for the major etiological agent for pharyngitis in our population. Serum opacity factor is a virulence determinant expressed by GAS, which mediate in adhesion of GAS to the mucosal epithelial cells. This study is aimed to detect the prevalence of GAS in causing Pharyngitis in our population and also to study the antibiotic resistance pattern and their ability to produce SOF of the isolates. A total of 430 children with pharyngitis were screened for GAS infection. GAS in 71 cases was confirmed by standard microbiological protocol. Antibiotic susceptibility testing was done by Kirby Bauer's disc diffusion test. Macrolide resistance was confirmed by minimum inhibitory concentration test. Serum opacity factor (SOF) was detected by tissue culture plate method. Out of total 71 GAS isolates, 38 (53.52%) were positive for SOF production. All the GAS isolated was completely sensitive to Penicillin, Amoxicillin, Cephalothin and cefuroxime but showed 28.1% resistance to Erythromycin, 11.26% to Clindamycin. 12.7% to Ofloxacin and 17% to Chloramphenicol. The present study showed significantly high rate of GAS pharyngitis infection in Chennai and Macrolide resistance is also on the rise which needs to be given attention.

**Key Words**: GAS, Serum opacity factor, Erythromycin resistance.

## INTRODUCTION

Group A Streptococcus accounts for the major etiological agent for pharyngitis in our population. Repeated infection with GAS can lead to fatal severe post streptococcal infectious sequalea like Rheumatic fever, Rheumatic heart disease and acute glomerulonephritis. Incidence of Rheumatic fever in India is around 6-11 cases/1000, while in other countries in US 0.6, Japan 0.7, Asia 0.4-21, Africa 1-17 cases per 1000 cases (Kumar et al., 2011).

Serum opacity factor is a virulence determinant expressed by GAS. It was first discovered in 1938 by Australians , Ward and Rudd. SOF has the ability to opacify serum, by disrupting the structure of high density lipoproteins resulting in the formation of large lipid vesicle to cause serum to be cloudy. SOF is a multifunctional protein by which it binds to various host proteins like fibronectin, fibrinogen and fibulin, which are involved in bacterial adhesion (Harry S. Courtney and Henry J Pownall, 2010)

This study is carried out to detect the prevalence of GAS causing pharyngitis and to study the antibiotic resistance pattern and their ability to produce SOF of the isolates.

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## MATERIALS AND METHODS

A total of 430 children were included in the study. Inclusion criteria: Study population included all children of 6 months to 14 years having pharyngitis. Exclusion criteria: The patients with chronic respiratory ailments and

those children whose parents were non-consenting were excluded from the study. Approval from institutional ethical clearance board was obtained. Informed Consent form was signed by the parent of the patient. A detailed case history which included the demographic, anthropometric, vaccination and clinical details about each patient was recorded.

***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 plated on Blood agar for isolation of beta haemolytic Streptococci. All the plates were incubated at 37°C over night. Significant colonies were identified by standard microbiological protocol. Antibiotic sensitivity testing was carried by Kirby Bauer's disc diffusion method, according to CLSI guidelines (CLSI). Antibiotics were used such as Penicillin, Ampicillin, Erythromycin, Clindamycin, Cephalothin, Cefuroxime, Ofloxacin and Co-trimoxazole.

***Beta hemolytic streptococci***

All Beta hemolytic streptococci were included for further study. Bacitracin sensitivity and PYRase test was done for all isolates for presumptive identification. All the positive strains were further grouped by antisera grouping kit, Group A, B, C, and G. The antigen extraction of group specific carbohydrate was done by enzymatic digestion method (Hi strep latex test kit from Hi Media). All identified beta haemolytic isolates were stocked in two sets , one in BHI with glycerol at -20$^0$ C and another in dry filter paper method.

***Serum Opacity factor detection***

Detection of SOF was done for all the GAS isolates. Isolates were first sub cultured in Brain heart infusion broth and incubated at 37°C for 24 hours, Centrifuged and take 10 µl of culture supernatant was taken in a micro titre plate. 100 µl of horse serum (Hi media) is added and incubate overnight at 37°C.100 µl of normal saline is added. Known positive and negative samples for SOF are taken as controls. The plates are read at 490 nm (Dwight R and Edward Kaplan, 1988).

**RESULTS**

A total of 71 GAS was isolated from 430 throat swabs collected from children with pharyngitis. All the GAS isolated were completely sensitive to Penicillin, Amoxicillin, Cephalothin and cefuroxime but showed 28.1% resistance to Macrolides, 11.26% to Clindamycin. 12.7% to Ofloxacin and 17% to Chloramphenicol as given in fig 1. 38 isolates (53.2%) out of 71 were positive for SOF test, fig 2.

**Figure 1. Antibiogram of GAS isolated from Pharyngitis**

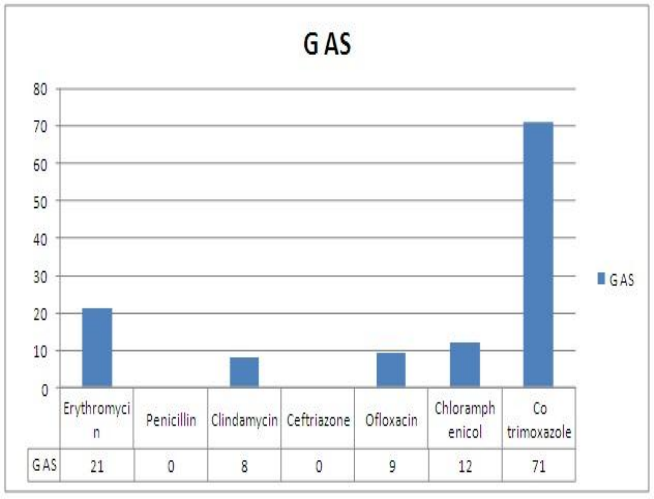
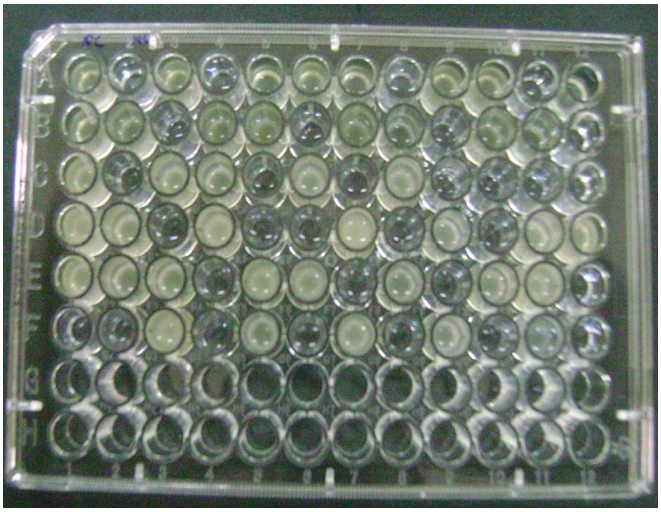



**Figure 2. Tissue culture plate method for Serum opacity factor detection**



**DISCUSSION**

The prevalence of GAS pharyngeal infection in this population is 16.5%, significantly higher than the report from North India( 1.3%) (Kumar *et al*., 2012). This could be explained as the study population were symptomatic cases from hospital, while other studies comprises of screening children from communities. Studies from Chennai showed pharyngitis caused by GAS (17.07%) which was almost same as the findings in present study (Charmaine A.C Lloyd 2006). In this study Macrolide resistance was 28.1%, which was in par with report by Capoor (29.4%) in New Delhi, but was higher than reports form Chennai (9.04%) by SE Jacob (Capoor MR *et al*., 2006, Charmaine AC Lloyd *et al*., 2006).

In our present study, SOF production was found to be 53% which is lesser than 83.3% reported by Baskara et.al from cases of pyoderma (Baskaran K *et al*., 2013). Various other reports give 34-50% SOF positivity. The difference in SOF positivity can be due to variation in M proteins. According to Fischer's test P value calculator for the checking association of Erythromycin resistance and SOF formation, P value is 0.79 which is not statistically significant.

Emm typing after DNA sequencing, is an efficient tool for epidemiological studies, but screening for SOF-production is still relevant, as it provides increased discrimination of clonal differences within *emm* types.

## CONCLUSION

According to present their significantly high rate of GAS pharyngitis in Chennai and Macrolide resistance is also on the rise which needs to be given attention. SOF positivity is seen greater than 50% of GAS isolated from pharyngitis supports that it is a virulent factor in enabling GAS infection.

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## REFERENCES

Baskaran K. Joseph Puspa Innocent and D.Prathiba G. Serum Opacity factor as a tool in the detection of Streptococcal pyoderma cases in Beta haemolytic Streptococcal infection. *Scholar Research Library Der pharmacia Lettre*. 2013; 5(1): 77-79.

Bernard Beall. Characterization of group A streptococci (Streptococcus pyogenes): correlation of M-protein and emm-gene type with T-protein agglutination pattern and serum opacity factor. *Journal of Medical microbiology*. 2006; 55(Pt 2): 157-64.

Capoor MR, Nair D, Deb M, Batra K, Aggarwal P. Resistance to Erythromycin and rising penicillin MIC in Streptococcus pyogenes in India. *Japan J infect Disease.* 2006; 59(5): 334-6

Charmaine AC. Lloyd, Swarna E. Jacob & Thangam Menon. Pharyngeal carriage of group A streptococci in school children in Chennai. *Indian J Med Res*. 2006; 124: 195-198.

CLSI guidelines,2013,M02-A11 and M07-A9, page 112-117.

Dwight R. and Edward Kaplan. Micro technique for Serum opacity factor Characterization of group A Streptococci adaptable to the use of Human sera. *Journal of Clinical Microbiology*. 1988; 2025-2030.

Harry S. Courtney and Henry J Pownall. The structure and function of Serum opacity factor: A unique Streptococcal virulence Determinant that targets high density lipoproteins. *Journal of Biomedicine and Biotechnology*. 2010: ID 956071, 16.

Jacob SE, Lloyd CAC, Menon T. cMLS and M phenotypes among *Streptococcus pyogenes*. *Indian Journal of Medical Microbiology*. 2006; 24: 147-148.

Kumar A, Batnagar A, Gupta S et al. SOF gene as a specific marker for detection of *Streptococcus pyogenes* causing Pharyngitis and Rheumatic heart disease. *Cell. Mol. Biology*. 2011; 57(1): 26-30,