

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.510.109>

## Epidemiology of *Streptococcus pyogenes* in Pyogenic Infections in Gulbarga, India

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### ABSTRACT

To study the prevalence of *Streptococcus pyogenes* in general population of different ages, and strata of socio-economic groups. This was a cross-sectional prospective study carried on the general population of poor socio-economic status over one year, by procuring data from the total 129 patient records of OPD patients. The clinical settings including history, examination and the (throat swab, pus cervical swab, sputum ear-discharge cultures) with anti-microbial sensitivity testing performed under standard conditions. In this study, *Streptococcus pyogenes* was isolated, of Out of the 129 samples screened, *S. pyogenes* was isolated from 51 samples indicating that 39.53% of pyogenic infections are being caused by *S. pyogenes*. The incidence of *S. pyogenes* was found to be very high among males than females. Out of the 89 samples collected from males, 43 yielded *S. pyogenes*, indicating an incidence of 48.31%. While in the case of females out of 40 samples only 8 samples showed the presence of *S. pyogenes* indicating an incidence of only 20%. The incidence was also high in pus (50%) and throat swab (48%) samples. While the same was very low in cervical swabs (10%) and ear discharge (11%). The antibiogram pattern indicated an alarming situation, in that among “*S. pyogenes* isolates were resistant to multiple antibiotics all the 51 isolates were resistant atleast to three antibiotics. The multiple resistances ranged from 3 to 8 antibiotics. Among the 51 isolates, as many as 17 (33%) were resistant to 5 antibiotics and 14 (27%) to 6 six antibiotics respectively. The study showed *S. Pyogenes* and also showed significant amount of resistance against commonly used antibiotics which were earlier sensitive probably due to injudicious use of antibiotics without undergoing routine culture and sensitivity.

#### Keywords

*Streptococcus pyogenes*,  
Pharyngitis,  
multiple  
resistances.  
Blood agar,  
Bacitracin,  
penicillin.

#### Article Info

Accepted:  
30 August 2016  
Available Online:  
10 October 2016

### Introduction

*Streptococcus pyogenes* has persisted and is now resurging as an important hospital and

community acquired pathogen. *Streptococcus pyogenes* is one of the most

frequent pathogens of humans. It is estimated that between 5-15% of normal individuals harbor the bacterium, usually in the respiratory tract, without signs of disease. *S. pyogenes* is the most pathogenic bacterium in the genus *Streptococcus*; it is the agent that causes bacterial pharyngitis, impetigo, scarlet fever, rheumatic fever and a host of other infections including severe invasive diseases (Facklam, 2002). Streptococcal infection in the form of scarlet fever has been described since centuries. Epidemics of both benign and fatal disease were reported, including an epidemic in the American colonies in 1736, which resulted in the death of nearly 4000 people. In the 19th century the lethality of GAS infections dramatically increased in both Europe and North America (Baxter and McChesney, 2000; Stevens *et al.*, 1989; Katz and Morens, 1992). The most common infections caused by *S. pyogenes* are grouped as the non-invasive streptococcal disease upper respiratory tract (pharyngitis) and skin (impetigo) infections (Mascini *et al.*, 1999; Stanley *et al.*, 1995; Sneath *et al.*, 1986; Efstratiou *et al.*, 1994; Ross, 1990; Stevens, 1992). Invasive soft tissue infection (Symptoms include pain at the site of infection, fever, malaise, nausea, vomiting and diarrhea, dizziness, confusion and a flat rash over large parts of the body). (Mascini *et al.*, 1999; Stanley *et al.*, 1995; Sneath *et al.*, 1986; Efstratiou *et al.*, 1994; Ross, 1990; Stevens, 1992) and non-suppurative sequelae 'Two serious diseases may develop as separate to *S.pyogenes* infections, Rheumatic fever and Acute glomerulonephritis. (Mascini *et al.*, 1999; Stanley *et al.*, 1995; Sneath *et al.*, 1986; Efstratiou *et al.*, 1994; Ross, 1990; Stevens, 1992).

The various virulence factors are involved in adherence of *S.pyogenes* (Bernnet *et al.*, 1998), M proteins (Gregory *et al.*, 2002),

Capsule and C5<sub>a</sub> peptidase (Dale *et al.*, 1996), Steptolysins (Kilian, 1998), Pyrogenic exotoxins (Berner *et al.*, 2000), Hyaluronidase, Streptokinase, Lipoproteinase and Deoxyrihonuclease (Kilian, 1998). In the present study a total of 129 samples were subjected for occurrence of *S.pyogenes* and we have recorded 51 *S.pyogenes* isolates. The incidence of *S.pyogenes* was highest in sputum, pus and throat swab. The incidence in sputum (60%) and throat swab (48%) allow us to speculate that pharyngitis might be endemic to the Gulbarga region. The emergence of *S. pyogenes* as a resurgent pathogen is because of its ability to cause a variety of diseases and also because of the increase in the trends in antibiotic resistance worldwide (Holmstrom *et al.*, 1990; Seppala *et al.*, 1992; Weiss *et al.*, 1997). In the present study the antibiogram of the *S. pyogenes* isolates was studied using eleven antibiotics including those which are routinely prescribed as a treatment model for *S. pyogenes* infection in general and pharyngitis, sore throat in particular. Bacitracin sensitivity was used as preliminary diagnostic aid and was found to be satisfactory. All the isolates have shown resistance to all the antibiotics in varying degree 42 out of the 51 isolates (82.35 %) showed resistance to erythromycin, the drug of choice to penicillin allergic patients. The results indicate a vast difference compared to earlier reports (Holmstrom *et al.*, 1990; Seppala *et al.*, 1992; Weiss *et al.*, 1997). Isolates showing resistance to 6 of the antibiotics. Three isolates (5.88%) showed resistance to 8 antibiotics out of the total 11. However, none of the isolates was resistant to all the 11 antibiotics used.

The present investigation was undertaken with a main objective of studying the epidemiology of *Streptococcus pyogenes* strains from socio-economic background

and characterization of the *S.pyogenes* and further to study the antibiogram by standard Kirby-bauer disk diffusion method to the commonly used antibiotics.

## Materials and Methods

### Sources of sample collection

The period of study span from January 2004 to August 2004. Patients admitted to Basaveshwara Hospital, KBN Hospital and Teaching Hospital of MRM College Gulbarga, constituted the sources of samples. The samples were taken on the basis of preliminary infection on skin lesion, throat infection, and other pyogenic infections. The samples pus, sputum swabs, from pyogenic sites from clinical patients” were collected in screw cap bottles/ test tubes and transported into the laboratory. The enriched cultures were subjected to preliminary microscopic observation by simple staining, Gram’s staining and other Morphological observations.” For isolation of the etiological agent *Streptococcus pyogenes* the Cary Blair medium was used as an enrichment medium (Luechtefield, 1981) and was obtained from hi-Media, Bombay. “For further characterization of *Streptococcus pyogenes* two more media were used, Blood agar and PYR Agar” (L. Pyrollidiony/acrylamide). Dehydrated media were recomposed as for the manufacturer’s specifications (Hi Media).

### Bacitracin susceptibility test

Bacitracin and Taxo AO differentiation disc (0.09-0.05 µg/disc) are used for the presumptive identification and differentiation of Lance field Group A (*Streptococcus pyogenes*) from other - Hemolytic Streptococci. “Maxted showed that group A Streptococci was sensitive to bacitracin might therefore be used as rapid diagnostic agent for group ‘A’

Streptococci”. The bacitracin Disc Technique was considered to be simplest and most practical for the routine clinical laboratory. The bacitracin sensitivity Triptose Blood agar.

### Antibiotic susceptibility testing

As the *Streptococcus pyogenes* isolates were subjected to antibiotic susceptibility testing commercially available antibiotic discs (Hi Media, Bombay) (10 - 30 mg) were used. These antibiotics were selected based on the survey in Gulbarga and also based on the literature that these are choice antibiotics used for the treatment of *Streptococcus pyogenes*. The method adopted is the Kirby-Buer’s Agar Disc Diffusion assay (Bauer, 1996). The antibiotic susceptibility discs and the Muller Hinton Agar were obtained from Hi Media Laboratories Mumbai, India. The antibiotics used in the study were ampicillin (10 mg) (30 mg), vancomycin (10 mg) (30 mg) Erythromycin (10 mg) (30 mg) Chloramphenicol (10 mg) (30 mg) ceftriaxone (10mg) (30 mg) cefaclor (10mg) (30 mg) clarithromycin (10 mg) (30 mg) clindamycin (10 mg) (30 mg) Levofloxacin (10mg) (30mg) ofloxacin (10mg) (30mg) Chephotaxime (10mg)(30mg).

## Results and Discussion

In the present study a total of 129 samples were subjected for occurrence of *S.pyogenes* and we have recorded 51 *S.pyogenes* isolates. The sex wise distribution shows males are significantly more susceptible to *S.pyogenes* infections indicated by 48% recoveries from males and 20% from females. The present investigation also made clear that children and young adults below 20 years and 20-40 years are more susceptible to *S.pyogenes* infection. The incidence of *S.pyogenes* was highest in the adults of age group 20-40 with an incidence

of 43.7%. Though the incidence of *S.pyogenes* in the children's and young adults above 40, the results of age groups 40-50 years are not conclusive as the sample size is small. Samples were collected from various body sites viz., pus, sputum, throat swab, cervical swab and ear discharge. The incidence of *S.pyogenes* was highest in sputum, pus and throat swab. The incidence in sputum (60%) and throat swab (48%) allow us to speculate that pharyngitis might be endemic to the Gulbarga region. The emergence of *S. pyogenes* as a resurgent pathogen is because of its ability to cause a variety of diseases and also because of the increase in the trends in antibiotic resistance worldwide (Holmstrom *et al.*, 1990; Seppala *et al.*, 1992; Weiss *et al.*, 1997). In the present study the antibiogram of the *S. pyogenes* isolates was studied using eleven antibiotics including those which are routinely prescribed as a treatment model for *S. pyogenes* infection in general and pharyngitis, sore throat in particular. Bacitracin sensitivity was used as preliminary diagnostic aid and was found to be satisfactory. All the isolates have shown resistance to all the antibiotics in varying degree 42 out of the 51 isolates (82.35 %) showed resistance to erythromycin, the drug of choice to penicillin allergic patients. The results indicate a vast difference compared to earlier reports (Holmstrom *et al.*, 1990; Seppala *et al.*, 1992; Weiss *et al.*, 1997). Though clindamycin is recommended for recurrent *S. pyogenes* pharyngitis (Bisno *et al.*, 2002; Hasenbein *et al.*, 2004) our results show a 82.35% resistance to clindamycin. *S. pyogenes* isolates from our study showed a remarkable resistance to ofloxacin (86.27%) and cefaclor (86.27%). Cephalexin and clarithromycin seem to be suitable alternatives for the patients not responding to erythromycin as the resistance shown by *S. pyogenes* in the current study is only 13.72% and 21.56% to cephalexin and

clarithromycin respectively. Multiple antibiotic resistances was also seen among the 51 *S.pyogenes* isolates. 17 isolates (33.33%) showed resistance to 5 Out of the 11 antibiotics used, followed by 14 (27.41%) isolates showing resistance to 6 of the antibiotics. Three isolates (5.88%) showed resistance to 8 antibiotics out of the total 11. However, none of the isolates was resistant to all the 11 antibiotics used. During the period from January 2004 to August 2004, 129 clinical samples were collected, from these a total of 51 strains of *S.pyogenes* have been isolated from the patients admitted to Basveshwar hospital, M.R.M collage and K.B.N hospital, infected with pyogenic infections. Five types of sources of samples were used pus, sputum, cervical swab, throat swab, and ear discharge. The isolates were further characterized and confirmed by microscopic, cultural and biochemical investigations and bacitracin sensitivity tests. Further, antibiogram pattern of the *S. pyogenes* isolates to some of the most commonly employed antibiotics were also investigated.

“Out of the 129 samples screened, *S. pyogenes* was isolated from 51 samples indicating that 39.53% of pyogenic infections are being caused by *S. pyogenes* (Table-2 and Fig. 1). The incidence of *S. pyogenes* was found to be very high among males than females. Out of the 89 samples collected from males, 43 yielded *S. pyogenes*, indicating an incidence of 48.31%. While in the case of females out of 40 samples only 8 samples showed the presence of *S. pyogenes* indicating an incidence of only 20 %. The breakup of *S. pyogenes* isolates from different samples is presented in Table-3 and Fig.2. Highest incidence has been found to be in sputum where in 15 samples out of 25 yielded *S. pyogenes* indicating an incidence of 60.0%.

The incidence was also high in pus (50%) and throat swab (48%) samples. While the same was very low in cervical swabs (10%) and ear discharge (11%). The age wise distribution of *S. pyogenes* incidence is depicted in Table-4 and Fig. 3. The results indicated highest incidence among young and the middle aged people less than 50 years of age. The patients in the age group of 20-40 years yielded the highest incidence of 50 %. While the age group 40-50 indicated an incidence of 43.7% followed by the age group <20 years showing 40 % Incidence of *S. pyogenes*. The incidence was very low in individuals above 50 years of age. The age group 50-60% indicated an

incidence of only 11%, and in the patients above 60 years *S. pyogenes* was present in 20 % of the cases.

**Antibiotic susceptibility testing**

The results of the susceptibility of the 51 isolates of *S. pyogenes* to the 11 most commonly prescribed antibiotics for pyogenic infections are represented, The antibiogram pattern indicated an alarming situation, in that among “*S. pyogenes* isolates were resistant to multiple antibiotics all the 51 isolates were resistant atleast to three antibiotics.

**Table.2** Sex wise Incidence of *S.pyogenes*

Sex	No. of Samples	No. of <i>S.pyogenes</i> isolates	% of incidence
Male	89	43	48.31
Female	40	8	20
<b>Total</b>	<b>129</b>	<b>51</b>	<b>39.53</b>

**Table.3** Incidence of *S.pyogenes* in different samples

Sample Collected	No. of Samples	No. of <i>S.pyogenes</i> isolates	% of incidence
Pus	40	20	50.0
Sputum	25	15	60.0
Throat swab	25	12	48.0
Cervical swab	20	2	10.0
Ear discharge	19	2	11.0
<b>Total</b>	<b>129</b>	<b>51</b>	<b>39.50</b>

**Table.4** Age-wise incidence of *S.pyogenes*

Sample Collected	No. of Samples	No. of <i>S.pyogenes</i> isolates	% of incidence
<20	25	10	40.0
20-40	60	30	50.0
40-50	16	7	43.7
50-60	18	2	11.0
>60	10	2	20.0
<b>Total</b>	<b>129</b>	<b>51</b>	<b>39.50</b>

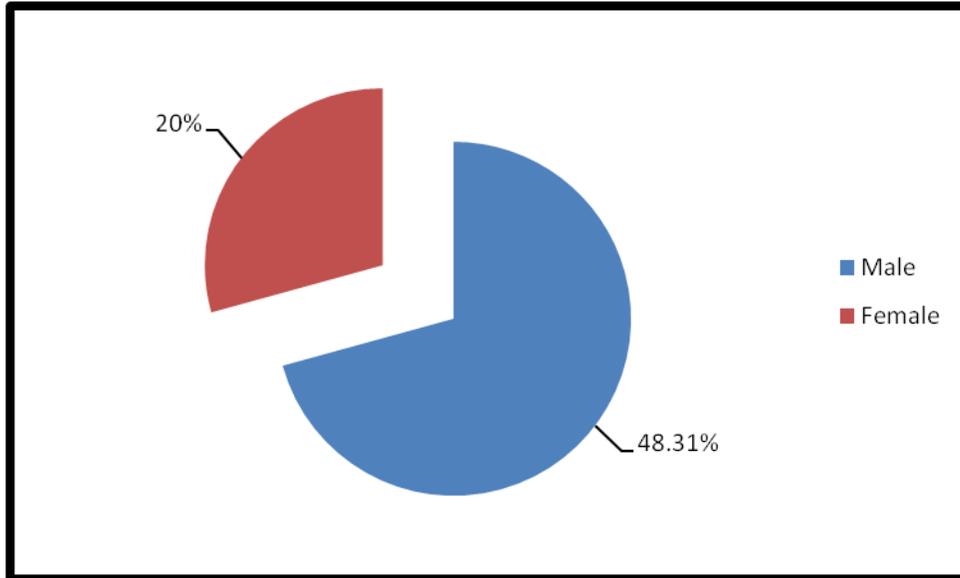
**Table.6** Incidence of multiple antibiotic resistances among *S.pyogenes*

No. of Antibiotics	Isolates shown V	% of Resistant
3	5	9.80
4	4	7.84
5	17	33.33
6	14	27.45
7	8	15.69
8	3	5.88
<b>Total</b>	<b>51</b>	<b>100.00</b>

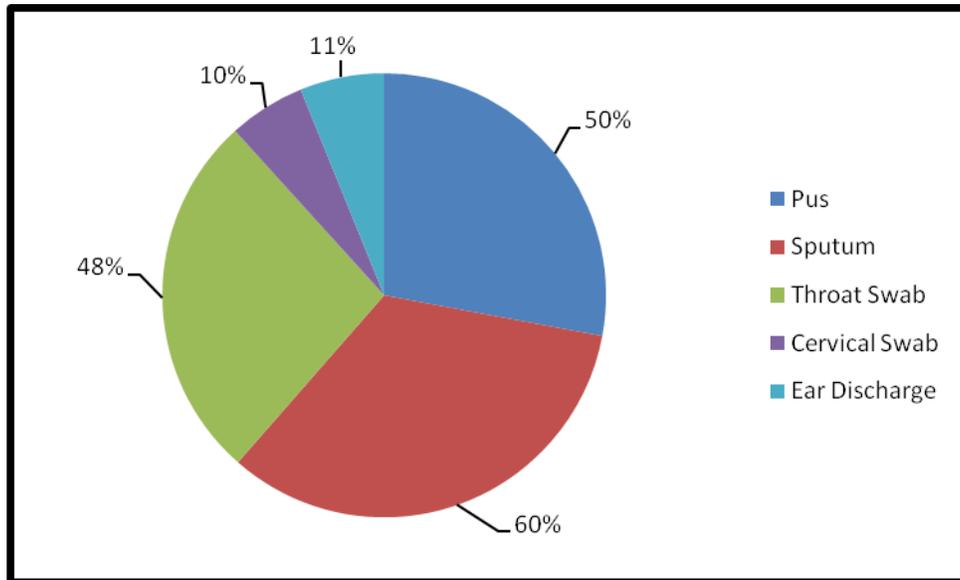
**Table.7** Incidence of antibiotic resistance among *S.pyogenes* isolates

Antibiotics	No. of Resistivity	No. of Sensitivity	% of Resistant
Ampicillin	15	36	29.41
Vancomycin	40	11	78.43
Erythromycin	42	9	82.35
Chloroamphenicol	14	37	27.45
Ceftriame	21	30	41.17
Cefaclor	44	7	86.27
Clarithromycin	11	40	21.56
Clindamycin	42	9	82.35
Levofloxacin	15	36	29.41
Cephotaxime	7	44	13.72
Ofloxacin	44	7	86.27

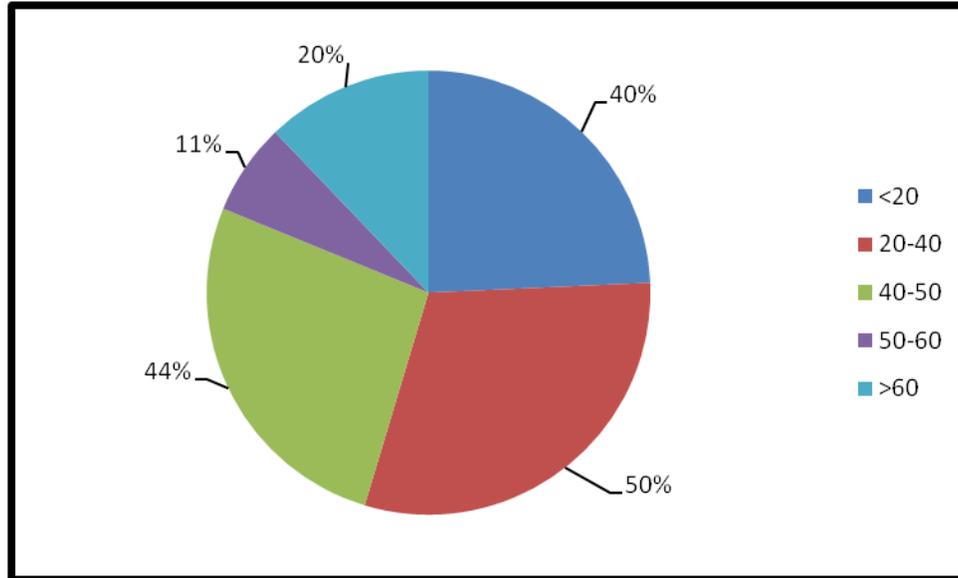
**Fig.1** Sex wise incidence of *S. pyogenes*



**Fig.2** Incidence of *S.pyogenes* in different samples



**Fig.3** Age wise incidence of *S.pyogenes*.



The multiple resistances ranged from 3 to 8 antibiotics. Among the 51 isolates, as many as 17 (33%) were resistant to 5 antibiotics and 14 (27%) to 6 six antibiotics (Table-6). Among the 11 antibiotics, the highest incidence of resistance (86.27%) among *S.pyogenes* isolates was observed against Cefaclor and Ofloxacin and 82.35% resistance against erythromycin and clindamycin. Resistance also was very high (78.43%) against vancomycin. However, in respect of other antibiotics low (13.72%) to moderate (41.17%) resistance was observed (Table-7).

In conclusion, the findings in our study were consistent with the view that *Streptococcus pyogenes* infection in general population should not be neglected. This study highlights the regular screening and the importance of regular surveillance to keep *S.pyogenes* in check and by treating early with appropriate antibiotics by routine culture and sensitivity and also highlights upcoming drug resistance to the commonly used antibiotics which may be due to injudicious and excessive use of antibiotic

therapy without following proper antibiotic policy.

#### Acknowledgements

Authors are grateful to Prof. S.M Gaddad, Department of Microbiology, Gulbarga University, Gulbarga.-585016.karnataka state, India. For his valuable suggestions. Another faculty Dr. C.T. Shivananveur Department of microbiology, Gulbarga University, Gulbarga for his valuable suggestions.

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#### How to cite this article:

Sanjay Rathod, Muzaaheed, Venkat M. Shinde, H.P. Jai Shanker Pillai. 2016. Epidemiology of *Streptococcus pyogenes* in Pyogenic Infections in Gulbarga, India. *Int.J.Curr.Microbiol.App.Sci*. 5(10): 1030-1038. doi: <http://dx.doi.org/10.20546/ijcmas.2016.510.109>