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## Original Research Article

Prevalence and antibiotic susceptibility of *Streptococcus pyogenes* isolated from pyoderma in a tertiary care hospital, Hyderabad, South IndiaL N Rao Sadanand<sup>1</sup>, Pendru Raghunath<sup>1,\*</sup><sup>1</sup>Dept. of Microbiology, Dr. VRK Women's Medical College, Hyderabad, Telangana, India

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## ABSTRACT

**Introduction:** Pyoderma is a common acute superficial bacterial skin infection which is highly contagious. In the great majority of cases, pyoderma is caused by *Streptococcus pyogenes*, *Staphylococcus aureus*, or both. The present study was carried out to determine the prevalence and antibiotic susceptibility of *S. pyogenes* isolated from pyoderma in Dr. VRK Women's Teaching hospital.

**Materials and Methods:** Swabs or pus samples were collected from 250 patients attending Dermatology, outpatient department (OPD) of Dr. VRK Women's Teaching hospital. Samples were inoculated onto 5% sheep blood agar plates and incubated for 24 h at 37°C in a candle jar. BHS isolates were phenotypically identified by standard microbiological techniques, all the isolates presumptively identified as BHS were tested for Bacitracin susceptibility. Presumptive identification of a strain as a Group A Streptococcus (GAS) was also made by PYRase test. Presumptively identified GAS isolates were serogrouped by Lancefield grouping using a commercially available latex agglutination test. *S. pyogenes* isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method.

**Results:** BHS were isolated from 30% of samples. Prevalence of BHS was more among 0-10 years age group (38%). BHS were isolated more frequently from males (38.8%). *S. pyogenes* were isolated from 52 (20.8%) samples. All 52 *S. pyogenes* isolates were found to be susceptible to Penicillin G, amoxicillin, ceftriaxone, azithromycin and vancomycin. Erythromycin and clindamycin showed good activity with sensitivity rates of 92.3% & 96.1%, respectively. Resistance to tetracycline (59.6%) and chloramphenicol (23.1%) was commonly seen in *S. pyogenes*.

**Conclusion:** This study reports the prevalence and antibiotic susceptibility of *S. pyogenes* isolated from pyoderma in Dr. VRK Women's Teaching hospital. Results of this study suggests the peak incidence of pyoderma in children aged 0 to 10 years and male preponderance. Our study also reports high prevalence of tetracycline and chloramphenicol resistance in *S. pyogenes*.

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## 1. Introduction

*Streptococcus pyogenes* is responsible for a wide variety of skin and soft tissue infections (SSTIs) worldwide. This bacterium causes impetigo, erysipelas, cellulitis, necrotizing fasciitis, and myonecrosis. Pyoderma, impetigo, and impetigo contagiosa are interchangeable terms used

to describe purulent lesions of the skin. Pyoderma is very common acute bacterial infection of the skin infection and it is highly contagious. It is characterized by pustules and crusted erosions. Pyoderma is transmitted by direct contact and patients with pyoderma can easily spread the infection to their close contacts. Pyoderma is prevalent in children of 2-5 years of age.<sup>1</sup> Previous studies have identified several risk factors for impetigo viz., poor hygiene, low socio-economic status, crowding and underlying scabies.<sup>2,3</sup>

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Adults may develop impetigo either by contact with children or by fomites.<sup>4</sup> In children, the incidence of impetigo is greatest during the summer months due to the close contact.<sup>5</sup> Lesions are mainly formed on head & neck (65.4%), followed by hands (19.6%), trunk and legs (7.5% each).<sup>6</sup> In pyoderma, most common symptoms are skin lesions, however, in few patients mild lymphadenopathy is also observed.<sup>7,8</sup> Up to 5% of the patients might develop a complication of pyoderma i.e. acute Post-streptococcal Glomerulonephritis (PSGN). Only few serotypes 1, 4, 12, 25 and 49, known as nephritogenic strains are known to cause PSGN.<sup>9</sup>

Most cases of pyoderma are caused by *S. pyogenes*, *Staphylococcus aureus*, or both. *S. pyogenes* causes pyoderma in tropics and pharyngitis in temperate regions.<sup>10</sup> Bullous impetigo, the second type of disease presentation, is caused exclusively by *S. aureus*.<sup>11–13</sup> Prevalence of *S. aureus* and *S. pyogenes* from pyoderma has varied over time.<sup>14</sup> In recent decades, *S. aureus* especially methicillin-resistant *S. aureus* (MRSA) has been responsible for pyoderma worldwide especially in temperate regions where disease is less common.<sup>15</sup> In contrast pyoderma is very common in tropical regions, carries the greatest risk of sequelae<sup>16</sup> and *S. pyogenes* is still remained as dominant pathogen in tropics.<sup>17</sup>

At any point of time, over 140 million people are affected with pyoderma; among them 100 million are children.<sup>16,18</sup> A research study has demonstrated a male predominance.<sup>6</sup> There is limited information on microbiology of pyoderma from high burden contexts and antibiotic susceptibility pattern.<sup>17</sup>

The objectives of this study were:

1. To determine the prevalence of group A Streptococcal pyoderma in patients visiting Dr. VRK Women's Teaching Hospital & Research Centre.
2. To explore the associations of this microbiology with age & sex.
3. To study the antimicrobial susceptibility pattern of *S. pyogenes* isolates from pyoderma.

## 2. Materials and Methods

This is a cross-sectional study, carried out in the Dr VRK Women's Teaching hospital & Research Centre, a tertiary care hospital, at Aziznagar from January 2015 to March 2016. As many as 250 patients attending Dermatology, outpatient department (OPD) of Dr. VRK Women's teaching hospital were included in this study. Study participants have not received any antibiotic therapy before sample collection.

### 2.1. Sample collection and processing

Samples were collected from the base of the skin lesion by means of sterile swabs. In case of pustular lesions, the material was collected with a sterile loop after rupturing

the pustule with a sterile needle. The demographic profile was taken from each case. Samples were immediately transported at 4°C in a temperature monitored cooler to the Clinical Microbiology Laboratory, where they were processed within 2 hours. Swabs or pus samples were inoculated onto 5% sheep blood agar plates and incubated for 24 h at 37°C in a candle jar, which can provide an atmosphere of 5% CO<sub>2</sub>. Culture plates negative for β-haemolytic colonies were incubated for additional 24 hours to allow the growth of slow growers. Beta-haemolytic streptococci (BHS) isolates were phenotypically identified by standard microbiological techniques: which include β-haemolytic activity on sheep's blood agar, Gram stain revealing Gram positive cocci, and negative catalase test.

All the isolates presumptively identified as BHS were tested for Bacitracin susceptibility. BHS strains inoculated on sheep blood agar plates. Test was performed using 0.04 units Bacitracin discs (Himedia Laboratories, Mumbai, India) as per standard protocol. Bacitracin disc was placed over the medium with the help of sterile forceps and the plates were incubated at 37°C overnight. Isolates with a zone of inhibition ≥ 15 mm diameter were considered as sensitive. Presumptive identification of a strain as a Group A Streptococcus (GAS) was also made on the basis of production of the enzyme L-pyrrolidonyl-beta-naphthylamide (PYRase test). Presumptively identified GAS isolates were serogrouped by Lancefield grouping by a commercially available Streptex™ Rapid kit (Thermo Fisher Scientific, India).

Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method. Briefly, 10<sup>5</sup> CFU/mL of bacterial suspensions were inoculated on sheep blood Mueller-Hinton agar plates and incubated in 5% CO<sub>2</sub> for 24 to 48 hours at 37°C. *S. pyogenes* isolates were subjected to antibiotic susceptibility testing by the following antibiotic discs with respective concentration. Penicillin (10 unit), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Amoxicillin (25 µg), Erythromycin (15 µg), Clindamycin (2 µg), Tetracycline (30 µg) Clarithromycin (15 µg), Azithromycin (15 µg) and Vancomycin (30 µg). Clinical Laboratory and Standard Institute (CLSI) guidelines were followed for interpretation of diameters of zone of inhibition.

## 3. Results

A total of 250 patients attending Skin outpatient department (OPD) of Dr. VRK Women's teaching hospital were enrolled from January 2015 to March 2016. Among them, 142 (56.8%) were males and 108 (43.2%) were females. In this study, the subjects were divided into four groups based on their age. Majority of the patients belonged to 0-10 years age group (92) followed by 11- 20 years age group (77) (Table 1). BHS were isolated from 75 (30%) of 250 patients (Table 1). Prevalence of BHS was more among 0-10 years age group (38%), followed by 11-20 years age group

(29.9%) (Table 1).

**Table 1:** Age wise distribution of BHS isolates

Age group (Years)	Total number of cases	No of cases positive for BHS	Positive percentage (%)
0-10	92	35	38
11-20	77	23	29.9
21-30	43	10	23.25
≥31	38	07	18.42
Total	250	75	30

BHS were isolated more frequently from males, 48 (38.8%) of 142 specimens compared to females, 27 (25%) of 108 specimens (Table 2). A total of 52 (69.3%) of 75 BHS isolates were susceptible to Bacitracin and identified as GAS by latex agglutination test. In this study, *S. pyogenes* were isolated from 52 (20.8%) of 250 samples. As many as 23 (30.6%) of 75 BHS isolates were resistant to Bacitracin and presumptively identified as non-group A BHS (NGABHS). By serogrouping, they were further differentiated into group B *Streptococci* (GBS), group C *Streptococci* (GCS) and group G *Streptococci* (GGS) (Table 3).

**Table 2:** Sex wise distribution of BHS isolates

Sex	Total number of cases	No of cases positive for BHS	Positive percentage (%)
Males	142	48	33.8
Females	108	27	25

**Table 3:** Lancefield grouping of BHS isolates

Serogroup	Number of BHS isolates positive	Positive percentage (%)
A	52	69.2
B	05	6.6
C	07	9.3
G	11	14.68

All 52 *S. pyogenes* isolates were found to be susceptible to Penicillin G, amoxicillin, ceftriaxone, azithromycin and vancomycin (Table 4). Erythromycin and clindamycin showed good activity with 48 (92.3%), 50 (96.1%) isolates, respectively displaying susceptibility. As many as 40 (76.9%) *S. pyogenes* isolates were susceptible to chloramphenicol (Table 4). Only 21 (40.4%) *S. pyogenes* isolates were susceptible to tetracycline (Table 4).

#### 4. Discussion

In this study, BHS were isolated from 30% of patients. This result is in agreement with the previous study by Raghunath and colleagues,<sup>19</sup> which has reported the prevalence of 33.8%. However, Mehta et al.<sup>20</sup> reported higher prevalence

**Table 4:** Susceptibility rates of *streptococcus pyogenes* as determined by Kirby-Bauer disc diffusion method

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
Penicillin G	0	0	100
Amoxicillin	0	0	100
Ceftriaxone	0	0	100
Erythromycin	7.7	0	92.3
Azythromycin	0	0	100
Clarithromycin	5.8	3.8	90.4
Clindamycin	3.9	0	96.1
Chloramphenicol	23.1	0	76.9
Vancomycin	0	0	100
Tetracycline	59.6	0	40.4

(52.8%) of BHS from pyoderma cases. NGABHS were isolated from 9.2% of the samples. Sharma & Bhatia<sup>21</sup> have reported the prevalence of NGABHS as 15.3%. In this study, BHS were isolated more frequently from 0-10 years age group (38%). This is in agreement with the previous studies,<sup>1,22</sup> which reported the peak incidence of impetigo in children aged 2 to 5 years. In the present study, BHS were isolated more frequently from males (38.8%) compared to females (25%). Male preponderance has also been observed in many other studies.<sup>6,20,23,24</sup> Higher prevalence of pyoderma among males could be due to greater involvement of males in outdoor activities, thus exposing them to trauma and infection.

In our study, *S. pyogenes* were isolated from 20.8% of samples. Brahmadathan & Koshi<sup>25</sup> reported the prevalence rate of pyoderma as defined by the isolation of GAS was 10.1% with monthly variations from 2.1% to 17.1%. Bowen et al.<sup>2</sup> conducted a systematic review and reported that in children the median prevalence was 12.3%, and pooled prevalence 16.6%.

In our study, we observed that all strains were susceptible to penicillin G. Similar results had been reported in many countries, namely Morocco,<sup>26</sup> France,<sup>27</sup> and Germany.<sup>28</sup> In addition to penicillin G, amoxicillin and ceftriaxone were fully active against *S. pyogenes*. This result is in agreement with other research reports from Central, Eastern, and Baltic European countries,<sup>29</sup> Turkey,<sup>30</sup> Nepal,<sup>31</sup> where no resistance has been detected to  $\beta$ -lactams among *S. pyogenes* isolates. All *S. pyogenes* isolates tested in this study were susceptible to vancomycin. These results are in line with other studies reported in other countries.<sup>32,33</sup> For patients who are allergic to penicillin, erythromycin and other macrolides were considered as good alternatives.<sup>34</sup> In our study, no resistance was detected for azithromycin where as 7.7% of resistance to erythromycin. This result is in agreement with a research study from UK and Ireland in 2006, where erythromycin resistance was reported as 10.8%, which increased over time.<sup>35</sup> Erythromycin resistance rates vary in different geographic areas.<sup>36–38</sup> In

this study, clindamycin showed good activity with low rate of resistance (3.9%). Similar results were reported from Japan (1.4%),<sup>39</sup> Germany (1.1%).<sup>28</sup> In this study, 76.9% of *S. pyogenes* isolates were susceptible to chloramphenicol. Camara et al.<sup>40</sup> reported that Chloramphenicol was sensitive in 82.1% of isolates with the disk diffusion method, Most of the *S. pyogenes* (59.6%) isolates were resistant to tetracycline. This result is in line with the previous reports from Dakar,<sup>40</sup> Poland,<sup>41</sup> Iran<sup>42</sup> and South Korea.<sup>43</sup>

## 5. Conclusion

This study reports the prevalence and antibiotic susceptibility of *S. pyogenes* isolated from pyoderma in a tertiary care hospital, Hyderabad, South India. Results of this study suggests the peak incidence of pyoderma among children aged 0 to 10 years and male preponderance. Our study also reports high prevalence of tetracycline and chloramphenicol resistance in *S. pyogenes*.

## 6. Source of Funding

None.

## 7. Conflict of Interest

All the authors declare that there is no conflict of interest.


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