A Study of Bacteriological Isolates of Pyoderma and Its Carrier Sites

KEYWORDS
Pyoderma, Staphylococcus, Antibiotic susceptibility, Phage typing

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ABSTRACT
Pyoderma is one of the commonest clinical conditions encountered in Dermatological practice. The etiological agents are Staphylococcus aureus, Streptococcus pyogenes and others. Staphylocci are from normal skin and may be a source of infection. In the present study we tried to correlate the features of pyoderma cases with normal skin of axilla and anterior nares

Aims and objectives: To isolate and identify the bacterial organisms from clinical samples of patients with pyoderma, To study the antibiotic susceptibility pattern of isolates and phage typing of the Staphylococcal isolates

Materials and methods: Bacterial flora from 100 cases of pyoderma and normal skin of anterior nares and axillary skin were analyzed and tested for biochemical reactions, antibiotic sensitivity and phase typing

Results: Out of the 100 cases studied, 67 were males and 33 were females, with a high incidence in 0 to 10 years age group. Staphylococcus was the commonest organism isolated in the pyoderma as well as normal skin showing resistance to Penicillin. The range of biological activity of coagulase positive Staphylococci from lesions is high when compared to carrier sites where the proportion of coagulase negative is high. Autogenous infection of 18.3% was observed in the present study.

Conclusion: The present study gives an indication of present pattern of bacterial isolates of pyoderma. And also elaborates the resistance pattern of antibiotics.

Introduction
Pyoderma is one of the commonest clinical conditions encountered in Dermatological Practice. Various factors like poverty, malnutrition, overcrowding and poor hygiene stated to be responsible for its incidence in lower socio-economic group. Climatic conditions also play a major role. The etiological agents implicated in pyoderma are Staphylococcus aureus, Streptococcus pyogenes and others. Staphylocci are from normal skin and may be a source of infection.

Materials and methods
The material for the present study was collected from out-patients who attended Department of Dermatology, King George Hospital, Visakhapatnam during the period from Jan, 2012 to April, 2013.

Inclusion criteria:
Patients having erosive skin lesions and purulent discharge.

Exclusion criteria: non-purulent lesions and allergic reactions.

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One hundred cases of pyoderma, who had not been on prior antibiotic treatment were selected for this study.

For testing biochemical reactions, strains were inoculated into peptone water and a broth culture was prepared. The

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appropriate testing medium was inoculated with the broth culture. The strains isolated were subjected to catalase test slide, coagulase test, tube coagulase test, urease test, phenolphthalein phosphatase test, DNase test, mannitol fermentation test and O/F test.

All the strains of S.aureus isolated from various samples were tested for antibiotic sensitivity as per standard disc diffusion technique. All the coagulase positive strains were sent for phage typing to the department of Microbiology, Moulana Azad Medical College, Delhi.

Results

Out of the 100 cases studied, 67 were males and 33 were females, with a high incidence in the 0-10 years age group. Out of the 100 cases studied 41 cases were impetigo, 17 cases were Folliculitis, 11 cases of furunculosis, 9 cases of ecthyma, 12 cases of infected scabies and 10 cases of infected ulcers. A total of 100 strains were isolated which includes 64 strains of Staphylococci, followed by 10 strains of coagulase negative Staphylococci, 4 strains of beta haemolytic Streptococci, 3 strains of Escherichia coli, 4 strains of Klebsiella spp. Mixed infection of aureus and beta haemolytic Streptococci seen in 5 cases and 15 samples showed no growth. Out of 85 strains of organism isolated, 28 strains of Staphylococci, 4 strains of beta haemolytic Streptococci, 2 strains of CONS were isolated from bullous impetigo. 13 strains of Staphylococci and 1 strain of CONS were isolated from folliculitis, 8 strains of Staphylococci and 3 strains of CONS were isolated from furunculosis. 7 strains of Staphylococci and 1 strain of CONS were isolated from ecthyma lesions. From infected scabies, 2 strains of Staphylococci, 2 strains of CONS, 2 strains of E.coli, 2 strains of Klebsiella and 3 strains of Staphylococci & beta haemolytic Streptococcus isolated. From the infected ulcers, one strain of Staphylococci, 1 strain of CONS, 1 strain E.coli, 2 strains Klebsiella and 2 strains of Staphylococci & Streptococci were isolated (Table-1). Out of the 25 strains that could be typed 14 strains belonged to mixed group and 9 strains belonged to group II. Remaining 2 strains belonged to group I. Taking the individual phage types, GI 29 were isolated in 2 cases, GII 47 in 6 cases and GII 42/47 in 3 cases. Among the mixed group GI 29, GII 42/47 in 1 case, GI 29, GII 47 in 4 cases, GI 29, GII 42/47/85 in 4 cases and GI 29, GII 47/85 in 5 cases were isolated. Antibiotic sensitivity pattern of the strains of coagulase positive Staphylococci isolated, all the 64 (100%) were sensitive to Amoxycylav, 61 (95.3%) to Vancomycin, 59 (92.1%) to Gentamicin, 58 (90.6%) to Cefpodoxime, 57 (89%) to Azithromycin, 51 (79.7%) to Cefoxitin and all strains were resistant to Penicillin. All the 10 strains of coagulase negative Staphylococci were found to be sensitive to Amoxycylav, Vancomycin and Gentamicin, 8 strains were sensitive to each Cefpodoxime & Azithromycin and 4 strains were sensitive to each Penicillin & Cefoxitin. All the 9 strains of beta haemolytic Streptococci isolated were sensitive to Amoxycylav, Cefpodoxime and Vancomycin. Three strains were sensitive to Cefoxitin, Azithromycin and Penicillin each (67%). Antibiotic sensitivity pattern of Gram negative bacilli isolated from lesion showed 3 strains of Escherichia coli were sensitive to Ceftriaxone, Ceftazidime, Amikacin and Imepenem. Two strains of E.coli were sensitive to Ciprofloxacin, Ceftaxime and Gentamicin (66.6% each). All the 4 strains of Klebsiella isolated were sensitive to Amikacin and Imepenem. Three strains of Klebsiella were sensitive to Ciprofloxacin, Ceftriaxone, Ceftazidime and Gentamicin (75% each). Two strains were sensitive to Ceftriaxone (50%).

Organisms isolated from anterior nares

From 100 swabs the Staphylococci were the predominant isolates (75) of which 33 coagulase positive and 42 negative. Out of which 67 were purely isolated and 8 mixed isolates, Staphylococcus & Diphtheroids (4), Staphylococcus & beta haemolytic Streptococci (4). 25 swabs were sterile after repeating the culture again. All the coagulase positive strains were resistant to Penicillin.

Of the coagulase negative strains 39 strains (92.8%) were resistant to Penicillin. On comparison between the coagulase positive and coagulase negative strains no significant difference in the sensitivity pattern is seen for the antibiotics tested. Organisms isolated from skin of 100 patients. Out of the 100 swabs collected from the normal skin (axillary area) of pyoderma cases 77 isolates were Staphylococci, 10 isolates were aerobic spore bearers, 10 were mixed isolates, Diphtheroids and Staphylococci (7), Gram negative bacilli and Staphylococci (3) and 3 were Gram negative bacilli. Among the 87 strains of Staphylococci 25 (29%) were coagulase positive and 62 (71%) were coagulase negative. Of the 25 coagulase positive Staphylococci all were resistant to Penicillin. Among 62 strains of coagulase negative Staphylococci 59 (95.1%) were resistant to Penicillin.

Comparative analysis of characteristics of coagulase positive strains from lesions, nose and skin

Strains isolated from lesions showed highest percentage characteristics among strains isolated from lesions followed by nose and skin. The strains isolated from skin exhibited lowest activity. This difference is significant with reference to all the properties. Comparison between coagulase positive staphylococci isolated from cases and carrier sites in antibiotic resistance showed all the 236 strains of Staphylococci showed high degree resistance to Penicillin and moderate resistance to Cefoxitin and Azithromycin in coagulase positive Staphylococci. The strains from all the sources were sensitive to Amoxycylav & Vancomycin (Table-2).

Phage types pattern of Staphylococci from Lesions, anterior nares and skin areas

Showed identical phage type in lesion, anterior nares and skin was found in 4 cases, phage type GI 29 in 3 cases and GI 29, GII 47 in 1 case. Identical phage type of GI 29 and GII 47 was found in 4 cases of lesion and anterior nares. One identical phage type of GI 29 was found in 1 case of lesion and skin. The percentage of incidence of identical phage type was 18.3% (Table-3).

Discussion

Among the 100 cases of pyoderma taken for the present study 41% was bullous impetigo cases which was consistent with Parveen Thind et al.(42%), followed by folliculitis 17% and furuncle 11%. Pyoderma was found to be of common occurrence among the males of all age groups. In the present study high incidence was recorded among males of 0-10 age group with 67%. Similar findings were reported by Shashi Gandhi et al.5, 61.2% Varsha et al.(2014) 54% Among the organisms isolated from pyoderma Staphylococci predominated, 59% of cases singly and along with streptococci in 5% of cases. Similar observations were made by K V Ramana et al.(2008); Y Bhawan et al., (2011); Paudel U et al.(2013). Beta haemolytic Streptococci was next common isolation from cases and carrier sites in antibiotic resistance showed all the 236 strains of Staphylococci showed high degree resistance to Penicillin and moderate resistance to Cefoxitin and Azithromycin in coagulase positive Staphylococci. The strains from all the sources were sensitive to Amoxycylav & Vancomycin (Table-2).
correlating with the findings of S.Kulkarni et al10., DNase production was found in 93.7% of coagulase positive strains. Mannitol fermentation was reported to be useful in detecting pathogenic Staphylococci by J Dinesh Kumar et al11., (2009) and Pamela J Yeh et al., (2011).Present study is correlating with the above studies showing 84.3% man-nitil fermentation.In the present study several strains were found to be positive for beta-haemolysis. Among the co-agulase positive strains 73.4% were found to be haemo-lytic. Coagulase test has been recognized as the routine laboratory test for the detection of pathogenic Staphy-loccoci and in the present study also it showed that it is still the best test to detect the pathogenic Staphylococci. In our study sensitivity of all beta haemolytic Streptococci was consistent with Mohammed I. Fatani et al12(2002) who reported100% sensitivity to Penicillin and Oxacillin. All the strains of Esherichia coli were sensitive to Ceftriazone Cefazidine, Amikacin and Imepenem. Mohammad I.Fatani et al12 (2002) reported 100% sensitivity to Ceftaixime, Cef-triaxone & Gentamicin. All the strains of Klebsiella were sensitive to Amikacin and Imepenem, 75% were sensitive to Ciprofloxacon, Ceftriaxone, Cefazidime and Gentamicin. And 50% were sensitive to Cefaxime. Suresh K Malhotra et al.13 reported 50% sensitivity to Ceftaixime and Gen-tamicin, which is correlating with our study.

Organisms isolated in anterior nares
From the anterior nares of these cases, the most commonly isolated organism was Staphylococci in 75% of cases, (67 strains singly and 8 strains along with other organisms). Carriage of Staphylococci in anterior nares was reported in many studies. Kumar P et al.14 reported 82.9%, Ramani T V and Jayakar P A 15reported nasal carriage of 85%.High incidence of resistance to Penicillin (100%) was observed in strains from nose. All strains were sensitive to Amoxycilav and Vancomycin. Mukesh Palsingh et al16, (2013) reported 100% sensitivity to Amoxycilav from carriers. Wide spread resistance to Penicillin among nasal isolated Staphylococci was reported by Bhawani Y et al 97.5% the present study correlates with this study.

Organisms isolated from normal skin in axillary region
Among the100 strains isolated 87 strains of Staphylococci 29% were coagulase positive and 71% were coagulase negative (Table-15). Y Bhawani et al17, reported 34% of co-agulase positive Staphylococci over the skin. In the present study the incidence of coagulase positive Staphylococci over the skin was much lower. Characteristic properties of coagulase positive Staphylococci isolated from normal skin and their sensitivity pattern was correlated with Y.Bhawani et al17, 2011 who reported 96.5% sensitivity to Penicillin.

Comparison and correlation of staphylococci isolated from lesions, anterior nares and normal skin in cases
Out of the total 236 strains of Staphylococci isolated from various sources in the study, 74 were from lesions, 75 from nose and 87 from normal skin. Of them 86.4% from lesion, 44% from anterior nares and 29% from skin are coagulase positive. The incidence of coagulase positive Staphylococci in the lesion was significantly higher P≤ 0.01 as Staphylo-cocci is much higher.

The range of biological activity of coagulase positive Staphylococci from lesions is high when compared to car-rier sites where the proportion of coagulase negative is high. Comparatively low range of activity is seen in coagu-lase positive strains of anterior nares and still less activity was observed by coagulase positive strains from normal skin, in that descending order. This high biological activity of strains from lesions may be due to their active participa-tion in pathogenesis compared to other strains which are quiescent at the carrier sites.

The comparative analysis of resistance pattern of coagu-lase positive Staphylococci from lesions and carrier sites revealed that all the strains were resistant to Penicillin. There is a difference in the resistance pattern to Cefoxitin with strains isolated from lesion showing highest resistance fol-lowed by strains isolated from nose. All the strains tested in the two comparable groups were sensitive to Amoxycilav and Vancomycin An attempt was made to identify whether or not the source of infection in these cases is from their own carrier sites by matching the strains of Staphylococci isolated from these cases from three sites (lesion, nose and skin) by their identical phage types. In 4 cases identical phage type in lesion, anterior nares and skin were found, phage type GI 29 in 3 cases and GI 29, GII 47 in 1 case. Identical phage type of GI 29 and GII 47 were found in 4 cases of lesion and anterior nares. One identical phage type of GI 29 is found in 1 case of lesion and skin. In these cases the anterior nares might be the source of infection, although it may also possible that these strains in the ante-rior nares and skin may be acquired from lesion also. Sanjay M.Wavare et al18, (2012) reported that most common group was mixed phage group (17%) followed by phage group I (13.55%). The present study correlates with this study, reporting an autogenous infection of 18.3%. The possible explanation for low incidence of autogenous in-fections among Indians could be, due to high incidence of extraneous cross infection in these patients with poor hy-giene and socio-economic status.

Summary & Conclusion
Swabs were collected from the lesion, anterior nares and normal skin of axillary region from 100 clinically diagnosed cases of pyoderma and cultured for bacterial flora. High incidence (41%) of bullous impetigo is observed among males of 0 to 10 years age group with a common occur-rance is on the face. Staphylococcus is the commonest organism in the causation of pyoderma along with other strains. It was also found to be the commonest organ-ism in the anterior nares and axial skin. Pattern of co-agulase positive Staphylococci from lesions and carrier sites revealed that all the strains were resistant to penicil-lin. All the strains from the test were sensitive to Vanco-mycin and Gentamicin. All the strains isolated from an-terior nares and axial skin were sensitive to Amoxycilav, Vancomycin and Gentamicin. No significant difference is noted between coagulase positive and coagulase nega-tive Staphylococci strains in their sensitivity pattern. Inci-dence of coagulase positive Staphylococci is significantly high (Ps 0.01) in lesion when compared to anterior nares and skin. Coagulase positive strains isolated from lesions have exhibited significantly high range of all the patho-genic characters when compared to the coagulase positive strains from nose and skin (Ps 0.01). All the strains of beta haemolytic Streptococci were sensitive to Amoxycilav, Cef-odoxime and Vancomycin. All the strains of E.coli were sensitive to Ceftriaxone, Cefazidime, Amikacin and Im-e penem. All strains of Klebsiella were sensitive to Amikacin and Imepenem.

The phage typing of coagulase positive Staphylococci isolated from lesions revealed that Group I and Group III phages were able to lyse most of the strains. No par-ticular phage type were found to be predominant. Out of the 6 strains which were typable from anterior nares, 4 strains belonged to Group I and 2 strains belong to
Pyoderma cases are more common in males, mostly affecting children in their first decade of life. Face was most commonly affected site. Staphylococcus aureus was the commonest causative agent, and the emergence of MRSA in the community is a warning sign. With the knowledge of likely causative organisms and their resistance pattern, the suitable antibiotic therapy can be started and avoid unnecessary medication with ineffective drugs.

### TABLE-1: Distribution of organisms isolated in 100 cases of pyoderma

<table>
<thead>
<tr>
<th>Clinical type</th>
<th>No. of patients</th>
<th>S. aureus</th>
<th>CONS</th>
<th>E. coli</th>
<th>K. brettschneideri</th>
<th>Staphylococcus aureus</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buloous impetigo</td>
<td>41</td>
<td>29</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Folliculitis</td>
<td>17</td>
<td>13</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Furuncle</td>
<td>11</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythema</td>
<td>9</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infected scars</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Infected ulcer</td>
<td>10</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Total: 100

### TABLE-2: Comparison between coagulase positive Staphylococci isolated from cases and carrier sites in antibiotic resistance

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Staphylococci strains from lesions (64)</th>
<th>Staphylococci strains from nose (33)</th>
<th>Staphylococci strains from skin (25)</th>
<th>X²p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>64</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>64</td>
<td>100</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>38</td>
<td>60</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>38</td>
<td>60</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Cefapridom</td>
<td>38</td>
<td>60</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>38</td>
<td>60</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Cefprozil</td>
<td>38</td>
<td>60</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

### TABLE-3: Distribution of identical Phage types pattern of Staphylococci from Lesions, anterior nares and skin

<table>
<thead>
<tr>
<th>Areas</th>
<th>Total no. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior nares</td>
<td>4</td>
</tr>
<tr>
<td>Anterior Skin</td>
<td>4</td>
</tr>
<tr>
<td>Lean</td>
<td>4</td>
</tr>
<tr>
<td>Skin</td>
<td>4</td>
</tr>
</tbody>
</table>

### REFERENCES