

## Clinico-bacteriological study of pyoderma with special reference to methicillin resistant *Staphylococcus aureus*

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### Abstract

Pyoderma is one of the common skin infections seen in dermatology clinics in India. The changing pattern of etiological agents of pyoderma and the emergence of antibiotic resistant strains are posing a significant problem in deciding empirical therapy. With this background, the present study was carried out to determine the bacteriological profile of pyoderma and to assess the antibiogram of the isolates. Thirty patients with purulent skin lesions attending Dermatology outpatient department were studied. The swabs from the lesions were subjected to Gram stain and culture on MacConkey and blood agar plates. The isolates were identified by standard microbiological methods. Antibiotic susceptibility testing was done as per CLSI guidelines. Screening for methicillin resistance was done by cefoxitin disc diffusion method. Primary pyoderma constituted 86.7% and secondary pyoderma 13.3% of cases. Common types of pyoderma seen were impetigo, folliculitis, furuncle and cellulitis (16.7%) each. *S. aureus* was the most common isolate (56.8%) followed by coagulase negative *Staphylococci* (10.8%). Majority of *S. aureus* and *Enterococcus species* were found resistant to ciprofloxacin (88% and 100%) respectively. *Enterococcus species* was 100% resistant to aminoglycosides (High level aminoglycoside resistance). *Enterobacter species*, *Citrobacter species* and *Klebsiella species* showed 100% resistance to ciprofloxacin and cephalosporins. Majority of the *Pseudomonas* isolates were resistant to cefotaxime and ceftriaxone (100%). Methicillin resistance was seen in 14.3% of *S. aureus* and 50% of coagulase negative *Staphylococci*. Though there was not a major difference noted with respect to the etiological agents of pyoderma, an alarming resistance pattern to commonly used antibiotics is noted in the present study. Hence periodic screening for the bacteriological profile and sensitivity pattern of pyoderma isolates is mandatory.

**Keywords:** Methicillin resistance; Pyoderma; *Staphylococci*.

### Introduction

Pyoderma is one of the most common and most challenging problems encountered in clinical practice.<sup>(1,2)</sup> It is defined as purulent skin disease and represents infections in the epidermis and dermis or in hair follicle.<sup>(3)</sup> Humid climate, poverty, malnutrition, overcrowding and poor hygiene have been implicated in the development of pyoderma.<sup>(3-5)</sup> Pyoderma are classified into primary (pyoderma occurring without predisposing cause or skin lesion) and secondary pyoderma (pyoderma in which an existing skin lesion becomes secondarily infected).<sup>(6)</sup> Primary pyoderma comprises of impetigo, folliculitis, furuncle, carbuncle, ecthyma, sycosisbarbae and cellulitis. Secondary pyoderma constitutes tropic ulcer, infected scabies and various dermatosis infected with organisms.<sup>(1,7)</sup> According to the set up from where the infection is contracted, pyoderma may be community acquired or hospital acquired infection.<sup>(8)</sup> A skin and soft tissue infection is considered as community acquired if the infection arose outside the health care setting and there is no history of surgery, hospitalization, catheterization, any other invasive procedure in the previous year and no history of antibiotic usage in the previous two months or methicillin resistant *Staphylococcus aureus* (MRSA) isolation in the past. It is considered hospital associated or acquired if the infection occurred during the patient's

stay in the hospital or the specimen was collected after 48 hours of admission.<sup>(9)</sup>

Pyoderma is most commonly caused by *Staphylococcus aureus* followed by group A  $\beta$  hemolytic *Streptococci*, *Klebsiella species*, *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus species* and coagulase negative *Staphylococci*.<sup>(1)</sup> Indiscriminate use of topical and systemic antibiotics has lead to development of antibiotic resistance among bacteria.<sup>(10)</sup> Among Staphylococcal isolates, resistance to penicillin was noted within 4 years of introduction of penicillin and is due to production of enzyme called  $\beta$  lactamase (penicillinase).<sup>(6)</sup> In 1960, methicillin, the first  $\beta$  lactamase stable semi synthetic penicillin was introduced.<sup>(6)</sup> *Staphylococcus aureus* strains which are resistant to penicillinase stable beta lactams are referred as methicillin resistant *Staphylococcus aureus* (MRSA).<sup>(11)</sup> Resistance to methicillin was developed among *Staphylococci* due to the gene *mecA* which codes for penicillin binding protein PBP2a.<sup>(12)</sup> The virulence of MRSA is due to the Pantone -Valentine leukocidin factor which is dermonecrotic and leukocidal toxin.<sup>(12)</sup> MRSA can cause infection ranging from simple furuncles to life threatening necrotizing fasciitis and pyomyositis.<sup>(12)</sup> MRSA also have remarkable ability to develop resistance to a variety of antibiotics including penicillins, cephalosporins, aminoglycosides, macrolides and quinolones.<sup>(11)</sup> MRSA was recognized initially in the

health care setup. In 1980s MRSA spread in the community.<sup>(8)</sup> From then community acquired MRSA is increasingly being reported in skin and soft tissue infections in India and globally.<sup>(9)</sup>

There is also considerable variation reported in the antibiotic susceptibility pattern of the other organisms isolated from pyoderma with a trend towards increasing resistance.<sup>(9)</sup> Hence, timely recognition and prompt bacterial diagnosis with antimicrobial sensitivity is imperative for the effective management and treatment of pyoderma.<sup>(2)</sup> Because the culture and sensitivity results are available at an approximate interval of 72 hrs, the majority of pyoderma cases are treated by empirical therapy followed by streamlining of antibiotic therapy, once the culture and sensitivity reports are in hand.<sup>(8)</sup> The changing pattern of etiological agents of pyoderma and the emergence of antibiotic resistant strains are posing a significant problem in deciding empiric therapy.<sup>(13)</sup>

If suitable empirical drug is not selected, it will result in treatment failure and also promote the development of drug resistance among bacteria. An appropriate empirical therapy can be decided by framing periodic antibiotic policies.<sup>(4,14)</sup> To form antibiotic policy, the knowledge regarding regional antibiotic sensitivity and resistance patterns of the prevalent bacteria is of immense importance.<sup>(8)</sup> With this background the present study was undertaken to know the clinico- bacteriological profile of pyoderma and to study the antibiogram of the isolates.

## Materials and Methods

The present cross sectional study was carried out in the Department of Microbiology from 13<sup>th</sup> May 2015 to 13<sup>th</sup> July 2015. Institutional Ethical Committee clearance was obtained for the study.

**Inclusion criteria:** Patients having skin lesions with purulent discharge attending Dermatology Outpatient department of the college were included in the study.

**Exclusion criteria:** Patients who refused to give consent and who were on antibiotics or had taken antibiotics one week prior to time of sample collection were excluded from the study.

**Sample size:** 30 patients.

**Sampling method:** Simple random sampling.

**Statistical analysis:** Results expressed in terms of percentages.

After obtaining informed consent from the patient, demographic details of the patient were collected. The clinical diagnosis of the lesion was noted. Specimen was collected aseptically from the skin lesion using two sterile swabs. One swab was used for making a smear for Gram stain and the other swab for inoculation on to the 5% sheep blood agar and MacConkey agar plates [Hi Media Mumbai, India].<sup>(1)</sup>

The inoculated plates were incubated aerobically at 37°C for 24-48 hours. The isolates were identified by standard microbiological methods.<sup>(15,16)</sup>

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines [CLSI] using control strains.<sup>(17)</sup>

The following antibiotic discs were tested and were procured from Hi Media Mumbai India.

Penicillin (10 U), ampicillin (10µg), amoxicillin-clavulanic acid (30µg), piperacillin-tazobactam (100/10µg), cefepime (30µg), cefixime (5µg), ceftriaxone (30µg), ceftazidime (30µg), cefotaxime (30µg), linezolid (30µg), teicoplanin (30µg), rifampicin (5 µg) ciprofloxacin (15µg), erythromycin (15µg), clindamycin (2µg), cotrimoxazole (1.25/23.75µg), gentamicin (10µg and 120µg), amikacin (30µg), tetracycline (30µg), chloramphenicol (30µg) and imipenem (10µg).

Staphylococcal isolates were tested for methicillin resistance by ceftoxitin (30µg) disc diffusion method as per CLSI guidelines.<sup>(17)</sup>

## Results

**Table 1: Age wise distribution of cases**

Age in years	Number (%)
0-20	8 (26.7)
21-40	13 (43.3)
41-60	2 (6.7)
Above 61	7 (23.3)
Total	30 (100)

Table 1 shows that, majority of the cases belonged to 21-40 years age group (43.3%). Males constituted 66.7% of study group and females 33.3% of study group. Primary pyoderma Constituted 86.7% and secondary pyoderma 13.3%.

**Table 2: Types of primary pyoderma noted in the study group**

Type of primary pyoderma	Number (%)
Impetigo	5 (19.2)
Folliculitis	5 (19.2)
Furuncle	5 (19.2)
Cellulitis	5 (19.2)
Diabetic foot ulcer	3 (11.5)
Abscess	2 (7.8)
Ecthyma	1 (3.9)
Total	26 (100)

Table 2 shows that, impetigo, folliculitis, furuncle and cellulitis constituted majority of primary pyoderma cases, 19.3% each.

**Table 3: Types of secondary pyoderma noted in the study group**

Type of secondary pyoderma	Number (%)
Infected dermatitis	1 (25)
Infected burns	1 (25)
Infected wound	1 (25)
Infected kerion	1 (25)
Total	4 (100)

**Table 4: Bacteria isolated in the present study**

Bacteria	Number (%)
Staphylococcus aureus	21 (56.8)
Coagulase negative Staphylococci	4 (10.8)
Pseudomonas species	3 (8.1)
Citrobacter species	3 (8.1)
Enterobacter species	2 (5.4)
Escherichia coli	2 (5.4)
Enterococcus species	1 (2.7)
Klebsiella species	1 (2.7)
Total	37 (100)

Table 4 shows that, *S. aureus* was the most common isolate (56.8%) followed by coagulase negative Staphylococci (10.8%). Single bacterium was isolated in 24(80%) cases and multiple bacteria in 6(20%) cases.

**Table 5: Clinico- bacteriological profile of pyoderma**

Clinical type of pyoderma (Number)	S.aureus	Coagulase negative Staphylococci (CONS)	Enterococci species	Pseudomonas species	Enterobacter species	Citrobacter species	Klebsiella species	E.coli
	Number	Number	Number	Number	Number	Number	Number	Number
Folliculitis(5)	4	1	-	-	-	-	-	-
Furuncle(5)	5	-	-	-	-	-	-	-
Cellulitis(5)	-	1	1	3	1	1	-	1
Impetigo(5)	4	1	-	-	-	-	-	-
Diabetic foot ulcer(3)	1	1	-	-	-	2	1	1
Abscess(2)	2	-	-	-	-	-	-	-
Ecthyma(1)	1	-	-	-	-	-	-	-
Secondary pyoderma(4)	4	-	-	-	1	-	-	-
Total (30)	21	4	1	3	2	3	1	2

Table 5 shows that, majority of folliculitis and impetigo were caused by *S.aureus* (80%) followed by CONS (20%). *S.aureus* was isolated in all furuncle cases (100%).

All Gram positive cocci were sensitive to vancomycin, linezolid and teicoplanin (100%). Coagulase negative Staphylococci (100%) and 70% of *S.aureus* were found sensitive to clindamycin and chloramphenicol respectively. Majority of *S.aureus* and *Enterococcus species* were found resistant to ciprofloxacin (88% and 100%) respectively. *Enterococcus species* was 100% resistant to aminoglycosides (High level aminoglycoside resistance). Eighty percent of *S.aureus* and 50% of coagulase negative Staphylococci showed resistance to erythromycin.

*Enterobacter species*, *Citrobacter species* and *Klebsiella species* showed 100% resistance to ciprofloxacin and cephalosporins, whereas, 50% of *E.*

*coli* were found sensitive to these drugs. *Enterobacter species* and *Citrobacter species* showed 50% resistance to imepenem. Majority of the *Pseudomonas* isolates were resistant to cefotaxime and ceftriaxone (100%) followed by cefepime, ceftazidime and piperacillin - tazobactam (66.7%) each.

**Table 6: Methicillin resistance among Staphylococcal isolates**

Staphylococcus species	Methicillin resistant Number (%)	Methicillin sensitive Number (%)	Total Number (%)
<i>S.aureus</i>	3 (14.3)	18 (85.7)	21 (100)
CONS	2 (50)	2 (50)	4 (100)

Table 6 shows that, 14.3% of *S. aureus* and 50% of coagulase negative Staphylococci were methicillin resistant.

**Table 7: Resistance pattern of methicillin resistant *Staphylococci* in the present study**

<i>Staphylococcus species</i>	Erythromycin	Clindamycin	Gentamicin	Amikacin	Tetracycline	Chloramphenicol	Cotrimoxazole	Ciprofloxacin	Ofloxacin
MRSA	100%	33.3%	33.3%	33.3%	33.3%	33.3%	33.3%	100%	100%
MR CONS	50%	00	00	00	50%	00	100%	00	00

MRSA: Methicillin resistant *Staphylococcus aureus*; MR CONS: Methicillin resistant coagulase negative *Staphylococci*.

Table 7 shows that, all MRSA strains were resistant to erythromycin and fluoroquinolones (100%) and all MR CONS isolates were resistant to cotrimoxazole (100%).

## Discussion

Pyoderma is quite common in India and constitutes a major portion of the cases in dermatology clinics.<sup>(18)</sup> In the present study pyoderma was found more in 21-40 years age group (43.3%) where as Gandhi, et al have reported high incidence in <10 years age group and Ramana, et al in >40 years age group.<sup>(2,4)</sup> This variation may be attributed to various facts that predisposes to pyoderma like poverty, overcrowding, poor hygiene, illiteracy and malnutrition.<sup>(3-5,19)</sup> In the present study pyoderma was found more in male patients (66.7%). Similar results were reported by others.<sup>(2,4)</sup> Male preponderance may be due to more involvement in the manual work and in turn more prone to trauma.<sup>(12)</sup> In the present study majority of the cases were primary pyoderma (86.8%). Similar results were found by others.<sup>(10,20)</sup> The reason for higher rate of primary pyoderma may be due to the fact that, we selected patients from outpatient department which usually represent community acquired pyoderma and majority of community acquired pyoderma are primary pyoderma. Secondary pyoderma is most commonly seen in hospital acquired pyoderma.<sup>(12,18)</sup> The common clinical types of pyoderma in the present study were impetigo, folliculitis, furuncle and cellulitis (16.7%) each. Many of the authors have reported impetigo followed by folliculitis as the most common clinical type.<sup>(1,3,4,7,10,14)</sup> Whereas Ramana, et al have reported abscess as the most common type followed by furunculosis and Patil, et al found folliculitis and furunculosis as the predominant types.<sup>(2,5)</sup> The pattern of skin diseases varies from place to place and can be attributed to differing climatic, cultural and socioeconomic factors in the study group.<sup>(19)</sup> In the present study, *S.aureus* was the most common isolate both in primary and secondary pyoderma. Similar findings were reported others.<sup>(1,3,4,7,10,12,14,20,21)</sup> The reason may be that normally *Staphylococcus species* colonizes the skin and also contain many virulence factors affecting skin.<sup>(22)</sup> *Streptococcus species* was not isolated in the present study similar to study by Ramana, et al.<sup>(2)</sup> But others have reported  $\beta$  haemolytic *Streptococci*.<sup>(4,12,18,20,23)</sup> Due to small sample size in the

present study we might have missed the Streptococcal isolates.

The variation in the pattern of etiological agents may be attributed to the region of the study, environmental factors, type of population studied, hygiene and nutritional status.<sup>(19)</sup>

In the present study, multiple isolates were seen in cellulitis and diabetic ulcer cases (Table 5) and Gram negative bacilli were the predominant bacteria isolated in both conditions. While deciding empirical therapy for cellulitis and diabetic ulcer, antibiotics whose spectrum of activity involves both Gram positive and Gram negative bacteria need to be considered.

The emergence of antibiotic resistance is significantly posing a severe threat to public health worldwide.<sup>(7)</sup> In the present study, all Gram positive cocci were found sensitive to vancomycin linezolid and teicoplanin. *S. aureus* showed 90% resistance to penicillin. Similar results were reported by others.<sup>(14,21)</sup> Approximately 88% and 83% of the Staphylococcal isolates showed resistance to fluoroquinolones and to macrolides respectively. Others have reported lesser resistance rates.<sup>(10,14,21)</sup> Higher rate of resistance against ciprofloxacin and erythromycin found in the present study may be due to frequent prescription of these antibiotics because they are available in oral form and misuse of these drugs in the community because of over the counter availability of drugs, which increases the selection pressure and thus the development of resistance.

In the present study, *S. aureus* were found sensitive to cephalosporin 81% followed by tetracycline 80%, chloramphenicol 75%, amikacin 73%, clindamycin 70% and gentamicin 64.7%. Similar results with respect to amikacin, clindamycin and chloramphenicol were reported by others.<sup>(10,18)</sup> In the present study 65% of *S.aureus* were resistant to Cotrimoxazole whereas Ghadage, et al have reported 72% sensitivity rate.<sup>(10)</sup> Gandhi, et al had reported similar sensitivity pattern to vancomycin, gentamicin and cephalosporins.<sup>(4)</sup> Amoxycylav sensitivity in the present study was 40% where as Gandhi, et al had reported 94.35% sensitivity rate.<sup>(4)</sup>

It is noted from the present study that for empirical treatment of *Staphylococcus aureus* strains, penicillin group of drugs, beta lactam and beta lactamase inhibitor combination, erythromycin and ciprofloxacin are not recommended.

In the present study, all coagulase negative *Staphylococci* (CONS) isolates showed 100% sensitivity to clindamycin, gentamicin and amikacin and 75% sensitivity to ciprofloxacin. Malhotra, et al. reported maximum sensitivity to amikacin 77.7% and 52.4% sensitivity to ciprofloxacin.<sup>(18)</sup>

*S.aureus* isolates were found more sensitive to cephalosporins (80%) as compared to CONS isolates (50%). In the present study, 14.3% of *S.aureus* isolates were methicillin resistant. Similar results were reported by some authors.<sup>(1,4,6)</sup> whereas, few authors found lesser incidence.<sup>(5,12,20)</sup> This shows that, MRSA which are known to cause more hospital acquired pyoderma are spreading in the community.<sup>(9,12,23)</sup> In the present study, 50% of coagulase negative *Staphylococci* were methicillin resistant whereas Malhotra, et al had reported 1.64%.<sup>(18)</sup> This shows that, methicillin resistance is increasing among CONS isolates. This will make the treatment of pyoderma still more difficult because CONS are the second most common bacteria isolated in pyoderma and they colonize the skin.

All methicillin resistant isolates in the present study were found sensitive to vancomycin, linezolid. Similar results were reported by others.<sup>(12)</sup> All MRSA strains were found resistant to erythromycin (100%) and ciprofloxacin (100%). MRSA showed 33.3% resistance to gentamicin. Lalremruata, et al had reported 80% resistance to gentamicin and 90% to ofloxacin.<sup>(23)</sup> The reason for higher rate reported in the other study may be because, the study involved mainly hospital acquired pyoderma and hospital acquired MRSA isolates are known to be more drug resistant. In the present study 33.3% sensitivity is noted among MRSA isolates to chloramphenicol, cotrimoxazole, tetracycline and clindamycin. Unlike MRSA strains, methicillin resistant coagulase negative *Staphylococci* (MR CONS) showed 100 % sensitivity to fluoroquinolones, aminoglycosides, clindamycin and chloramphenicol.

In the present study, all Gram negative bacilli except *E.coli* (50%), showed maximum resistance to ampicillin, amoxyclav, cefotaxime and ceftriaxone and to ciprofloxacin. Gandhi, et al. had reported maximum resistance to cefepime (80%), amoxicillin (75%) and to ciprofloxacin (50%).<sup>(4)</sup> Majority of Gram negative bacteria except *Klebsiella species* were found sensitive to amikacin. *Pseudomonas species* showed maximum resistance to third generation cephalosporin (100%), piperacillin – tazobactam (66.7%) and ciprofloxacin (50%). It was found in the study that, for Gram negative bacteria, ampicillin, amoxyclav, cephalosporins and to some extent ciprofloxacin are not helpful as empirical drugs.

The present study highlights the alarming rise in resistance pattern among bacteria causing pyoderma in the study locality. Limitation of the present study is the small sample size. The inferences drawn in the present study need to be confirmed by a larger study.

## Conclusion

Pyoderma has become a significant cause of skin infections. In the present study changing patterns are noted in the causative agents of pyoderma and also an alarming resistance pattern to commonly used antibiotics. Hence, an antibiotic policy, based on periodic screening for the bacteriological profile of pyoderma and sensitivity pattern is mandatory. The emergence of MRSA and MR CONS in the community as shown in the present study is a warning sign. An awareness should be created among general practitioners and among public regarding judicious use of antibiotics.

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