

## Research Article

# Incidence of drug resistance bacterial isolates from different Pyoderma cases attending at tertiary care hospital of Central Nepal

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### ABSTRACT

**Background & Objectives:** Pyoderma is defined as any purulent skin disease and represents infections in epidermis and dermis including hair follicles. Thus, this study was conducted to find out incidence and bacteriological profile of pyodermas as well as to determine their antibiotic susceptibility test of isolated bacteria.

**Materials and Methods:** The samples were collected in dermatological OPD and sent the collected samples immediately to bacteriology laboratory for culture and sensitivity tests from December 2018 to May 2019 A.D. Antimicrobial susceptibility testing was done using Kirby-Bauer disc diffusion method and the results were interpreted according to the CLSI guidelines. Quality control of the test was done by standard ATCC strains of *S.aureus* (ATCC 25923), *E.coli* (ATCC 25922).

**Results:** The incidence of pyoderma was 3.24%. Maximum cases were detected in the age group below 13 years of age. Primary pyoderma (61.8%) was more frequent when compared to secondary pyoderma (38.2%). The highest number of organism isolated was *S. aureus* (24.7%) from different cases of pyoderma followed by *E. coli* (8.2%), CONS (6.1%) and *Klebsiella pneumoniae* (5.1%) whereas other organisms were found in less number. Gram positive bacteria mainly *S. aureus* and other isolates for the cause of pyoderma were mostly resistant to penicillin (100%), ciprofloxacin (75.0%) and clindamycin (79.2%). Gram negative *E. coli* (8.2%) was second most bacterial isolates and others GNB isolates were (14/63) where resistance pattern were seen more (75.0%) in each ceftriaxone and imipenem.

**Conclusion:** Incidence of pyoderma was low among the patients visiting to the Dermatology Department. Antibiotic resistance is increasing day by day; therefore, routine culture and sensitivity tests are essential to guide proper treatment procedure.

**Keywords:** Bacterial isolates, drug resistance, pyoderma

### INTRODUCTION

Pyoderma is a term used to describe any skin infection involving the formation of pus. It can be caused by bacteria, fungi or other

Mahaseth, S et al.,

microorganisms and can affect people of all ages [1]. Common symptoms of pyoderma include redness, swelling and the presence of pustules or abscess on the skin. It is common among preschool and school going children [2, 3].

Primary pyodermas are impetigo, folliculitis (sycosis barbae), furuncle, carbuncle, ecthyma and erthyrasma. Secondary pyodermas constitute tropic ulcer, infected pemphigus, infected contact dermatitis, infected scabies and various other dermatoses infected with organisms [1, 2, 3]. In majority of cases, pyoderma is commonly caused by Gram positive bacteria, but occasionally it may cause by Gram negative organisms. In most of the cases, infection is caused by a single organism, but mixed infections may also occur [4]. Among Gram positive organisms, *Staphylococcus aureus* is the commonest pathogen, which may be either methicillin-sensitive (MSSA) or methicillin-resistant (MRSA), followed by *Streptococcus pyogenes*, *Corynebacterium* species, coagulase negative *Staphylococcus* (CONS), *Haemophilus influenzae*, *Escherichia coli*, *Pseudomonas* species, *Proteus* species, *Klebsiella* species and anaerobic bacteria are occasionally isolated from skin lesions of pyodermas [5].

Climatic conditions also play a role in the occurrence of the disease [6, 7]. In tropical countries, summer and rainy seasons are the periods of maximum occurrence of pyodermas. Higher incidence of pyoderma in underdeveloped countries is multifactorial and attributed to the factors like poverty, malnutrition, warm weather, humidity, poor hygiene, overcrowding and immunosuppression. Pyoderma is best prevented by attention to adequate personal hygiene [8]. Isolation of patient with open

draining *Staphylococcal* infections, strict hand washing procedures, good nursery techniques and careful handling of patients are important in the reduction of transmission of infection. Nepal is a diverse country topographically and in terms of the ethnicity which may have a significant influence on the prevalence of pyoderma in Nepal. The Terai region of the country has a tropical climate and has more influence in the prevalence of disease. Proper diagnosis of the disease as in any infection is always based on clinical examination and confirmed by bacteriological culture by isolation and identification of the causative organisms.

Although easily treatable, pyodermas are known for their chronicity, recurrence and multitude of complications. Therefore, timely recognition and prompt bacterial diagnosis with antimicrobial sensitivity is imperative for the effective management of pyoderma. Indiscriminate use of antibiotics in clinical practice has led to the emergence of antimicrobial drug resistant strains of bacteria which has significantly eroded the utility of established antibiotics therapy and posed a serious threat to public health worldwide [9]. MRSA is an important health care associated pathogen. Many of these isolates are becoming multidrug resistant. All  $\beta$ -lactams including carbapenems and cephalosporins, piperacillin, tazobactam etc. are ineffective against MRSA. Knowledge of prevalence of MRSA and their current antimicrobial profile becomes necessary in the selection of appropriate empirical treatment of this infection. Vancomycin along with newer agents like linezolid, daptomycin and quinupristin/dalfopristin are the drug of choice for treating multidrug resistant *Staphylococcal* infections particularly those caused by MRSA [10]. The current prospective hospital based study was

Mahaseth, S et al.,

conducted at the Department of Microbiology, College of Medical Sciences, Bharatpur to study the incidence of pyodermas along with drug resistance patterns of bacterial isolates from different cases of pyodermas.

## **MATERIALS AND METHODS**

This cross sectional prospective study was conducted in the Department of Microbiology, College of Medical Sciences-Teaching Hospital (COMS), Bharatpur, Chitwan, Nepal. Out of 2994 cases visited in the Dermatology outpatient department (OPD), the new cases of different pyoderma was 97 selectively enrolled for the current research over the period starting from December 2018 to May 2019 A.D. All clinically diagnosed pyoderma cases of all age groups and either sex were included whereas patients had taken antimicrobial treatment either local or systemic during the last two weeks were excluded. Therefore, ninety seven patients of clinically diagnosed cases of pyoderma meeting the inclusion and exclusion criteria were included in the study after taking informed consent. Ethical permission was obtained from Institutional Review Committee of COMS, Bharatpur. [Ref No:2019-016]

After taking complete dermatological examination, the specimen was collected after cleaning the area around the lesion with 70% ethyl alcohol. Crusts of lesions were lifted and material was taken from underneath with the help of two sterilized swabs. In case of intact pustular lesion, material was collected after rupturing the pustule with sterile needle. The swabs were labeled, numbered and transported immediately to the bacteriology laboratory of Department of Microbiology for further processing.

## **Sample processing**

All the samples were collected aseptically with the help of two sterile cotton swabs from each lesion, which were processed for isolation and identification of bacterial pathogens, according to the standard microbiological techniques. Gram stain preparations were made from one swab, and culture plates were inoculated from another swab. Each sample was inoculated on blood agar and MacConkey agar. All the above media and reagents were obtained from HiMedia, Mumbai, India. The media were prepared according to the manufacturers' instructions. The plates were incubated at 37°C for 24 hours. The plates were observed for growth in the following day but incubation was extended to 48 hours if there was no bacterial growth within 24 hours. Isolated colonies were subjected to Gram staining and biochemical tests for identification. Identification was carried out according to the standard microbiological guideline by using different biochemical tests like catalase test, coagulase test, mannitol test, oxidase test, urease test, TSI tests as per requirement [11].

## **Antibiotic susceptibility testing**

Anti-microbial susceptibility test was carried out on isolated and identified colonies using commercially prepared antibiotic disk (HiMedia) on Mueller Hinton agar plates by the disk diffusion method, according to the Clinical Laboratory Standards Institute (CLSI) guidelines.

Antibiotics used in our study were penicillin-G (10unit), amoxicillin (100µg), azithromycin (15 µg), clindamycin (2µg), amikacin (30µg), gentamicin (10µg), cefotaxime (10µg), ceftriaxone (30µg), ciprofloxacin (5µg), and vancomycin (30µg), imipenem (10µg),

Mahaseth, S et al.,

piperacillin (100µg), tobramycin (10µg). MRSA detection was done using oxacillin disc (1µg), and using Mueller Hinton agar with 2% NaCl. The plates were incubated for 24 hours at 37°C, and zone diameter was measured. If zone diameter was ≥13 mm, it was considered as MSSA and if it was ≤10mm, then it was considered as MRSA [12].

**RESULTS**

Out of 2994 cases visited in the Dermatology outpatient department (OPD), the new cases of different pyoderma was 97, therefore the incidence was 3.24%. The male to female ratio was found to be 2.16:1, the current

research showed the occurrence of pyodermas was more in males (68.33%) than in females (31.67%) as depicted in table 1.

In our study, primary pyoderma (61.8%) was more frequent than secondary pyoderma (38.2%). In primary pyoderma, there were significantly high number of cases seen as 35.0% cases of furunculosis followed by 21.6 % cases of superficial folliculitis and 16.6% cases of impetigo. Secondary pyoderma showed the highest number of cases from dermatitis 24.3%, followed by 21.6% infected dermatophytosis and 16.2% scabies. Most of the pyodermas were having more predilections among children below 13 years

**Table 1: Sex distribution of the primary pyoderma**

Cases	Male	Female	Total
	No / percentage	No/percentage	
Impetigo	8 (80.0)	2 (20.0)	10
Superficial folliculitis	9 (69.23)	4 (30.77)	13
Furuncle	14 (66.67)	7 (33.33)	21
Carbuncle	2 (66.67)	1 (33.33)	3
Abscess	4 (50.0)	4 (50.0)	8
Cellulitis	4 (80.0)	1 (20.0)	5
Total	41	19	60

**Table 2: Patterns of various pyoderma cases (n=97)**

Primary Pyoderma	Age Below 13 years	Age 13-50 years	Age Above 50 years	Total No	Percentage
Furuncles	11	3	7	21	35.0
Folliculitis	4	2	7	13	21.6
Impetigo	6	1	3	10	16.6
Abscess	4	2	2	8	13.3
Cellulitis	2	1	2	5	8.3
Carbuncles	2	0	1	3	5.0
<b>Total</b>	<b>29</b>	<b>9</b>	<b>22</b>	<b>60</b>	<b>100</b>
Secondary Pyoderma					
Infected Eczema	2	0	0	2	5.4
Infected Scabies	3	1	2	6	16.2
Infected Dermatitis	5	1	3	9	24.3
Infected Dermatophytosis	2	4	2	8	21.6
Infected Herpes zoster	4	0	1	5	13.5
Infected ulcer	1	1	1	3	8.2
Infected pyoderma	1	1	2	4	10.8
<b>Total</b>	<b>18</b>	<b>8</b>	<b>11</b>	<b>37</b>	<b>100</b>

of age as seen in table 2.

On culture, 63 cases showed growth of single organism and 34 cases shown no growth from 97 cases of pyoderma. Different Gram positive (68.05%) and negative bacteria (34.92%) isolated from cases of primary and secondary pyoderma as shown in table 3. The

highest number of organism isolated was *Staphylococcus aureus* (24.7%) from different cases of pyoderma followed by *Escherichia coli* (8.2%), CONS (6.1%) and *Pseudomonas aeruginosa* (4.1%) whereas other organisms were found in less number.

Antibiotic susceptibility tests of all isolates

**Table 3: Bacteriological profile of culture positive organisms**

Bacteria Isolated		Number	Percentage
Staphylococcus aureus	Gram Positive Bacteria	24	24.7
Coagulase negative Staphylococcus(CONS)		6	6.1
Staphylococcus haemolyticus		4	4.1
Streptococcus pyogens		2	2.0
Micrococcus		5	5.1
Escherichia coli	Gram Negative Bacteria	8	8.2
Klebsiella pneumonia		5	5.1
Pseudomonas aeruginosa		4	4.1
Proteus vulgaris		2	2.1
Citrobacter species		2	2.1
Proteus mirabilis		1	1.0
No organisms isolated		34	35.0
<b>Total samples processed</b>		<b>97</b>	<b>100</b>

**Table 4: Antibiotics susceptibility patterns of Gram positive bacterial isolates**

Antibiotics	S. aureus	CONS	S. haemolyticus	S. pyogens	Micrococcus
Penicillin-G	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)
Amoxicillin	15 (62.5)	4 (66.6)	2 (50.0)	2 (100)	3 (60.0)
Ciprofloxacin	6 (25.0)	1 (16.6)	0 (0.0)	2 (100)	3 (60.0)
Azithromycin	14 (58.3)	2 (33.3)	1 (25.0)	2 (100)	2 (40.0)
Clindamycin	5 (20.8)	2 (33.3)	1 (25.0)	0 (0.0)	1 (20.0)
Gentamicin	20 (83.3)	2 (33.3)	1 (25.0)	2 (100)	2 (40.0)
Vancomycin	22 (91.6)	5 (33.3)	4 (100)	2 (100)	5 (100)

**Table 5: Antibiotics susceptibility patterns of Gram Negative bacterial isolates**

Antibiotics	E. coli	K. pneumoniae	P. aeruginosa	P. vulgaris	Citrobacter spp	P. mirabilis
Amikacin	6 (75.0)	2 (40.0)	2 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)
Cefotaxime	7 (87.5)	3 (60.0)	2 (50.0)	1 (50.0)	1 (50.0)	1 (100)
Ciprofloxacin	4 (50.0)	2 (40.0)	2 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)
Ceftriaxone	2 (25.0)	1 (20.0)	1 (25.0)	1 (50.0)	1 (50.0)	0 (0.0)
Imipenem	2 (25.0)	1 (20.0)	1 (25.0)	0 (50.0)	0 (0.0)	0 (0.0)
Gentamycin	6 (75.0)	3 (60.0)	2 (50.0)	1 (50.0)	2 (100)	0 (0.0)
Piperacillin	4 (50.0)	3 (50.0)	3 (75.0)	1 (50.0)	1 (50.0)	1 (100)
Tobramycin	6 (75.0)	3 (60.0)	3 (75.0)	2 (100)	1 (50.0)	1 (100)

were taken as per standard microbiological guideline from different cases of Pyoderma. Only antibiotic susceptibility tests of different antibiotics are depicted in the table 4 and 5. This current research showed that the Gram positive bacteria mainly *S. aureus* and other isolates for the cause of pyodermas were mostly susceptible to vancomycin (91.6%), gentamycin (83.3%), amoxicillin (62.5%) and azithromycin (58.3%) i.e. above 60.0%, whereas it showed more resistant to penicillin (100%), ciprofloxacin (75.0%) and clindamycin (79.2%) i.e. above 75.0%.

Among the Gram negative bacterial isolates, the most common *E. coli* was susceptible to amikacin, gentamycin and tobramycin (75.0%), cefotaxim (87.5%) i.e above 75.0% and each piperacillin and ciprofloxacin (50.0%). The resistance pattern of *E. coli* was seen in each ceftriaxone and imipenem (75.0%).

## DISCUSSION

Incidence rate of the new cases of different pyoderma was 3.24%. In different studies [13, 14] the prevalence was ranged from 2.55% to 29.8%. In a community-based study [15] among children, prevalence of pyoderma was 4.5%. The possibility of lower percentage might be that was a hospital based study rather than community based survey.

Pyoderma is one of the commonest clinical conditions encountered in dermatological practice [16]. In our study, we reported higher cases of pyoderma in males and in first decades of life, similar to observations made by Nagmoti et al in 1999 [17]. The highest numbers of cases (48.33%) were detected in the age group less than 13 years. Most of the pyodermas were having more predilections among children below 13 years of age. This

may be because children have lower immunity, and all of this has been observed in patients who have visited hospitals personally. In our study the male to female ratio was 2.16:1. The current research showed the occurrence of pyoderma was more in males (68.33%) than in females (31.67%). Our findings can be comparable to a study conducted by Nagmoti et al in 1999 [17] and Patil et al in 2006 [18] where male predominance was also observed. This male predominance of pyoderma could be due to more active life in this area compared to females.

Primary pyoderma (61.8%) was more frequent when we compared to secondary pyoderma (38.2%). In primary pyoderma, there were significantly high number of cases seen as 35.0% cases of furunculosis followed by 21.6 % cases of superficial folliculitis and 16.6% cases of impetigo. Secondary pyoderma showed the highest number of cases as 24.3% dermatitis, 21.6% infected dermatophytosis and 16.2% scabies. Our findings can be comparable to Lee et al [19] whose analysis of 127 patients with pyodermas found 71% of the primary pyodermas were due to *Staphylococcus aureus* in 1990. Another study by Gandhi et al [1] in 2012 reported that in males there were 58 cases of impetigo (54.72%), 52 cases of superficial folliculitis (65.39%), 11 cases of furuncle (84.62%) and 3 cases of carbuncle (100%) while in females there were 48 cases of impetigo (45.28%), 27 cases of superficial folliculitis (34.61%), 2 cases of furuncle (15.38%) and no cases of carbuncle. However, study by Malhotra et al in 2012 [20] in their study found that primary pyodermas accounted for 19.67% cases while 80.33% cases were of secondary pyoderma. Among the primary pyodermas, impetigo was the

commonest entity seen (14.75%). Ahmed et al in 1998 [21] in their clinical analysis showed that impetigo (27%) formed the largest clinical group. The occurrence of primary pyoderma was more because it is caused by the direct invasion of healthy skin, whereas secondary pyoderma originates in diseased skin as superimposed conditions.

Many researchers also documented that Gram positive bacteria play major role causing pyoderma [22] like in our investigation, the highest number of isolated organism were *Staphylococcus aureus* (24.7%) from different cases of pyodermas followed by *Escherichia coli* (8.2%), CONS (6.1%) and *Pseudomonas aeruginosa* (4.1%) whereas other organisms were found in less number. Gandhi et al in 2012 [1] also depicted similar results with commonest organism as *S. aureus* followed by Gram negative organisms. There has been high trend in the isolation of *S. aureus* as etiological agent in pyoderma but other species of *Staphylococcus* should not be overlooked, even it is isolated as low number [1].

Geoffrey et al in 2013 [23] reported *S. aureus* comprising 37.4% of total isolates, whereas Ramana et al in 2014 [24] reported *S. aureus* in 52.1% of total isolates, coagulase negative *Staphylococcus* in 19.2%, *Enterococci* in 11.4% cases, *Klebsiella* in 7.8% cases and diphtheroides in 2.8% cases while 6.4% cases were culture negative. Gram negative organisms, although it is less frequently grown as compared to Gram positive organism in both the groups [21], were relatively more frequent in the secondary pyoderma group compared to primary pyoderma group. Another study [25] had also reported a similar observation that Gram negative organism *E. coli* was isolated most frequently from pyodermas cases compared to other Gram negative bacteria.

Our finding showed that *S. aureus* and other isolates like CONS for the cause of pyoderma were mostly susceptible to amoxicillin (62.5%) azithromycin (58.3%) gentamicin (83.3%) and vancomycin (91.6%) i.e. above 60.0%, whereas it showed more resistant to penicillin (100%), ciprofloxacin (75.0%) and clindamycin (79.2%) i.e. above 75.0%. Many other studies [26] have reported Gram positive to be highly susceptible to aminoglycosides particularly to gentamicin. Our findings was similar to Wavare et al in 2012 [27] found a total of 59 strains of *S. aureus* isolated from 100 pus samples of pyoderma cases. Out of which 98.3% were resistant to penicillin and 81.35% were resistant to ampicillin.

Mathew et al in 1992 [6] showed that resistance to penicillin (79.3%), ampicillin (79.3%) and tetracycline (42.4%) by *S. aureus* was significant. Ramana et al in 2008 [24] tested antimicrobial susceptibility tests of isolates which revealed greater resistance against penicillin (100%) and ampicillin (92%). Vancomycin showed 91.6% sensitivity towards coagulase positive *S. aureus* in our study. It was similar to a study conducted by Gandhi et al in 2012 [1] with sensitivity of 99.3% but inconsistent to another study by Tambe et al in 2011 [28] where sensitivity was only 40% towards coagulase positive *S. aureus*. Amoxicillin was 62.5% sensitive towards coagulase positive *S. aureus* in our study which was comparable to study reported by Kar in 1996 [29] where it was 91.2% sensitive towards *S. aureus*. However, Gandhi et al in 2012 [1] reported that amoxicillin was only 34.84% sensitive towards coagulase positive *S. aureus*. In our study ciprofloxacin was sensitive in 25% cases. It was similar to studies conducted by Malhotra et al in 2012 [20] and Tambe et al in 2011 [28] where sensitivity of ciprofloxacin

Mahaseth, S et al.,

was only 52.4% and 40% sensitive towards coagulase positive *S. aureus* respectively. However, studies conducted by Patil et al in 2006 [18] and Gandhi et al in 2012 [1] where they were 82.8% and 74.19% sensitive towards coagulase positive *S. aureus* respectively.

Thus we speculate that this difference in sensitivity pattern may be because of scarce use of ciprofloxacin in routine management of pyoderma. *In view of the emergence of penicillin, tetracycline and erythromycin resistant strains of Staphylococcus aureus, vancomycin along with the newer agents like linezolid and quinupristin/dalfopristin is the drug of choice for treating multidrug resistant Staphylococcal infections particularly those caused by MRSA [23]. Linezolid is also a promising therapeutic option in an era of rapidly growing antibiotic resistance.*

Among the gram negative bacterial isolates, *E. coli* was susceptible to amikacin, gentamicin and tobramycin (75%) cefotaxime (87.5%) and each piperacillin and ciprofloxacin (50%), i.e above 75%. Resistance pattern were more seen in each ceftriaxone and imipenem with (75%). *Pseudomonas aeruginosa* showed a high susceptibility 75% to piperacillin and 50% to ciprofloxacin. It was similar to studies conducted by Singh A et al in 2015 [13] where *E. coli* was more than 80% susceptible to amikacin, tobramycin and ceftriaxone. *Pseudomonas aeruginosa* showed a high susceptibility 100% to polymyxin and moderate susceptibility 75% to piperacillin, levofloxacin and ciprofloxacin.

Limitation of this study was since the samples were taken from outpatients only; it may not reflect the real situation of community acquired pyoderma in the community.

## CONCLUSION

The highest number of organism isolated was *S. aureus* (24.7%). *Staphylococcus aureus* was mostly resistant to penicillin (100%), ciprofloxacin (75.0%) and clindamycin (79.2%). This research might assist clinicians to choose suitable antibiotics for pyoderma. Especially in the remote plane and hilly areas of Nepal where it is difficult to get culture and sensitivity tests report for proper treatment of poor patients. Ideally the choice of the antimicrobial agents would depend on the culture and sensitivity patterns in a particular region, but continuous monitoring of culture sensitivity report for antibiotics like amoxicillin with sulbactam/clavulanate, linezolid or ampicillin with sulbactam may be preferred. The changing trend of causative agents of pyodermas and their susceptibility pattern needs constant monitoring through prospective study in the future.

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