ORIGINAL RESEARCH PAPER
Volume-9 | Issue-4 | April-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ANTIBIOTIC SUSCEPTIBILITY PATTERN AND EMERGING RESISTANCE IN CASES OF PYODERMAS – A PROSPECTIVE STUDY AT TERTIARY CARE HOSPITAL ,NAGPUR.

Microbiology

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ABSTRACT

Introduction- Pyogenic skin infection (pyoderma) is the bacterial infection of skin and its appendages.S. aureus, Pseudomonas, E.coli, Proteus, Citrobacter, Enterobacter, Klebsiella spp. Etc are important pyogenic organisms most commonly isolated from the pyodermacases.Rapid emergence of multidrug resistance in most of the Gram positivebacterial isolates complicates the management of pyoderma and demonstrates the need for more judicious use of antimicrobial agents.**Aims and objectives-** 1)To study the bacteriological profile ofpyodermacasesin Nagpur district 2)To study the antibiogram of isolated organisms. **Material and methods-** This prospective study was conducted on 200 clinically suspected patients of pyodermas after taking informed consent. After taking proper history, samples were collected using sterile cotton swab stick and were processed according to standard microbiological guidelines for culture and antibiotic sensitivity patterns.**Results**Out of 178 culture positivity 63.5% showed the growth of S.aureus, 9.5% CONS, 4.5% S.pyogenes, 4.5% Enterococcus, 3.4% E.coli, 2.2% C. freundii, 10.1% Klebsiellaspp, 4.5% Proteus spp, and 7.9% Ps.aeruginosa. S.aureuswas the commonest organism isolated (63.5%), followed by Klebsiellaspp (10.1%).S. aureus was 100% sensitive to Linezolid and Vancomycin followed by Netilmicin (99.1%), Amikacin (96.4%), Tobramycin (87.6%), Gentamicin (86.7%).Ps. aeruginosawere most sensitive to Aztreonam (92.9%) followed by Imipenem and Pipericillin-Tazobactam (85.7%).Except for vancomycin and linezolid, resistance to all the antimicrobials tested is more among MRSA isolates than MSSA isolates.The present study indicated a relatively high rate of susceptibility among clinical isolates of MSSA to tetracycline, aminoglycosides and macrolides tested. **Conclusion-** As most of the cases were culture positive, pyodermas should not be ignored and should be treated. Despite various Gram negative bacterias, Staphylococcus aureusremains the most common bacteriaandshould be regularly assessed for its sensitivity patterns to decide the empirical drug in a particular area.

KEYWORDS

Pyoderma, sterile cotton swab stick, Staphylococcus aureus,aminoglycosides,Enterobacteriaceae.

INTRODUCTION

Pyogenic skin infection (pyoderma) is the bacterial infection of skin and its appendages.1Pyoderma constitutes a major portion of patients in dermatological clinics in India.2,3Primarypyodermas are caused by direct invasion of normal skin and have a characteristic morphology while Secondary pyodermas originate in the diseased skin as a superimposed condition like in scabies, pediculosis, wounds, insect bites, and eczema.[4]

S. aureus and S. pyogenes are the two important pyogenic organisms most commonly isolated from the pyoderma cases, the former being more common than the latter.2,3 Besides these, other organisms that are occasionally isolated from pyodermas are Enterococci, Pseudomonas, E.coli, Proteus, Citrobacter, Enterobacter, and Klebsiella spp.[2]

Diagnosis is mainly based on clinical examination correlated with laboratory investigations like examination of the Gram stained smear of the purulent material along with culture and isolation of the causative organism and its identification by various biochemical tests.[5]

Rapid emergence of multidrug resistance in most of the Gram positivebacterial isolates complicates the management of pyoderma and demonstrates the need for more judicious use of antimicrobial agents.[6,7]

MATERIALAND METHODS-

Type of study- Prospective study
Number of cases- 200

Inclusion criteria-All clinically diagnosed pyoderma cases of all age groups and either sex were included in the study.

Exclusion criteria-Patients having taken antimicrobial treatment (local or systemic) during the last 15 days were excluded from the study.

Sample collection- Samples were collected using sterile cotton swab stick after cleaning the area around the lesion with 70% ethyl alcohol after taking the informed verbal consent.

Transport of specimen- Specimens were transported and processed within 2 hours of collection by the standard microbiological technique.[8,9]

Following procedures were performed:
- Gram staining
- Bacterial culture
- Identification of pathogens
- Antimicrobial susceptibility testing of pathogens

RESULTS-

Table 1: Bacteriological analysis of pyoderma cases

Pathogens	No. of pathogens		Total (%)
	Primary pyoderma n=148 (%)	Secondary pyoderma n =52 (%)	n=200 (%)
Staphylococcusspp	91 (61.4)	22 (42.3)	113(63.5)
CONS	11 (7.4)	06(11.5)	17(9.5)
S.pyogenes	06 (4.1)	02 (3.8)	8(4.5)
Enterococcusspp	08 (5.4)	0	8 (4.5)
E. coli	6(4.1)	0	6(3.4)
Klebsiellaspp K. pneumoniae K. oxytoca	12(8.1) 12 00	06 (11.5) 03 03	18 (10.1)
Citrobacterspp Cit. koseri Cit. Freundii	04(2.7) 01 03	0	4(2.2)
Proteusspp P. mirabilis P. vulgaris	02 (1.3) 00 02	06(11.5) 05 01	8(4.5)
Ps. aeruginosa	04 (2.7)	1 0 (19.2)	14(7.9)

Out of 178 culture positivity 63.5% showed the growth of S.aureus, 9.5% CONS, 4.5% S.pyogenes, 4.5% Enterococcus, 3.4% E.coli, 2.2% C. freundii, 10.1% Klebsiellaspp, 4.5% Proteus spp, and7.9% Ps.aeruginosa. S.aureuswas the commonest organism isolated

(63.5%), followed by Klebsiellaspp (10.1%). The difference between organisms causing Primary pyoderma and Secondary pyoderma was not statistically significant (p>0.005)

Table 2.Antimicrobial sensitivity of gram positive cocci from pyogenic skin infections

Drugs	S. aureus n = 113 (%)	CONS n = 17 (%)	S.pyogenes n = 8(%)	Enterococcus n = 8 (%)
PEN	15 (13.3)	2(11.7)	4 (50)	1 (12.5)
AMP	-	-	-	1 (12.5)
CX	95 (84.1)	14 (82.3)	-	-
CPM	-	-	7 (87.5)	-
E	83 (73.4)	12 (70.6)	5 (62.5)	-
CD	95 (84.1)	13 (76.4)	6 (75)	-
GEN #	98 (86.7)	14 (82.3)	-	1 (12.5)
STP #	-	-	-	1 (12.5)
AK	109 (96.4)	15 (88.2)	-	-
TOB	99 (87.6)	14 (82.3)	-	-
NET	112 (99.1)	14 (82.3)	-	-
TE	95 (84.1)	12 (70.6)	-	-
CHL	45 (39.8)	6 (35.3)	5 (62.5)	-
CIP	65 (57.5)	15 (88.2)	-	-
COT	42 (37.1)	14 (82.3)	-	-
VA	113 (100)	17 (100)	8 (100)	6 (75)
LZ	113 (100)	17 (100)	8 (100)	6 (75)

P – Penicillin G, A – Ampicillin, CX – Cefoxitin, CPM– Cefepime E– Erythromycin, CD– Clindamycin, GEN – Gentamicin, STP- Streptomycin, AK – Amikacin, TOB – Tobramycin, NET – Netilmicin, TE – Tetracycline, CHL– Chloramphenicol, CIP– Ciprofloxacin, COT – Cotrimaxazole, VA – Vancomycin, LZ – Linezolid

- For Enterococcus spp, high level gentamicin (120 µg disk) and streptomycin (300 µg disk) was used. For all other organisms gentamicin (10 µg disk) was used.

Table 2 shows that allS. aureus was 100% sensitive to Linezolid and Vancomycin followed by Netilmicin (99.1%), Amikacin (96.4%), Tobramycin (87.6%), Gentamicin (86.7%). Aminoglycosides were followed by Clindamycin, Tetracycline anderythromycin in the range of 80% – 90% sensitivities. While lesser sensitive drugs were Ciprofloxacin (57.5%), Chloramphenicol (39.8%) and Cotrimoxazole (37.1%). It was least sensitive to Penicillin G (13.3%).

Out of total 113 isolates of S. aureus 95 (84%) were sensitive to cefoxitin and 18 were resistant. Therefore percentage of MRSA isolated was 16%. All isolates of S. aureus (100%) were Linezolid sensitive.

Among the CONS isolated, 100% were sensitive to Linezolid &Vancomycin whereas Aminoglycosides, Quinolones, Cotrimoxazole and Cephalosporins show sensitivity in range 80% - 90%. It was least sensitive to Penicillin G (11.7%).

S. pyogeneswas 100% were sensitive to Linezolid&Vancomycin followed by Cefepime (87.5%), Clindamycin (75%), Erythromycin and Chloramphenicol (62.5%), Penicillin G (50%).

Enterococci were most sensitive to Linezolid and Vancomycin (75%) while Penicillin G and Ampicillin showed sensitive (12.5%). 87.5% resistance to high level gentamicin and streptomycin was observed. Two out of eight (25%) isolates were resistant to Linezolid & Vancomycin.

Table 3.Antimicrobial sensitivity of Gram negative bacteriasfrom pyogenic skin infections

Drugs	E. coli n =6 (%)	K. pneumoniae n = 15 (%)	K. oxytoca n = 3 (%)	C. koseri n = 1(%)	C. freundii n = 3 (%)	P. vulgaris n = 4 (%)	P. mirabilis n = 4 (%)	Ps. aeruginosa n = 14 (%)
AMP	1 (16.7)	0	0	0	1 (33.3)	0	1 (25)	0
AMC	1(16.7)	2(13.3)	0	0	0	0	1 (25)	2 (14.3)
CXM	1(16.7)	3(20)	0	0	0	0	1 (25)	-
CX	5(83.3)	11(73.3)	1(33.3)	0	0	0	0	6
CTX	5(83.3)	12(80)	1(33.3)	0	0	0	0	-
CPM	5(83.3)	10(66)	0	1 (100)	1 (33.3)	0	2 (50)	10 (71.4)
CAZ	4(66.7)	8(53.3)	0	0	0	0	1 (25)	6 (42.9)
PI	6(100)	13(86.7)	1(33.3)	0	1 (33.3)	2 (50)	1 (25)	7(50)
PIT	6(100)	14(93.3)	3(100)	1 (100)	3 (100)	4 (100)	2 (50)	12 (85.7)
IPM	6 (100)	15 (100)	3 (100)	1 (100)	3 (100)	4 (100)	3(75)	12 (85.7)
AT	6(100)	15(100)	1(33.3)	0	1 (33.3)	4 (100)	2 (50)	13 (92.9)
GEN	6(100)	5(33.3)	2(66.6)	0	0	1 (25)	1 (100)	6 (42.9)
AK	6(100)	8(53.3)	3(100)	0	2(66.6)	1 (25)	0	7(50)
TOB	5(83.3)	6(40)	2(66.6)	0	0	1 (25)	0	5 (35.7)
NET	6(100)	14(93.3)	2(66.6)	1 (100)	0	1 (25)	1 (25)	5 (35.7)
TE	5(83.3)	2(13.3)	1(33.3)	0	0	0	0	-
CHL	4(66.7)	7(46.7)	1(33.3)	0	0	0	0	-
CIP	3(50)	8(53.3)	2(66.6)	0	0	2 (50)	1 (25)	10(71.4)
COT	1(16.7)	7(46.7)	0	0	1 (33.3)	0	0	2(14.3)

AMP – Ampicillin, AMC – Amoxyclav, , CXM – Cefuroxime, CX – Cefoxitin, CTX – Cefotaxime, CPM – Cefepime, CAZ – Ceftazidime, PI– Piperacillin, PIT – Piperacillin-tazobactam, IPM – Imipenem, AT – Aztreonam, GEN – Gentamicin, AK – Amikacin, TOB – Tobramycin, NET – Netilmicin, TE – Tetracycline, CHL– Chloramphenicol, CIP– Ciprofloxacin COT – Cotrimaxazole

Table 3.shows that all the isolates of E.coli showed 100% sensitivity to Pipericillin, Pipericillin-Tazobactam, Imipenem, Aztreonam, Amikacin, Gentamicin and Netilmicin. Followed by Tetracycline and Cephalosporins (60-80%). Least sensitive were Ampicillin and Amoxyclav Klebsiella spp. were most sensitive to Imipenem (100%) followed by AztreonamPipericillin, Pipericillin-Tazobactam.K. pneumoniae showed less sensitivity towards cotrimaxazole (46.7%), 3rd and 4th generation cephalosporins and Ciprofloxacin(53.3%) but was overall more sensitive than K. oxytoca. They showed 100% resistance to Ampicillin.

Citrobacter spp. were the most resistant of the Enterobacteriaceae. C. freundii isolates showed 100% sensitivity to Imipenem and Pipericillin-Tazobactam while, C. koseri showed 100% sensitivity to Imipenem, Pipericillin-Tazobactam, Netilmicin and Cefepime.

Both theProteus spp. showed increased resistance to all antibiotics with 100% resistance to Cefoxitin, Cefotaxim, Tetracyclin, Chloramphenicol, and Cotrimoxazole.

Ps. aeruginosawere most sensitive to Aztreonam (92.9%) followed by Imipenem and Pipericillin-Tazobactam (85.7%) each, and least sensitive to Amoxyclav, Cotrimoxazole, (14.3%) each besides being fully resistant to Ampicillin.

Table 4: Comparison of antibiotic resistance pattern among methicillinresistant and methicillin susceptible S. aureus isolates

Antibiotics	MSSA (n=95)	MRSA (n=18)
	Resistant Number (%)	
Penicillin	80(84.1)	18 (100)
Erythromycin	19(20)	11 (61.7)
Clindamycin	10 (10.5)	8 (44.4)
Gentamicin	5 (5.2)	10 (55.6)
Amikacin	0 (0)	4 (22.2)
Tobramycin	4 (4.2)	10 (55.6)
Netilmicin	0	1 (6.7)
Tetracycline	10(10.5)	8 (44.4)
Chloramphenicol	52(54.7)	16 (88.8)
Ciprofloxacin	31(32.6)	17 (94.4)
Cotrimoxazole	55(57.8)	16 (88.8)

From the table 4 it is evident that except for vancomycin and linezolid, resistance to all the antimicrobials tested is more among MRSA isolates than MSSA isolates. MRSA strains showed 100% and 88.8% resistance to penicillin and cotrimoxazole respectively. Resistance to ciprofloxacin was 94.4% while it was 32.6% in MSSA. Resistance to gentamicin was 55.6% among MRSA, while it was only 5.2% among MSSA isolates. Resistance to amikacin and erythromycin was 22.2% and 61.7% among MRSA isolates respectively, while it was nil and 20% respectively among MSSA isolates. Resistance to clindamycin was 44.4% in MRSA while it was 10.5% in MSSA.

Z test for difference between two proportions was applied to this table which showed that difference between resistance to all antimicrobials tested among MRSA and MSSA was statistically significant ($p<0.0001$).

DISCUSSION-

Aetiological agents

In the present study conducted on 200 cases the most common pathogenisolated was S.aureus(63.5%). Similar findings have been reported by other workers.2,10-13However, there was no significant difference between the isolation of Staphylococcus aureus in primary and secondary pyodermas, the percentage being 61.4% and 42.3% respectively with a $P > 0.005$ which correlates with the study of Paudel et al.14 In one study, even in chronic wound infections, Staphy lococcusaureus was isolated in 70.8% of cases, though more number of Gram-negative bacilli have been isolated from secondary pyodermas and chronic wound infections as compared to primary pyodermas.[14]

Isolation of Streptococci in the present study was 4.5% which is similar to that of Patil et al, 13where the isolation was 2.3%. However other studies2,10,11,6,13have shown a higher isolation rate. The reason behind this could be due to the change in the etiological agent or due to inhibition of Streptococcus pyogenes by secondary invasion of Staphylococcus aureus which is supposed to produce bacteriocins, toxic to Streptococci or due to bacterial interference.

In our study Enterococcusspp were isolated in 4.5% cases. In study conducted by Ramana et al6isolation rate of Enterococcusspp was 11.4%. As Enterococcus fecalis is a part of normal fecal flora, the isolation seen in this study may be due to contamination of the lesion or due to opportunistic infection.14 in our study, 9.5%were CONS, 3.4% E.coli, 2.2% C. freundii, 10.1% Klebsiellaspp, 4.5% Proteusspp, and7.9% Ps.aeruginosawere found which are compared to other studies.

In our study of the 50 cases of folliculitis, S. aureus was isolated from 29 cases (58%), S. pyogenes(2%), Klebsiellaspp. was isolated from 6 (12%) cases and Proteus spp. from 4 (8%) cases. This is comparable to Gandhi et al15 who isolated 71.1% caused by S. aureus (56) followed by β-hemolytic Steptococci (1.2%), 8.9% samples grew Klebsiella spp.

Similarly, 68.9% impetigocasesgrewS. aureus alone, while Gandhi et al15 isolated S. aureus from 81.1% impetigo cases. Patil et al 13from 58.9%cases.

Antibiotic susceptibility pattern

S. aureus was 100% sensitive to Linezolid and Vancomycin followed by Netilmicin (99.1%), Amikacin (96.4%), Tobramycin (87.6%), Gentamicin (86.7%). Aminoglycosides were followed by Clindamycin, Tetracycline anderythromycin in the range of 80% – 90% sensitivities. While Ciprofloxacin (57.5%), Chloramphenicol (39.8%) and Cotrimoxazole (37.1%) were found to be less sensitive drugs.

This is compared to other studies shown below. Penicillin is least sensitive (13.35) probably is due to the penicillinase producing strains. Similar findings have been shown by other workers. 2,10

Among the CONS isolated, 100% were sensitive to Linezolidand Vancomycin whereas Aminoglycosides, Quinolones, Cotrimoxazole and Cephalosporins show sensitivity in range 80% - 90%. It was least sensitive to Penicillin G (11.7%).Malhotra et al(2012)18 also suggested high(77.7%) aminoglycoside sensitivity.

All S. Pyogenesisolates were sensitive most of the antibiotics tested. Malhotra et al(2012)18 observed similar findings.

Enterococci were most resistant toPenicillin G and Ampicillin (12.5%).Paudel et al14(2013)also showed 100% resistance to these drugs.

Among Gram Negative bacterias(table 3), all were uniformly sensitive only to higher antibiotics like Imipenem, Piperacillin-Tazobactam, Aztreonam.Citrobacter spp. were the most resistant of the Enterobacteriaceae, Least effective drugs were Ampicillin and Amoxyclav. Proteus spp. showed increased resistance to all antibiotics with 100% resistance to Cefoxitin, Cefotaxim, Tetracyclin, Chloramphenicol, and Cotrimoxazole.

Study series	Percentage of sensitivity							
	P	Cip	GEN	Ak	Co	E	Va	Lz
Ghadage et al2	27	61	12	75	72	-	-	-
Nagmoti et al10	22	85	90	-	-	-	-	-
Kakar et al8	14.1	89.1	84.4	-	-	-	-	-
Patil et al 13	12.8	82.8	98.6	-	-	75.1	100	-
Ramana et al6	0	100	100	-	-	-	100	-
Tushar et al17	25.3	78.1	-	-	-	75.6	100	-
Malhotra et al18	-	52.3	66	100	-	-	-	-
Wavare et al19	-	-	91.5	-	30.5	69.5	-	-
Paudel et al14	-	84	-	-	76.8	85.5	-	-
Present study	13.3	57.5	86.7	96.4	37.1	73.4	100	100

P – Penicillin G, Cip– Ciprofloxacin, E– Erythromycin, GEN – Gentamicin, Co- Cotrimoxazole, Ak– Amikacin, VA – Vancomycin, LZ – Linezolid

Comparison of antibiotic susceptibility pattern

Percentage of MRSA isolated

In present study(table 4), percentage of MRSA isolated was 16%. Thus, showing that the incidence of MRSA from pyoderma cases was not very high. Similarfindings have been shown by several workers who have also shown a decreased incidence of MRSA.6,13,20

Table 20: Comparison of percentage of MRSA isolated

Study series	Percentage of MRSA isolated
Nagaraju et al20	10.9%
Patil et al13	1.4%
Ramana et al6	19.1%
Tushar et al17	10.4%
Malhotra et al18	20%
Gandhi et al15	20%
Present study	16%

Resistance pattern of MRSA & MSSA:

Comparison of antibiotic resistance pattern among MRSA & methicillin sensitive S. aureus (MSSA) isolates showed that resistance to fluoroquinolones as well as to other antibiotics tested was significantly higher in MRSA isolates than that in MSSA isolates ($p<0.0001$) table13. Similar findings were observed by Nishijima et al (2002)21andSubedi et al (2005).[22]

The MSSA isolates were susceptible to most of the antibiotics tested, although high degree of resistance was observed with penicillin (84.1%), co-trimoxazole (57.8%) and ciprofloxacin (32.6%) the antibiotics often used to treat general infections. The present study indicated a relatively high rate of susceptibility among clinical isolates of MSSA to tetracycline, aminoglycosides and macrolides tested.

CONCLUSION-

Staphylococcus aureus was the most common agent causing pyodermas followed by Gram negative isolates. S.aureuswas resistant to all antibiotics except for vancomycin and linezolid. Gram negative bacteria were also found resistant to imipenem.

CONFLICT OF INTEREST- none

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