



**CLINICO-BACTERIOLOGICAL STUDIES ON PYODERMA IN GULBARGA
REGION (KARNATAKA STATE) EMPHASES TO METHICILLIN
RESISTANT *STAPHYLOCOCCUS AUREUS*****VIVEK KULKARNI, Y.M.JAYARAJ*, C.T. SHIVANNAVAR,
SAGAR M. ARAI AND RAVI M***Department of PG Studies and Research in Microbiology Gulbarga University,
Gulbarga- 585106, Karnataka, India***ABSTRACT**

Pyoderma is one of the commonest conditions encountered in dermatological practices. Emergence and spread of multidrug resistant pathogens are posing a great challenge. The present study was undertaken to investigate the common causative agents and their antibiotic susceptibility pattern in pyoderma. 173 pus samples were collected from the out patients of Department of Dermatology, Government hospital, Gulbarga, Karnataka (South India). *Staphylococcus aureus* was the main aetiological agent of pyoderma in 95 cases and was a sole aetiological agent in 53 cases (30.64%), while in association with *Klebsiella* it was in 42 cases (24.28%). Species of Coagulase negative *Staphylococci*, *Streptococci*, *Klebsiella* and *Pseudomonas* were isolated in 12.42%, 7.84%, 5.88% and 3.27% of the cases respectively. Among the 95 strains of *Staphylococcus aureus* isolated, 37 (38.95%) were Methicillin resistant (MRSA) and 13 (13.68%) were Vancomycin resistant (VRSA). Thus emergence of drug resistance is becoming a threat and needs monitoring.

KEYWORDS: Pyoderma, antibiotic resistance, *Staphylococcus aureus*, *Klebsiella***Y.M.JAYARAJ**Department of PG Studies and Research in Microbiology Gulbarga University,
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INTRODUCTION

Pyoderma is quite common in India and constitutes a major portion of patients in dermatological clinics. The major pyodermal infections include Impetigo, Folliculitis, Ecthyma, Furuncle, Paronychia, Carbuncle and many others^{1,2,5,6,16}. Majority of the reports published before 2000 have listed *Staphylococcus aureus* and *Streptococcus pyogenes* as aetiological agents of pyoderma^{1,6,9}. A few reports highlighted the importance of β -haemolytic *Streptococci* in pyoderma⁷. The aetiological microbes in complicated infections are predominantly *S. aureus* and *Streptococci*, but often involve mixed Gram-positive and Gram-negative aerobic and anaerobic bacteria as well⁸. The Gram negative bacteria include *Klebsiella*, *Pseudomonas*, *Escherichia* and other coli forms^{6,16}. Emergence of multidrug resistant strains is a threat in medical practices. *Staphylococcus aureus*, the principal skin pathogen that has become resistant to choice of drugs like Methicillin¹² and Vancomycin. Methicillin resistance is quite frequent and at time exceeds 50% in tertiary care centres, however Vancomycin resistance has been comparatively low. In India the indiscriminate use of the antibiotic may alter the scenario. Therefore, regular survey for the incidence of *Staphylococcal* aetiology and their multidrug resistance pattern is necessary². Hence, in present investigation, 173 samples of pyoderma were selected on random basis; among the patients attending the outpatient department, belonging to all age groups and both sex.

MATERIALS AND METHODS

The samples were collected from the out patients attending the Department of Dermatology, Government hospital, Gulbarga, Karnataka (South India). The pus samples from pyoderma lesions were collected by using sterile swabs. The collected samples were transported to the laboratory under aseptic conditions using Nutrient and BHI broth. The swabs were inoculated on Nutrient Agar, Mannitol Salt Agar, McConkey Agar and Blood Agar and were incubated aerobically at 37⁰ C for 24 hours. All the isolates were identified based on conventional diagnostic procedure (cultural, morphological and biochemical characters)¹³. In all, 20 different antibiotics were used to study the antibiotic sensitivity test using standard Kirby-Bauer disc diffusion method (2006 CLSI guidelines). The antibiotics and their concentrations used in the study are as follows: Penicillin-G(10 units), Ampicillin(10 μ g), Amikacin(30 μ g), Clindamycin(2 μ g), Erythromycin(15 μ g), Tetracycline(30 μ g), Cefotaxime(30 μ g), Ceftazidime(30 μ g), Ceftriaxone(30 μ g), Cefepime(30 μ g), Methicillin(5 μ g), Vancomycin(30 μ g), Linezolid(30 μ g), Co-Trimoxazole (Trimethoprim/Sulphamethoxazole) (1.25/23.75 μ g) Amoxyclav (Amoxycillin/Clavulonic acid) (20/10 μ g), Imipenem(10 μ g), Gentamicin(10 μ g), Ciprofloxacin(5 μ g), Carbanicillin(100 μ g) and Nalidixic acid(30 μ g).

RESULTS

Table 1

Age and sex wise and economic status wise distribution of the 173 cases of Pyoderma.

Age and sex wise Distribution			
Age groups in years	No of cases	Male	Female
1-10	113	72	41
11-20	17	06	11
21-30	13	05	08
31-40	03	01	02
41-60	27	17	10
Total	173	101	72
Distribution Economic Status group			
Lower group	119	76	43
Middle group	54	25	29
Upper group	00	00	00
Total	173	101	72

Table 2

Types and Bacteriological aspects in type of the 173 cases of Pyoderma.

Sl. No.	Type of pyoderma	Number of cases (+culture/-culture)	Staphylococcus aureus		Coagulase negative Staphylococci		Streptococcus species	Klebsiella species	Pseudomonas species
			Single aetiology	Mixed with Klebsiella species	Single aetiology	Mixed with Klebsiella species			
1	Impetigo	64 (57/7)	38	4	6	1	4	3	1
2	Folliculitis	43 (38/5)	5	21	5	4	-	3	-
3	Ecthyma	19 (17/2)	-	3	4	2	6	-	2
4	Furuncle	13 (12/1)	2	6	-	2	-	2	-
5	Paronychia	19 (14/5)	6	4	1	-	2	-	1
6	Carbuncle	15 (15/0)	2	4	3	4	-	1	1
Total		173 (153/20)	53	42	19	13	12	9	5

Out of 173 cases, 113 cases were represented by children. The eldest was 52 years old while youngest patient was 2 year old. The number of male cases 101(58.38%) is higher than that of female cases 72(41.62%) among 173 pyoderma cases (Table 1). The maximum samples were from lower socio-economic status 119(68.78%) and few were from middle economy group 54(31.22%). The data on the types of pyoderma and bacterial isolates is presented in Table 2. Out of 173 cases of the pyoderma, 64 cases (37%) were Impetigo cases followed by Folliculitis 43(24.86%), Ecthyma 19(11%),

Paronychia 19(11%), Carbuncle 15(8.7%) and least in Furuncle 13(7.51%). Out of 173 study samples, bacteria were isolated from 153(88.44%) cases while 20 samples showed no growth. It is interesting to note that *Staphylococcus aureus* emerges as the main causative agent in all types of pyodermas, except Ecthyma. In 19 Ecthyma type of pyoderma cases yielded not a single case for *Staphylococcus aureus* as a single aetiology, however 6 cases yielded for *Streptococcus sps* as a single aetiology. The details of bacterial isolates in 153 cases are shown in Table 3.

Table 3
Bacterial isolates in 153 pyoderma cases.

Sl.No	Type of Bacteria isolated	Number of Bacteria isolated	Percentage
1	<i>Staphylococcus aureus</i>	53	34.64
2	Coagulase negative <i>Staphylococci</i>	19	12.42
3	<i>Streptococcus sps</i>	12	7.84
4	<i>Klebsiella sps</i>	9	5.88
5	<i>Pseudomonas sps</i>	5	3.27
6	<i>Staphylococcus aureus</i> with <i>Klebsiella sps</i>	42	27.45
7	Coagulase negative <i>Staphylococcus sps</i> with <i>Klebsiella sps</i>	13	8.5
Total		153	100

Note: Total *Staphylococcus aureus* - 95 (53+42)
Coagulase negative *Staphylococci* - 32 (19+13)
Klebsiella species - 64 (9+13+42)

Staphylococcus aureus was isolated as a single aetiological agent in 53 cases (34.64%), while in association with *Klebsiella* it was in 42 cases (27.45%). So, prevalence rate of *Staphylococcus aureus* from present study was 62.1% (95 cases). Coagulase negative *Staphylococci* were isolated in 19 (12.42%) of the cases, while in association with *Klebsiella* it was in 13 cases (8.5%). *Klebsiella* is isolated single aetiological agent in 9 cases (5.88%). So, the prevalence rate of *Klebsiella species* from present study was 64 (41.83%). *Streptococci* were isolated in 12 cases (7.84%) while *Pseudomonas* was isolated in 5 cases (3.27%). The distribution of types of

pyoderma on the human body and associated pathogen are given in Table 4. Maximum number of Impetigo cases (53) were taken from face and neck region. In cases of Folliculitis, Ecthyma, Furuncle, Paronychia and Carbuncle maximum numbers of samples were taken from the region of scalp, leg, hand, finger and face region respectively. Among the *Staphylococcus aureus* isolated from pyoderma cases, majority were isolated from the lesions of the upper parts of the body. Lower part of the body showed less isolates of *Staphylococcus aureus* but mixed aetiology was found.

Table 4
Body region wise distribution of 153 infected skin lesions (pyoderma) and bacteria isolated

Sl. No.	Type of pyoderma (No of samples)*	Number of Positive culture	Site of infection	No. of cases
1	Impetigo (64)	57	Face and Neck	38
			Hand, palm and fingers	12
			Trunk	02
			Leg and toes	04
			Abdomen	01
2	Folliculitis (43)	38	Face and Neck	02
			Hand, palm and fingers	01
			Leg and toes	02
			Scalp	33
3	Ecthyma (19)	17	Leg and toes	14
			Abdomen	01
			Buttock	02
4	Furuncle (13)	12	Face and Neck	03
			Hand, palm and fingers	06
			Leg and toes	03
5	Paronychia (19)	14	Hand, palm and fingers	11
			Leg and toes	03
6	Carbuncle (15)	15	Face and Neck	12
			Leg and toes	02
			Abdomen	01
Total		153		153

Note: * - one sample from one patient.

The Gram negative bacteria as single aetiology were mainly isolated from the lesions of lower parts of the body. In contrast *Klebsiella sps* as a mixed aetiology isolated from pyodermal lesions from neck, hand and scalp. The emphasis of the study was on aetiology and the strains of antibiotic susceptibility of Methicillin and Vancomycin resistant *Staphylococcus aureus*. A comparative evaluation of the antibiotic resistant pattern of all isolates to different antibiotics is given in Table 5. The results are evaluated in the discussion. The co-resistance pattern of MRSA and MSSA are given in

Table 6. It is evident that majority of MRSA exhibited resistance to other conventional antibiotics. Out of 95 isolates of *Staphylococcus aureus*, 37(38.95%) and 13(13.68%) were resistant to Methicillin and Vancomycin respectively. Among 32 isolates of coagulase negative *Staphylococci* only 5(15.63%) were resistant to Methicillin. But, they all were sensitive to Vancomycin, Linezolid and Amikacin. Interestingly rate of Vancomycin resistance in both MRSA and MSSA were found to be the same (13.51%), However, it is slightly higher in MSSA isolates (13.79%).

Table 5
Antibiotic resistant pattern of isolated pathogens

Sl.No	Antibiotic	Disc strength (in µg)	<i>Staphylococcus aureus</i> 95(%)	Coagulase negative <i>Staphylococci</i> . 32(%)	<i>Klebsiella species</i> 64(%)	<i>Pseudomonas species</i> . 5(%)
1	Penicillin-G	10 units	86(90.53)	24(75)	NT	NT
2	Ampicillin	10	70(73.68)	20(62.5)	39(60.94)	5(100)
3	Amikacin	30	14(14.74)	0	8(12.50)	1(20)
4	Clindamycin	2	29(30.53)	7(21.88)	NT	NT
5	Erythromycin	15	63(66.32)	13(40.63)	NT	NT
6	Tetracycline	30	17(17.89)	6(18.75)	29(45.31)	2(40)
7	Cefotaxime	30	68(71.58)	22(68.75)	39(60.94)	4(80)
8	Ceftazidime	30	68(71.58)	20(62.5)	37(57.81)	4(80)
9	Ceftriaxone	30	70(73.68)	21(65.63)	41(64.06)	4(80)
10	Cefepime	30	45(47.37)	18(56.25)	31(48.44)	3(60)
11	Vancomycin	30	13(13.68)	0	NT	NT
12	Linezolid	30	0	0	NT	NT
13	Co-Trimoxazole	1.25/23.75	28(29.47)	10(31.25)	NT	NT
14	Amoxyclav (Amoxycillin/Clavulonic acid)	20/10	NT	NT	26(40.62)	3(60)
15	Imipenem	10	NT	NT	4(6.25)	0
16	Gentamicin	10	NT	NT	41(64.06)	3(60)
17	Ciprofloxacin	5	35(36.84)	10(31.25)	33(51.56)	3(60)
18	Carbanicillin	100	NT	NT	12(18.75)	1(20)
19	Nalidixic acid	30	NT	NT	31(48.44)	2(40)
20	Methicillin	5	37(38.94)	5(15.62)	NT	NT

Note: NT- Not Tested

Table 6
Antibiotic resistant pattern of Methicillin resistant and sensitive staphylococci

Sl.No	Antibiotic	Disc strength (in µg)	Staphylococcus aureus (95)			Coagulase negative Staphylococci. (32)		
			MRSA (37)	MSSA (58)	Total (95)	MR (5)	MS (27)	Total (32)
1	Penicillin-G	10 units	100%	84.48%	90.53%	100%	70.37%	75%
2	Ampicillin	10	78.38%	70.69%	73.68%	80%	59.25%	62.5%
3	Amikacin	30	21.62%	10.34%	14.74%	0	0	0
4	Clindamycin	2	35.14%	27.59%	30.53%	20%	22.22%	21.88%
5	Erythromycin	15	64.87%	67.24%	66.32%	40%	40.74%	40.63%
6	Tetracycline	30	24.32%	13.79%	17.89%	20%	18.52%	18.75%
7	Cefotaxime	30	78.38%	67.24%	71.58%	80%	66.67%	68.75%
8	Ceftazidime	30	72.97%	70.69%	71.58%	60%	62.96%	62.5%
9	Ceftriaxone	30	75.68%	72.41%	73.68%	60%	66.67%	65.63%
10	Cefepime	30	56.76%	41.38%	47.37%	40%	59.26%	56.25%
11	Vancomycin	30	13.51%	13.79%	13.68%	0	0	0
12	Linezolid	30	0	0	0	0	0	0
13	Co-Trimoxazole	1.25/23.75	32.43%	27.58%	29.47%	40%	29.63%	31.25%
14	Amoxyclav (Amoxicillin/Clavulonic acid)	20/10	NT	NT	NT	NT	NT	NT
15	Imipenem	10	NT	NT	NT	NT	NT	NT
16	Gentamycin	10	NT	NT	NT	NT	NT	NT
17	Ciprofloxacin	5	37.84%	36.21%	36.42%	40%	29.63%	31.25%
18	Carbanicillin	100	NT	NT	NT	NT	NT	NT
19	Nalidixic acid	30	NT	NT	NT	NT	NT	NT

* NOTE: NT- Not tested, MRSA- Methicillin resistant Staphylococcus aureus, MSSA- Methicillin sensitive Staphylococcus aureus

DISCUSSION

As usual in this study also, cases of Impetigo (64) were more in number compared to other types of pyoderma cases^{1,6,16}. On other hand Rahul *et al* (2005) showed Folliculitis and Furuncles were the commonest primary pyodermas¹². In present study, the incidence of pyodermas in children below 10 years of age group (113) were higher than other age groups and is in correlation with the findings of earlier studies^{1,9,11}. Many of the earlier studies^{1,5,6,16} and our study revealed the incidence of pyoderma is more in males (101) than females (72) and it is in contrast with the report made by Ramani and Jayakar (1980)¹⁵. Majority of pyodermal cases belongs to lower socio-economic group. This is probably because more number of patients with lower income group visits to the OPD of Government hospital and this area is considered as socio-economically backward. The present study also shows the dominance of *Staphylococcus aureus* aetiology of pyoderma and is concordant to earlier studies too. On the contrary our study also support the decrease trend in *Streptococcal* aetiology of pyoderma reported^{12,16} in contrast to reports

before 2000^{1,6,9}. The Gram –ve aetiology of pyoderma revealed that *Klebsiella* is predominant one and majority *Klebsiella* were isolated from co-infection with *Staphylococcus aureus* (42) compared with prime single infection (9). But, earlier reports have reported less isolation rate of *Klebsiella*^{6,16}. This indicates the emergence of *Klebsiella* as an important co-pathogen in pyodermas. The present study also isolated coagulase negative *Staphylococci* (19), *Pseudomonas* (5) as a sole aetiological agent.

The aetiological agents of pyoderma and their antibiogram of the present are compared with the observations of earlier studies (Table 7). It was observed that 38.95% of isolates of *Staphylococcus aureus* were resistant to Methicillin (MRSA) and 13.68% of isolates to Vancomycin (VRSA). All the Methicillin resistant *Staphylococcus aureus* (MRSA) were also resistant to Penicillin (100%), while majority of them were resistant to other antibiotics in usage [Ampicillin-78.38%; Cefotaxime-78.38%; Ceftriaxone-75.68%; Ceftazidime-72.97% and Erythromycin-64.87%]. In contrast, Malhotra *et*

al (2010), recorded higher susceptibility rate of *S.aureus* to Gentamycin (66%) and Erythromycin (61.9%)¹⁶. Methicillin sensitive *Staphylococcus aureus* (MSSA) also showed a similar type of resistance pattern to other known antibiotics in usage. Even the coagulase negative *Staphylococci* exhibited significant resistance to antibiotics in usage including methicillin 5(15.63%), but all were

susceptible to Vancomycin, Linezolid and Amikacin. Isolates of *Klebsiella*, being the predominant co-pathogen, exhibited varying degree of resistance to antibiotics [Gentamycin-64.06%; Ceftriaxone-64.06%; Ampicillin-60.94%; Cefotaxime-60.94% and Ceftazidime-57.81%]. Previous workers also observed a similar phenomenon^{6,16}.

Table 7
Isolation of different bacteria and their antibiotic sensitivity and resistant pattern in various studies

Various Studies	RG Baslas et al. (1990)		Mathews MS et al. (1992)		Ghadage et al. (1999)		Rahul et al. (2005)		K.Malhotra et al. (2010)		Present study	
Bacteria isolated	<i>S. aureus</i> <i>β- haemolytic streptococci</i>		CPS, CNS, <i>Streptococcus pyogenes</i>		CPS, CNS, BHS, NHS, <i>Klebsiella</i> sps, <i>E.coli</i> , <i>Pseudomonas</i> sps, <i>Acetenobacter</i> sps, <i>citrobacter asps, proteus.</i>		<i>S. aureus</i> <i>s. pyogenes</i>		CPS, CNS, <i>Klebsiella</i> sps, <i>E.coli</i> , <i>Pseudomonas</i> sps, <i>citrobacter asps, proteus</i> and <i>Enterobacter</i> sps		<i>S. aureus</i> , CNS <i>Klebsiella</i> sps, <i>Pseudomonas</i> sps <i>Streptococcus</i> sps	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Antibiotic Sensitivity/ Resistant pattern of <i>Staphylococcus aureus</i>	CR-88% GEN-81.2% CN-67.3% E-59.6%	AMP- 72.6% P-71.8% C-52.3%	C-96.7% S-89.1%	P-79.3% AMP- 79.3% TE-42.4%	AK - 75% C - 62% CD- 61% CIP-61%	GEN-88% P - 79% NX- 61%	V -100% MET- 69% GEN- 69% CIP-58%	P-87.2% E-42.9% CIP- 17.2%	AK-100% GEN-66% E-61.9% CIP-52.3%	GF-57.2% CF-47.7%	LZ-100% V-86.32% AK-85.26% COT-70.53% CD-69.47%	P-90.53% CTR-73.68% AMP-73.68%
Antibiotic Sensitivity/ Resistant pattern of Coagulase negative <i>Staphylococci</i>			GEN-80% E-80% C-80%	P-100% TE-80% S-60%	AK-91% CIP-65% CD-65% C-61%	P-92% TE-55% S-59% GEN-44% E-42%			AK-77.7% GEN-66.6% E-55.6% AMP-44.5%	GF-66.7% E-55.6% AMP-44.5%	LZ-100% V-100% AK-100% TE-81.25% CD-78.12%	P-75% CTX-66.67% CTR-65.63% AMP-62.5%
Antibiotic Sensitivity/ Resistant pattern of other isolates	<i>β- haemolytic streptococci</i> CR-97.4% GEN-80.1% CN-68.3% E-57.6%	P-86.8% AMP-84% S-56.2% C-45.4%	<i>Streptococcus pyogenes</i> E-98.1% AMP-82.6% 90.7%	S-14.8% TE-14.8%	<i>Klebsiella</i> sps AK-77% CIP-58% S-40% C-35% <i>E.coli</i> AK-83% GEN-38% TE-33% <i>Pseudomonas</i> sps AK-72% CIP-42% GEN-34%	AMP-90% AMX-84% GEN-71% TE-71% AMP-90% AMX-83% CIP-71% AMP-94% C-85% TE-85%			<i>Klebsiella</i> sps AK-75% GEN-50% CIP-50%	GEN-75% CN-75% CF-50%	<i>Klebsiella</i> sps IPM-93.75% AK-87.5% CB-81.25% <i>Pseudomonas</i> sps AK-80% CB-80% TE-60%	GEN-64.06% CTR-64.06% AMP-60.94% AMP-100% CTX-80% CTR-80% CAZ-80%

*NOTE: CPS- Coagulase positive staphylococci, CNS- Coagulase negative staphylococci, BHS- *β- haemolytic streptococci*, NHS-non haemolytic streptococci. AK-Amikacin, AMP-Ampicillin, AMX-Amoxicillin, C-Chloramphenicol, CAZ-Ceftazidime, CB-Carbenicillin, CD-Clindamycin, CIP-Ciprofloxacin, CN-Cefalexin, COT-Co-Trimoxazole, CR-Cefaloridine, CTR-Ceftriaxone, CTX-Cefotaxime, E-Erythromycin, GEN-Gentamycin, GF-Gatifloxacin, IPM-Imipenem, LZ- Linezolid, MET-Methicillin, NX-Norfloxacin, P-Penicillin G, S- Streptomycin, TE-Tetracycline, V-Vancomycin .

CONCLUSION

The present study records the incidence of different types of pyoderma, the dominant being "Impetigo". *Staphylococcus aureus* continues to be the predominant pathogen. However, emergence of *Klebsiella* as a co-pathogen along with *Staphylococcus aureus* is alarming in pyoderma infections. The study emphasizes the dominance of *S aureus*, along with coagulase negative *Staphylococci* and

Klebsiella as a pathogen/ co-pathogen in skin infections. Majority strains of *Staphylococcus aureus* and other isolates were resistant to more than one antibiotic. However coagulase negative *Staphylococcus* were susceptible to Vancomycin, Linezolid and Amikacin. Hence there is a need for periodic studies on antibiogram and an antibiotic monitoring policy.

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