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Bacteriological study of pyodermas with an emphasis on Methicillin Resistant Staphylococcus Aureus (MRSA) at a tertiary care centre in North Karnataka

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Abstract
Background: Pyoderma is quite common skin infection and constitutes a major portion of patients in dermatological clinics. Many cases nowadays do not respond to the antibiotics that were previously sensitive. In order to successfully treat cases of pyodermas, sound knowledge is necessary regarding the etiology and their sensitivity patterns.
Aims: To study the causative organisms and their antimicrobial susceptibility pattern and prevalence of methicillin resistant Staphylococcus aureus (MRSA) among the patients.
Materials and Methods: A total of 500 patients with pyoderma who attended dermatology outpatient Department at VIMS hospital during the study period January 2017 to December 2017.
Statistical Analysis: Data was analyzed by SPSS Version 20.0 software.
Results: Staphylococcus aureus was isolated from 260 (52%) samples followed by coagulase negative Staphylococci in 105 (21%) samples. MRSA was reported in 42 (16.2%) cases.
Conclusion: This study gives an indication of the present pattern of bacteriological profile of Pyodermas in our tertiary care centre**.**

Keywords: Pyoderma, staphylococcus aureus, antibiotic susceptibility, methicillin resistant staphylococcus aureus

Introduction
Pyoderma or pyogenic infection of the skin, defined as 'any purulent skin disease', is one of the commonest conditions encountered in dermatological practice. [1] Majority of the pyodermas are caused by Staphylococcus aureus and Group A streptococci. The clinical spectrum is forever changing and there is increasing resistance to antibiotics. This is posing a big problem to the clinicians in the management [1].
Emergence and dissemination of Methicillin resistant Staphylococcus aureus (MRSA) is a global concern. MRSA is reported not only in those having risk factors but also in healthy individuals. MRSA are becoming multi-drug resistant. All beta-lactams including carbapenems, high-end cephalosporins, piperacillin, tazobactam etc are ineffective against MRSA. [3] The condition is known for its chronicity, recurrence, complications, and side effects. Therefore, timely recognition and correct bacterial diagnosis with antimicrobial susceptibility is crucial for the effective management and treatment of pyoderma. [4]
Patterns of isolates and antibiotic sensitivity may also vary regionally. Knowledge of prevalence of MRSA and their current anti-microbial profile becomes necessary in the selection of appropriate treatment of these infections. We could thereby reduce the economic burden of the treatment and sickness absentism.

Materials and Methods
Two swabs were taken from a representative lesion. Intact pustules were first cleaned with 70% alcohol. They were then ruptured with a sterile needle and the expressed pus was collected on two sterile cotton swabs.
In case of ulcers and crusted lesions, normal saline was used to clean the wound, while the surrounding normal skin was cleaned with 70% alcohol. Two sterile swabs were rubbed over the expressed pus or the advancing edge of the ulcer.

Smears were prepared using pus from the first swab and were stained by Gram's method. They were examined for the type and number of bacteria. The pus from the second swab was inoculated on blood agar and MacConkey's agar.
All isolates were tested for their antibiotic susceptibility by Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute guidelines. Methicillin resistance was detected using Cefoxitin discs. Those having a zone of inhibition </=21 mm for Cefoxitin were considered resistant to Methicillin (MRSA) and those having zone of inhibition >/=22 mm for Cefoxitin were considered sensitive to Methicillin. Pustules associated with acne, sterile pustules and patients with a history of using topical or systemic antibiotics in the past 2 weeks were excluded.

Statistical Analysis
Data was analyzed by SPSS Version 20.0 software. The data is represented in the form of frequency and percentages using tables and graphs.

Results
Primary pyoderma was commoner with 276 cases(55.4%) and secondary pyoderma accounted for 224 cases(44.6%). The ratio of primary to secondary pyoderma in this study was 1.24:1 (Table no.1).
Staphylococcus aureus was the most common organism isolated in 260 (52%) patients followed by coagulase negative staphylococci in 105 (21%) cases. Other organisms isolated include Streptococcus (9.2%), Citrobacter (7.4%), Klebsiella (6.4%), Pseudomonas (5.2%), Enterobacter (2.8%), Acinetobacter (6%), Proteus (0.8%), E.coli (0.6%), Serratia and Non-fermenting GNB in 0.4% cases. Multiple organisms were isolated in 37 cases (7.4%) (Table no.2).
Among 260 Staphylococcus aureus isolates, 218 (83.8%) were methicillin- sensitive and 42 (16.2%) were methicillin-resistant. Of 42 cases of methicillin-resistant**,** staphylococcus aureus, 38 (14.6%) were Community-acquired and 4 (1.5%) were Hospital-acquired. (Table no.3).
In our study, most of the Staphylococcus aureus strains were sensitive to Doxycycline (81.5%), followed by Tetracycline (75.4%), Erythromycin (62.7%), Gentamicin (48.8%) Co-trimoxazole (36.5%), Amikacin (32.7%).
Coagulase negative staphylococci were susceptible to Doxycycline (67.6%), Tetracycline (65.7%), Erythromycin (52.4%), Gentamicin (45.7%).
Streptococci were susceptible to Cefotaxim (63%), Tetracycline (65.7%), Gentamicin (56.6%). Among the gram negative organisms Klebsiella was susceptible mostly to Gentamicin (73%) followed by Amikacin (56.3%). Pseudomonas was susceptible to Gentamicin (65.4%) followed by Amikacin (53.8%) and Tetracycline (53.8%) (Table no.4). In our study, Staph. Aureus was found to be most resistant to Amoxyclav (56.9%) followed by Co-trimoxazole (50.8%) and Ciprofloxacin (41.5%). Most of the isolates of CONS were equally resistant to Co-trimoxazole (46.7%) and Erythromycin (46.7%) followed by Amoxyclav (42.9%). Streptococci were resistant to Amoxyclav (41.3%) and Co-trimoxazole (26.1%).
Among Gram negative organisms, Klebsiella was resistant to Amoxyclav (65.6%) followed by Ceftriaxone (43.8%) and Ciprofloxacin (34.4%). Pseudomonas was found to be resistant to Amoxyclav (73.1%) followed by Cefpodoxime Axetil (38.5%) and Ceftriaxone (34.6%) (Table no. 5).

Table 1: Distribution of the patients based on type of pyoderma

Type	Frequency	Percentage
Primary	276	55.4
Secondary	224	44.6
Total	500	100

Table 2: Organisms isolated among the patients

Organisms	Frequency	Percentage
Acinetobacter	6	1.2
Citrobacter	37	7.4
CONS	105	21
E.coli	3	0.6
Enterobacter	14	2.8
Klebsiella	32	6.4
Proteus	4	0.8
Pseudomonas	26	5.2
S.aureus	260	52
Serratia	2	0.4
Streptococcus	46	9.2
Non-fermenting GNB	2	0.4

Table 3: Distribution of patients with staph. Aureus based on Methicillin sensitivity (n=260)

Staph Aureus	Frequency	Percentage
MRSA	42	16.2
Community Acquired	38	14.6
Hospital Acquired	4	1.5
MSSA	218	83.8

Table 4: Antibiotic sensitivity of different isolates

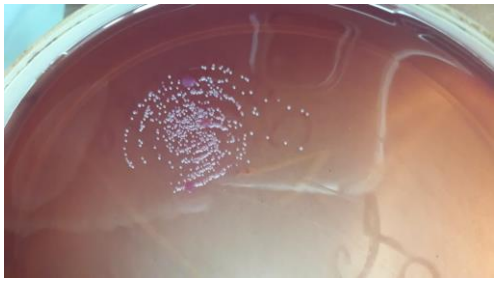
Antibiotic sensitivity of the common organisms of pyoderma												
	Staph. Aureus n=260	Streptococcus n=46	CONS n=105	Citrobacter n=37	Klebsiella n=32	Pseudomonas n=26	Enterobacter n=14	Proteus n=4	Acinobacter n=6	E Coli n=3	Serrratia n=2	NFGMB n=2
AC	18 (6.9)	20 (43.5)	29 (27.6)	5 (13.5)	6 (18.8)	2 (7.7)	0 (0.0)	3 (75.0)	1 (16.7)	1 (33.3)	0 (0.0)	0 (0.0)
AMP	4 (1.5)	4 (8.7)	1 (1.0)	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AZM	5 (1.9)	-	1 (1.0)	2 (5.4)	1 (3.1)	-	-	-	-	-	-	-
AK	85 (32.7)	22 (47.8)	31 (29.5)	22 (59.5)	18 (56.3)	14 (53.8)	9 (64.3)	2 (50.0)	1 (16.7)	3 (100)	1 (50.0)	1 (50.0)
E	163 (62.7)	12 (26.1)	55 (52.4)	11 (29.7)	8 (25.0)	2 (7.7)	5 (35.7)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
G	127 (48.8)	26 (56.6)	48 (45.7)	27 (73.0)	20 (62.5)	17 (65.4)	11 (78.6)	3 (75.0)	2 (33.3)	2 (66.6)	2 (100)	2 (100)
CF	50 (19.2)	8 (17.4)	40 (38.1)	17 (45.9)	15 (46.9)	12 (46.2)	8 (57.1)	3 (75.0)	2 (33.3)	0 (0.0)	1 (50.0)	0 (0.0)
LE	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
OF	1 (0.4)	2 (4.3)	0 (0.0)	2 (5.4)	7 (21.9)	3 (11.5)	3 (21.4)	3 (75.0)	1 (16.7)	1 (33.3)	0 (0.0)	0 (0.0)
CTX	42 (16.2)	29 (63.0)	15 (14.3)	13 (35.1)	10 (31.3)	9 (34.6)	9 (64.3)	2 (50.0)	0 (0.0)	2 (66.6)	1 (50.0)	0 (0.0)
CAZ	9 (3.5)	8 (17.4)	10 (9.5)	10 (27.0)	3 (9.4)	8 (30.8)	6 (42.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)
CTR	34 (13.1)	18 (39.1)	13 (12.4)	16 (43.2)	8 (25.0)	8 (30.8)	6 (42.9)	2 (50.0)	1 (16.7)	0 (0.0)	0 (0.0)	1 (50.0)
CEP	41 (15.8)	14 (30.4)	10 (9.5)	11 (29.7)	6 (18.8)	1 (3.8)	4 (28.6)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (50.0)
CPZ	20 (7.7)	23 (50.0)	10 (9.5)	14 (37.8)	7 (21.9)	12 (46.2)	8 (57.1)	3 (75.0)	0 (0.0)	2 (66.6)	2 (100)	1 (50.0)
DO	212 (81.5)	5 (10.9)	71 (67.6)	4 (10.8)	2 (6.3)	2 (7.7)	0 (0.0)	0 (0.0)	1 (16.7)	1 (33.3)	0 (0.0)	2 (100)
T	196 (75.4)	27 (58.7)	69 (65.7)	17 (45.9)	12 (37.5)	14 (53.8)	9 (64.3)	1 (25.0)	0 (0.0)	2 (66.6)	0 (0.0)	0 (0.0)
CO	95 (36.5)	-	35 (33.3)	1 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
LZ	2 (0.8)	-	4 (3.8)	-	-	-	-	-	-	-	-	-
PIT	0 (0.0)	-	0 (0.0)	1 (2.7)	-	-	-	-	-	-	-	-
IPM	2 (0.8)	1 (2.2)	4 (3.8)	3 (8.1)	4 (12.5)	0 (0.0)	1 (7.1)	1 (25.0)	-	-	-	-
PF	1 (0.4)	-	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.7)	-	-	-	-	-	-

Table 5: Antibiotic Resistance of Different Isolates

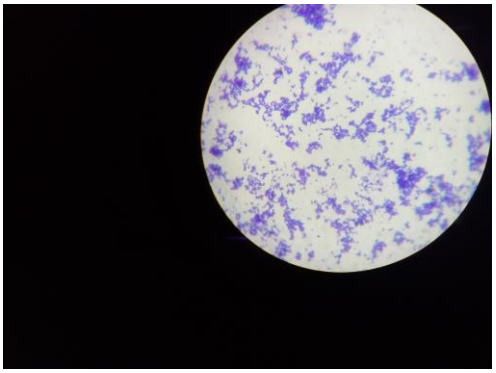
	S.aureus (260)	CONS (105)	Strept (46)	Citro (37)	Klebsiella (32)	Pseudo (26)
AC	148 (56.9)	45 (42.9)	19 (41.3)	26 (70.2)	21 (65.6)	19 (73.1)
Amp	55 (21.2)	8 (7.6)	1 (2.18)	7 (18.9)	4 (12.5)	4 (15.4)
AK	7 (2.6)	4 (3.8)	9 (1.9)	3 (8.1)	5 (15.6)	4 (15.4)
E	88 (33.8)	49 (46.7)	3 (6.5)	NT	NT	NT
G	35 (13.4)	25 (23.8)	7 (6.7)	7 (18.9)	8 (25)	2 (7.6)
CF	108 (41.5)	24 (22.9)	4 (3.8)	13 (35.1)	11 (34.4)	7 (26.3)
CTX	21 (8.1)	11 (10.5)	4 (3.8)	9 (24.3)	14 (43.8)	9 (34.6)
CAZ	19 (7.3)	4 (3.8)	1 (2.18)	7 (18.9)	4 (12.5)	3 (11.5)
CTR	11 (4.2)	11 (10.5)	3 (6.5)	9 (24.3)	10 (31.2)	5 (19.2)
CEP	18 (6.9)	7 (6.7)	NT	7 (18.9)	2 (6.2)	10 (38.5)
CPZ	14 (5.3)	2 (1.9)	NT	NT	9 (28.1)	3 (11.5)
DO	16 (6.1)	18 (17.1)	NT	9 (24.3)	6 (18.7)	5 (19.2)
T	34 (13.1)	30 (28.5)	2 (4.3)	3 (8.1)	3 (9.3)	1 (3.8)
CO	132 (50.8)	49 (46.7)	12 (26.1)	8 (7.6)	6 (1.8)	4 (15.4)

White opaque colonies showing staph. aureus on chocolate agar

Lactose-fermenting mucoid colonies showing klebsiella on MacConkey's agar



Lactose-fermenting opaque colonies showing staph aureus on MacConkey's agar



Gram positive cocci arranged in clusters - staph aureus

Discussion

- In our study, Staph aureus was the most common organism isolated (52%) followed by CONS (21%), streptococcus (9.2%), citrobacter (7.4%), klebsiella (6.4%) and pseudomonas (5.2%).
  Singh *et al* reported as staph aureus being the most common isolate (78.26%) followed by E.coli (4.79%), streptococci (2.17%) and CONS (1.74%). [1]
  Gandhi *et al* found staph aureus in 77.5% of isolates, beta-hemolytic streptococci in 3% isolates and klebsiella in 5% of the isolates. [5]
  Venniyil *et al* reported staph aureus (80.33%) as the most common found isolate. [3]
  Ghadage *et al* found staph aureus (67.34%) as the predominant species isolated followed by beta-hemolytic streptococcus (21.77%). [6]
  The present study is similar to the above studies with staph aureus being the most common organism isolated.
- In our study, out of 260 staph aureus isolates, 218 (83.8%) were methicillin sensitive and 42(16.2%) were methicillin resistant. Among 42 cases of MRSA, 38 (14.6%) were community-acquired and 4 (1.5%) were hospital-acquired.
  Venniyil *et al* reported 192 (80.33%) cases of staph aureus isolates, among which MSSA were 150 (78.12%) and MRSA were 42 (21.98%). Among MRSA, 33(78.57%) were community-acquired and 9 (21.41%) were hospital-acquired. [3]
  Malhotra *et al* reported staph aureus in 42.62% of cases. Among which, MSSA were 34.4% and MRSA were 8.2%. [2]
  Gandhi *et al* reported staph aureus in 155 (77.5%) isolates, among which 124 (80%) were MSSA and 31 (20%) were MRSA. [5]
  Majority of these studies show MSSA to be more commoner than MRSA and CA-MRSA to be more commoner than HA-MRSA, similar to our study.
- In our study, most of the Staphylococcus aureus strains were sensitive to Doxycycline (81.5%), followed by Tetracycline (75.4%), Erythromycin (62.7%), Gentamicin (48.8%), Co-trimoxazole (36.5%), Amikacin (32.7%) and most of them were resistant to Amoxyclav (56.9%) followed by Co-trimoxazole (50.8%) and Ciprofloxacin (41.5%).
  Coagulase negative staphylococci were susceptible to Doxycycline (67.6%), Tetracycline (65.7%), Erythromycin (52.4%), Gentamicin (45.7%) and resistant to Co-trimoxazole (46.7%), Erythromycin (46.7%) followed by Amoxyclav (42.9%).
  Streptococci were susceptible to Cefotaxim (63%), Tetracycline (58.7%), Gentamicin (56.6%) and resistant to Amoxyclav (41.3%) and Co-trimoxazole (26.1%).
  Among the gram negative organisms, Klebsiella was susceptible mostly to Gentamicin (73%) followed by Amikacin (56.3%) and resistant to Amoxyclav (65.6%) followed by Ceftriaxone (43.8%) and Ciprofloxacin (34.4%).
  Gandhi *et al* reported Staph aureus to be more sensitive to Vancomycin (99.35%), followed by Ceftriaxone (99.19%), Cefoperazone/sulbactum (99.19%), Gentamicin (96.77%), Amoxyclav (94.35%), Doxycycline (89.5%), Ciprofloxacin (74.19%), Cefuroxime (60%), Erythromycin (58.06%), Co-trimoxazole (50.32%), Amoxicillin (34.84%), and Cefixime (40%) respectively.
  In this region, based on our study results, Staph aureus was found to be still susceptible to conventional mode of treatment. Most of the strains were found to be resistant to Amoxyclav followed by Co-trimoxazole.

Conclusion

Staphylococcus aureus is the commonest cause for pyodermas followed by CONS. The prevalence of MRSA among these patients is significant, suggesting a need for more stringent regulations for the use of antibiotics.
Commonly used antibiotics like Doxycycline, Tetracycline, Erythromycin and Co-trimoxazole are working well. Organisms are developing resistance to Amoxyclav and Ciprofloxacin.
On account of the high prevalence of pyoderma, changing pattern of causative micro-organisms, and the indiscriminate use of antibiotics leading to altered antibiotic susceptibility pattern, there is a constant need to obtain more information about etiological agents and their antibiotic susceptibility, predisposing factors, modes of transmission, and effective methods for control through constant monitoring.
Our study will assist clinicians in suitable and judicious selection of antibiotic by using antibiotic sensitivity data and prevent the emergence of drug resistant strains.

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