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Study of Methicillin Resistant Staphylococcus Aureus in Pyodermas: an original research article

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ABSTRACT

OBJECTIVES:

1.To study the frequency of Methicillin resistant staphylococcus aureus (MRSA), the antimicrobial susceptibility patterns of (MRSA) and methicillin-sensitive Staphylococcus aureus (MSSA) isolates from the cases of primay and secondary pyodermas and the biofilm formation by Staphylococcus aureus isolated from pyodermas.

INTRODUCTION:

Pyodermas are one of the most commonly encountered dermatological problem that can have different presentations.They can broadly be classified into two types: Primary pyodermas and Secondary pyodermas. Staphylococcus aureus, a Gram-positive cocci, the leading cause of skin and soft tissue infections worldwide, can acquire resistance to antibiotics.

In Methicillin-resistant Staphylococcus aureus (MRSA), methicillin resistance arises from the mecA gene, produces an altered penicillin binding protein (PBP 2a) with a lower affinity for beta lactam group. MRSA is classified into two types, community-acquired (CA-MRSA) and hospital-acquired (HA-MRSA).

MATERIALS AND METHODS:

Patients with pyodermas, who attended the Dermatology and Venereology services of a tertiary care hospital from February 2016 to December 2016 were included in the study.

Sample: Fine needle aspirates (whenever possible) or sterile swabs from the pyoderma lesions.

Storage: stored at room temperature and were sent to the Microbiology laboratory within 4 hours.

Procedure:

For isolation of S. aureus, the obtained sample was cultured and incubated on 5 % Sheep blood Agar and MacConkey agar followed by gram staining and biochemical reactions.

MRSA detection was done using Cefoxitin disc (30μg) diffusion method and oxacillin screen agar method.

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The antibiotic susceptibility testing was performed by Kirby Bauer Disc Diffusion according to Clinical and Laboratory Institute (CLSI 2016 M100- S24) guidelines against selective drugs.

RESULTS:

A total of 178 samples of pyoderma were collected and sent for pus culture and antibiotic susceptibility testing to the microbiology laboratory.109 samples yielded S. aureus. Out of which 22 were MRSA.

Out of 109 S. aureus isolates, biofilm formation was seen for the 59 isolates, all of them were biofilm formers.

CONCLUSION: The prevalence of MRSA from pyoderma samples was found to be quite high and this may be attributed to over-the-counter/ irrational use of antibiotics esp. an increased resistance to those antibiotics which were once very effective. Resistance was not seen to those antibiotics which are usually prescribed in a hospital setting or are not over-the-counter available .

Keywords: Staphylococcus aureus, Methicillin resistant Staphylococcus Aureus (MRSA), pyodermas, antibiotic susceptibility testing, biofilms

INTRODUCTION:

Pyodermas are one of the commonest dermatological problems that can have a myriad of presentations varying from folliculitis, impetigo, furuncles, carbuncles to abscesses and cellulitis. They can be of two types: Primary pyodermas when the lesion develops over an apparently normal skin eg: impetigo, follicultis, furuncle, carbuncle, ecthyma, erthyrasma, and sycosis barbae, and secondary pyodermas when the lesions develop over a previously diseased skin, due to any other cause eg: infected contact dermatitis, infected scabies, trophic ulcer, infected pemphigus and various other dermatoses infected with organisms. *Staphylococcus aureus* is the leading cause of skin and soft tissue infections worldwide. [1] Staphylococci are Gram-positive cocci about 0.5 – 1.0 μm in diameter that grow in clusters, pairs and occasionally in short chains. The clusters arise because staphylococci divide in two planes. The configuration of the cocci helps to distinguish micrococci and staphylococci from streptococci, which usually grow in chains.

Staphylococcus aureus can be differentiated from other species of staphylococci by coagulase test. *S aureus and S intermedius* are coagulase positive. All other staphylococci are coagulase negative. *S. aureus* has a remarkable ability to acquire resistance to antibiotics. Due to acquisition of plasmid-encoded beta lactamase, penicillin was no longer effective. Changes in the bacterial surface receptors is the factor that is responsible for staphylococcal resistance against β lactamase resistant penicillins such as methicillin and cloxacillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961. It has become resistant to the antibiotics commonly used to treat staphylococcal infections. Over the past few decades epidemics of MRSA has increased in frequency throughout the world . Many hospitals have had to institute control measures to contain the spread of infection. New

clones of MRSA may follow changes in the antibiotic usage.

Resistance to antibiotics develops when an organism acquires a gene that allows the microbe to inactivate the antibiotic. This may occur spontaneously as a genetic mutation or involves the acquisition of mobile genetic elements such as a plasmid, transposon, integron or gene cassette. [2]

In MRSA, methicillin resistance arises from the mecA gene, a component of mobile genetic element, the staphylococcal cassette chromosome (SCC mec) that results in production of an altered penicillin binding protein (PBP 2a) with a lower affinity for β lactam group. MRSA is classified into two types, community-acquired (CA-MRSA) and hospital-acquired (HA-MRSA). All of the following conditions have to be met to label MRSA as CA-MRSA: [3]

(i) MRSA is isolated from the culture obtained as an outpatient or in first 3 days of hospitalization (≤ 72 hours),

(ii) no clinical culture with MRSA in the last 6 months,

(iii) no hospitalization or surgery within last 1 year, and

(iv) no hemodialysis.

In the year before the present hospitalization, if the patient had any one of the following, the MRSA will be labeled as HA-MRSA: [3]

(i) hospitalization,

(ii) surgery,

(iii) residency in a long term care facility and hemodialysis/ peritoneal dialysis, and

(iv) indwelling percutaneous devices or catheters at present admission.

An important characteristic of *S. aureus* is its capacity to form biofilms. [4] Biofilms are a group of microorganisms attached to a surface and covered by an exopolysaccharide matrix. Bacteria inside bio-films display an increased resistance to chemotherapeutics and host immune defenses, leading to persistent and difficult-to-treat infections. [5]

MATERIALS and METHODS: Patients with pyodermas, who attended the Dermatology and Venereology outdoor services of Sir Sundar Lal Hospital, BHU from February 2016 to December 2016 were included in the study. In all patients ELISA for HIV was done.

Sample: Fine needle aspirates (whenever possible) or aseptically collected samples using sterile swabs from the pyoderma lesions.

- Storage: stored at room temperature and were sent to the Microbiology laboratory within 4 hours.
- Procedure:
- For isolation of *S. aureus,* the obtained sample was cultured on 5 % Sheep blood Agar and MacConkey agar incubated at 37 ^{0}C for 24-48 hours.
- The culture isolate obtained was then identified by gram staining and biochemical reactions. The colonies were large (2- 4mm diameter), circular, convex, smooth, shiny, opaque and easily emulsifiable.
- MRSA detection was done using Cefoxitin disc (30μg) diffusion method and oxacillin screen agar method. [6]

  The antibiotic susceptibility testing was performed by Kirby Bauer Disc Diffusion according to Clinical and Laboratory Institute (CLSI 2016 M100- S24) guidelines. [7]

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The antibiotic discs were ordered from Hi Media Pvt. Ltd, Mumbai.
The antibiotic susceptibility testing was performed against selective drugs, as shown in table 1.

Table 1. Drugs against which antibiotic susceptibility testing was performed

MSSA	MRSA
penicillin (10 units	penicillin (10 units
amoxicillin with clavulanic acid(30 mcg)	azithromycin(15mcg) Erythromycin (15mcg)
cefadroxil	levofloxacin (5µg),
azithromycin(15mcg)	co-trimoxazole(25µg),
erythromycin (15mcg)	linezolid (30µg),
levofloxacin (5µg),	vancomycin
netilmicin (30µg)	clindamycin (2µg)
mupirocin (200µg).	piperacillin-tazobactam (1.25/ 23.75µg)
Fusidic acid (10µg)	netilmicin (30µg)
	fusidic acid(10µg)
	mupirocin (200µg)

The first 59 isolates were tested for the formation of biofilm. Isolates were initially identified by standard microbiological techniques including Gram stain, catalase test and coagulase test. All cultures were maintained on trypticase soy agar, Difco. Known additional reference strains of S. epidermidis ATCC 35984 (high slime producer) *S. epidermidis* ATCC 35983 (moderate slime producer) and S. epidermidis ATCC 12228 (non-slime producer), obtained from American type culture collection (ATCC, Rockville, Md.), were also included in the study.

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In the present study, we screened all the 59 isolates for their ability to form biofilm by TCP method as described by Christensen et al [8] with a modification in duration of incubation which was extended to 24 hours.

Isolates from fresh agar plates were inoculated in respective media and incubated for 18 hour at 37^{o}C in stationary condition and diluted 1in100 with fresh medium. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates (Tarson, Kolkata, India) were filled with 0.2 ml aliquot of the diluted cultures and only broth served as control to check sterility and non-specific binding of media.

The tissue culture plates were incubated for 24 hours at 37°C. After incubation content of each well was gently removed by tapping the plates. The wells were washed four times with 0.2 mL of phosphate buffer saline (PBS pH 7.2) to remove free-floating 'planktonic' bacteria. Biofilms formed by adherent 'sessile' organisms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent staphylococcal cells usually formed biofilm on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria were determined with a micro ELISA auto reader (model 680, Bio-rad) at wavelength of 570 nm (OD570 nm). These OD values were considered as an index of bacteria adhering to surface and forming biofilms.

Experiment was performed in triplicate and repeated three times, the data was then averaged and standard deviation was calculated. To compensate for background absorbance, OD readings from sterile medium, fixative and dye were averaged and subtracted from all test values. The mean OD value obtained from media control well was deducted from all the test OD values.

Classification of bacterial adherence

For the purpose of data calculation, we used classification based on OD values obtained for individual strains of *S. aureus.*

Classification of bacterial adherence by TCP method

Mean OD values with <0.120, 0.120-0.240 and >0.240 were classified as non-former, moderate-biofilm former and high biofilm former.

RESULTS:

A total of 178 samples of pyoderma were collected and sent for pus culture and antibiotic susceptibility testing to the microbiology laboratory. Out of these, 96 constituted primary pyodermas and 82 were cases of secondary pyodermas. Among primary pyodermas 45 cases were furuncle, 24 were folliculitis, 15 were impetigo, 9 were cellulitis, 2 were carbuncle, and 1 was ecthyma. 84 cases were of pyodermas that developed secondary to diseases like insect bite reaction (10cases), scabies(13 cases), milaria rubra (8 cases), discoid eczema (9 cases), tinea (2 cases), pompholyx (12 cases), psoriasis (2 cases), fissured feet (4 cases), stasis dermatitis (3 cases), lichen simplex chronicus (2 cases), intertrigo (1 case), stasis dermatitis (3 cases), trophic ulcer in hansen's disease (6 cases), hidradenitis suppurativa (2 cases), pyoderma gangrenosum (2 cases), keloid (3 cases) and at the site of trauma (3 cases).

The age of the patients in our study ranged from 1year to 68 years. Maximum number of patients were found in the age group of 10-25 years, followed by 25-40 years, followed by 40-50 years, least number of patients were found in 0-10 age group and above 50 years. There were 100 males and 78 females. 135 patients belonged to rural areas and 43 patients were from urban areas. 14 patients had diabetes mellitus (8 with furuncle, 2 carbuncle, 3 folliculitis, 1 cellulitis), 2 of these had recurrent history of furuncle and folliculitis. 5 were on treatment for diabetes mellitus and 9 were referred to endocrinology department for deranged plasma glucose. No patient had positive ELISA for HIV.

Out of the 178 samples collected, 109 samples were that of *S.aureus*. Out of the 109 samples, 87 samples were MSSA, 22 samples were that of MRSA thus MRSA constituted 20.18% of all the S. aureus isolates . 35 were other isolates that included other bacterias (Table 2).In 22 patients, the samples did not show any growth.

Table 2. Various bacteria isolated from pyoderma lesions and their numbers

Bacteria	**Number of samples (%)**
Micrococci	12 (6.74%)
Streptococcus	3 (1.68%)
Acinetobacter	2 (1.12%)
Pseudomonas aeruginosa	9 (5.05%)
Enterococcus fecalis	7 (3.93%)
Eischerichia coli	3 (1.68%)
Staphylococcus epidermidis	1 (0.56%)
Coagulase negative S. aureus(CONS)	2 (1.12%)
Proteus mirabilis	4 (2.24%)
Morganella morganii	1 (0.56%)
Diphtheroids	1 (0.56%)
Mixed commensals	2 (1.12%)

Antibiotic resistance to different antibiotics is shown in table 3.

Table 3. Antibiotic resistance of different bacteria isolated in the study

ANTIBIOTICS	All bacteria (n, %)	MSSA (n, %)	MRSA (n, %)
Penicillin	100%	100 %	100%
Amoxyclav	121, 67.23%	86, 78.89%	9, 8.25%
Moxifloxacin	117, 65%	83, 76.14%	19, 17.43%
Cotrimoxazole	78, 43.34%	59, 54.12%	18, 16.2%
Levofloxacin	51, 28.34%	41, 37.61%	8, 7.20%
Ciprofloxacin	36, 20.22%	25, 22.93%	9, 8.25%
Erythromycin	30, 16.85%	18, 16.51%	10, 9.17%
Azithromycin	25, 14.04%	18, 16.51%	7, 6.30%
Piperacillin- tazobactam	22, 12.22%	10, 9.17%	8, 7.20%
Netilmicin	2, 1.11%	1, 0.9%	1, 0.9%
Linezolid	0	0	0
Clindamycin	0	0	0
Vancomycin	0	0	0
Fusidic acid	0	0	0
Mupirocin	0	0	0

Biofilm formation Out of 109 *S. aureus* isolates, biofilm formation was seen for the first 59 isolates, all of them were biofilm formers, 46 of which were high biofilm former and 13 were moderate biofilm former (Table 4).

Table 4. Biofilm formation by the bacteria

Mean OD Value	Interpretation	Number of samples
<0.12	Non biofilm former	0
0.12 – 0.240	Moderate biofilm former	13
>0.240	High biofilm former	46

DISCUSSION:

In the present work, The prevalence of primary pyoderma (53.3%) and that of secondary pyodermas was (46.06%) which was similar to other studies. [10] [9][11] The maximum frequency among primary pyodermas was of furunculosis(24.4%) followed by folliculitis (11.1%), impetigo (7.2%), cellulitis (3.8%), carbuncle (1.1%) which was similar to other studies [10] [12] [9], but was different from other studies where impetigo was found to be the most common primary pyodermas followed by folliculitis and furunculosis. [14] [11][13][15][16] Among cases of secondary pyodermas, it was most frequently associated with scabies followed by pompholyx, similar to other studies where scabies was the most common.

On microbiological testing, out of 178 samples of pyoderma, 109 samples (61.6%) were found to be *S. aureus* .MSSA were 87 in number i.e. the prevalence was 79.8%. Twenty two

Staphylococcus aureus isolates were found to be MRSA. Thus, the prevalence of MRSA was 20.18%; 19 of 22 MRSA isolates were CA-MRSA and 3 were HA-MRSA.

In previous Indian studies on pyodermas , the prevalence of MSSA and MRSA out of *S. aureus* was as follows (Table 5):

Table 5. Prevalence of MSSA and MRSA out of S. aureus in previous Indian studies on pyoderma

Study	MSSA	MRSA
Present study	79.8%	20.18%
Furtado et al. 2014 [9]	88.66% (CA-MSSA) 82% (HA-MSSA)	11.33% (CA-MRSA) 18% (HA-MRSA)
Venniyil et al. 2016 [10]	78.12%	21.98%
Malhotra et al. 2012 [13]	83.33%	9.83%
Patil et al. 2006 [12]	98.5%	1.4%
Sharma et al. 2015 [19]	72.97%	27.02%
Vijaymohan et al. 2014 [18]	69.2%	30.8%
Thind et al. 2010 [17]	90.4%	9.6%
Nagaraju et al. 2004 [20]	89.1%	10.9%
Lalremruata et al. 2014 [21]	76.19%	23.8%
Bhat et al. 2016 [22]	39.32%	61%

The antibiotic susceptibility pattern was as follows: 100% resistance was seen to penicillin in both MSSA and MRSA. Among MSSA , maximum resistance was seen to penicillin followed by amoxyclav (78.89%), moxifloxacin (76.14%), cotrimoxazole (54.12%), levofloxacin (37.61%), ciprofloxacin (22.93%), erythromycin (16.51%), azithromycin (16.51%), piperacillin- tazobactam (9.17%), and netilmicin (0.9%).

Among MRSA , maximum resistance was seen to penicillin followed by moxifloxacin (17.43%), cotrimoxazole (16.20%),erythromycin (9.17%), ciprofloxacin (8.25%), amoxyclav (8.25%), levofloxacin (7.2%), piperacillin- tazobactam (7.2%) azithromycin (6.30%), and netilmicin (0.9%).

No resistance was seen to clindamycin, vancomycin, linezolid, fusidic acid and mupirocin in either MSSA or MRSA group, in the present study. In a few Indian studies, there was no resistance reported to linezolid and vancomycin [12][14][17][22][23], similar to our study. No resistance was reported to fusidic acid and mupirocin. [24]

Resistance of MSSA to fluoroquinolones such as moxifloxacin (76.14%), ciprofloxacin(22.93%), levofloxacin (37.61%) was also quite high in our study. Low susceptibility (8.3- 14.6%) was noted to fluoroquinolones (ciprofloxacin and levofloxacin) in another study. [11][13]

Bacterial biofilm has long been considered as a virulence factor contributing to infection associated with various medical devices and causing nosocomial infection. [25][26]

S. aureus can form biofilms on damaged or wounded skin in animal models [27][28], and staphylococcal biofilms have been found in human skin lesions. [27][29][30] In one study, all the *S.aureus* isolates from skin and soft tissue infections were able to form biofilms under most of the tested conditions, suggesting that biofilm formation is essential for skin infections. [5]

CONCLUSION:

The prevalence of MRSA from pyoderma samples was 20.8%, which is quite high. Such high percentage of MRSA may be attributed to over-the-counter/ irrational use of antibiotics.

In comparison with other Indian studies we found that the prevalence of MRSA, the type of pyoderma and age groups most affected by pyodermas differs considerably in various parts of India.

Also, the present study concludes that MRSA prevalence is high and over-the-counter/irrational use of antibiotics may have led to an increased resistance to those antibiotics which were once very effective.

Resistance was not seen to few antibiotics and those were the ones which are usually prescribed in a hospital setting or are not easily available at local or village drugstores.

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