



Research Article

Bacterial profiling of isolated MRSA strains from wounds in tertiary care hospital, Chennai, Tamil Nadu, India

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Abstract

Staphylococcus aureus being the normal microflora of the body are much easily prone to become a vital wound pathogen as is easy to invade and colonise the broken skin that exposes an optimal environment for their growth when a wound occurs. As they are the most prevalent microbes in wound infections, indiscriminate use of antibiotics has increased the virulence and variations in resistance of these strains resulting in frequent occurrence of MRSA. This study thus aimed to scrutinise the incidence of MRSA in diverse wound infection types against their susceptibility pattern to different antibiotics. Microbes were profiled from the wound infections in 289 patients using various samples like pus, swab and tissue. From the isolated microbes, MRSA isolates was screened and antibiotic susceptibility testing using routine disk diffusion method was performed. The wound microbial profiling indicated incidence of *Staphylococcus aureus* (58.55 %) to be the predominant pathogen with an incidence of 40.44 % MRSA isolates. These isolates were susceptible to vancomycin, levofloxacin and amikacin. Routine screening for antibiotic susceptibility of MRSA strains is required to determine the effective antibiotic regime to be adopted in the hospital.

Keywords: Staphylococcus aureus, MRSA, beta lactam antibiotics, wound infections

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1. Introduction

A break in the skin and exposure of subcutaneous tissue following the loss of integrity of the skin, thus providing a moist, warm and nutritive environment optimal for microbial colonization and proliferation is called wound [1]. The colonization of wound with infectious pathogens will result in pathogenesis of wound to wound infections invading the neighbouring tissues. In developing countries like India, large number of people die daily of preventable and curable diseases such as wound infections.

Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70 - 80 % mortality [2, 3]. Thus, the importance of wound infections, in both economic and human terms, should not be underestimated [4]. In a study, patient with an infected wound was found to take 6 - 10 days more than the wounds that will heal

without infections [5]. Staphylococci and streptococci species are the most common wound pathogens that have a role to play in clinically infected chronic wounds. They act synergistically to invade the tissue even if they themselves do not penetrate far into the deeper wound compartment [6] However, chronic infected wounds are polymicrobial, thus consists of mixed aerobic and anaerobic pathogens. Although the competition for cohabitation on intact skin appears to decrease the virulence of an individual species, the polymicrobial nature of the open wound is likely to provide opportunities for synergism that result in infection and delayed healing.

Nevertheless, there is an observation of species - specific effect on wound infections. This becomes explicit in the cases of beta haemolytic streptococcal infections, in particular that of *Streptococcus pyogenes*, which are pathogenic at numbers that are significantly lower than many

other species. Other species isolated at the same time, for example - *Staphylococcus aureus*, *Proteus* sp. and *Escherichia coli*, may have a positive effect by provoking inflammatory response that in turn accelerates wound repair by stimulating blood flow [7, 8].

The aim of the current study is to isolate the wound causing infections in our hospital for scrutinising for the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) and for analysing the effective antimicrobials for prescription on understanding the antibiotic susceptibility pattern. This study will thus involve bacterial profiling of wound infections, antibiogram for the selected isolates and screening for MRSA.

2. Material and methods

A total of 289 patients with wound infection attending as outpatient and inpatient in Kilpauk Medical College and Hospital, Chennai were included in the study conducted in the period from 2009 to 2010.

Collection of specimens:

1. **Pus:** The area over the abscess was wiped with sterile saline or 70 % alcohol and the pus was aspirated into a sterile test tube using a sterile syringe and needle
2. **Swab:** The wound was wiped with sterile saline and the swab was rolled along the leading edge of the wound and was transferred into a sterile test tube. Two swab specimens were collected, one for smear examination and one for culture inoculation.
3. **Tissue bits:** For chronic wounds, the wound area was wiped with sterile saline and tissue pieces were collected into sterile saline in a sterile test tube using sterile punch biopsy forceps.

Specimen processing

On reaching the laboratory, smears were prepared from one of the swabs and/or purulent material on a clean glass slide. Tissue specimens were ground or minced using sterile scissors and forceps before processing. Smears were routinely observed using Gram's stain for initial identification. The specimens were inoculated onto blood agar and Mac-Conkey agar plates and incubated aerobically at 37° C for 18- 24 hours. The microbes were identified based on colony morphology, Gram's stain, motility and

biochemical reactions. Information from these primary plates in conjunction with the oxygen requirements, Gram's stain and colonial morphology of a pure isolate provides presumptive identification of anaerobic organisms.

Antimicrobial susceptibility testing

Routine disk susceptibility testing of the aerobic isolates was performed by Kirby-Bauer method on Mueller-Hinton agar medium obtained from Himedia. 25 ml of freshly prepared sterile medium was poured into a Petri dish of 90 mm diameter to obtain a thickness of 4 mm.

Preparation of 0.5 McFarland's turbidity standard for inoculum preparation:

0.05 ml of 1 % barium chloride solution was added to 9.95 ml of 1% sulphuric acid in a test tube with constant stirring to maintain a uniform suspension. The barium sulphate suspension was transferred in the range of 4 to 6 ml into a screw-capped tube of same size as those used for diluting the bacterial inoculum. The tube was tightly sealed and stored in refrigerators. Before each use, it was shook vigorously until all the deposits were raised into a uniform suspension.

Preparation of inoculum and inoculation: [9]

Morphologically, similar colonies from an agar medium were touched with a wire loop and were inoculated into a test-tube containing 1.5 ml of nutrient broth. The tube was incubated at 35° C until it is matched in density with 0.5 McFarland's standard, which corresponds to 150 million organisms per ml. Within 15 minutes of preparation of the suspension, a sterile cotton wool swab was used to streak plate the MHA tri-directionally and was left for 3 to 5 minutes before placing the antibiotic disks. **Antibiotic disks:** The antimicrobial susceptibility test for *Staphylococcus aureus* and coagulase negative *Staphylococcus* sp. include the use of Penicillin 10 U, Erythromycin 15 µg, Cotrimoxazole 25 µg, Oxacillin 1 µg, Cefotaxime 30 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg and Amikacin 30 µg disks. Vancomycin 30 µg disk was used only for Oxacillin resistant strains in the next stage of antibiogram. For beta-haemolytic streptococci the antimicrobial susceptibility testing

includes Penicillin 10U, Erythromycin 15 µg, Cotrimoxazole 25 µg and Cephalexin 30 µg disks.

The plates were inverted and incubated at 35° C to 37°C for 16 to 18 hours [10] Plates were read on a black non-reflecting background against an illuminated with reflected light.

The size of the zones of inhibition were interpreted by referring to the NCCLS table -2, Volume 20: 1 (2000) (zone diameter interpretive standards) and recorded as susceptible, intermediate or resistant.

Detection of MRSA strains -screening for MRSA

Oxacillin disk (1 µg):

Disk diffusion tests were performed with 1 µg of Oxacillin disk, which was placed on MHA plate. The zone of inhibition is determined after 24 hrs of incubation at 37° C. The zone size is interpreted according to CLSI guideline.

Susceptible	>13 mm
Intermediate	11-12mm
Resistant	< 10 mm

Cefoxitin disk (30 µg):

The test was performed with 30 µg of Cefoxitin disk placed on Muller Hinton agar plate without NaCl supplementation. The zone of inhibition is determined after 24 hrs of incubation at 37°C. The zone size is interpreted according to CLSI guidelines.

Susceptible	>19mm
Resistant	< 20mm

Quality and control strains used for MRSA detection:

ATCC *Staphylococcus aureus* 43300 (Positive control)

ATCC *Staphylococcus aureus* 25923 (Negative control).

Statistical analysis

Statistical analyses were carried out using SPSS package and Epi-info software with the help of a statistician. The proportional data of the cross sectional study was tested using Pearson's chi-square analysis test and binomial proportion test.

3. Results

Swabs obtained from 289 patients with wound infections attending surgical, orthopaedic, burns, OG, IMCU and plastic surgery departments as OP

and IP were studied from March 2009 to Feb 2010 to identify the bacteriological profile of wound infection, antimicrobial susceptibility pattern of the organisms isolated and for the prevalence of MRSA. Study included patients of both sexes and upto 80 years of age. Male patients constituted 143 (49.48%) and female patients constituted 146 (50.51%) in the age group of 3 months to 80 years. In all age groups except 11-20 and 21-30 the sex distribution was predominantly male. Bacterial isolates was found in 164 (56.74%) patients. The isolation rate was significantly higher in female (51.21%) compared to male (48.78%) (Table– 1). The predominant isolates were Gram-positive bacteria 96 (58.53%). The most frequently isolated microorganisms were *Staphylococcus aureus* 89 (54.26 %) followed by *Klebsiella pneumoniae* (24.39 %), *Pseudomonas aeruginosa* 22 (13.41 %), *Escherichia coli* 5 (3.04 %), Enterococci 5 (3.04 %), coagulase-negative *Staphylococcus* sp. 2 (1.21 %), *Acinetobacter* sp. 1 (0.60 %) (Table– 2).

Out of the 111 burn wound isolates, 63 (56.75%) were *Staphylococcus aureus* and 2 (1.8 %) coagulase-negative *Staphylococcus* sp (Table-3). The microorganisms isolated from 26 specimen of surgical site infections were 11 isolates of *Staphylococcus aureus* (42.30%), 7 isolates of *Klebsiella pneumoniae* (26.92%), 3 isolates of *Pseudomonas aeruginosa* (11.53%), 3 isolates of Enterococci (11.53%) and 2 isolates of *Escherichia coli* (7.6%). Out of 22 cutaneous abscess isolates 13 (59.09%) were *Staphylococcus aureus*, 2 (9.09%) were *Klebsiella pneumoniae*, 5 (22.72%) were *Pseudomonas aeruginosa* and 2 (9.09%) were *Escherichia coli*. Out of 5 traumatic wounds, 2 (40%) were *Staphylococcus aureus*, 2 (40%) were *Klebsiella pneumoniae* and 1 (20%) were *Pseudomonas aeruginosa*.

Out of the 89 isolates of *Staphylococcus aureus*, 45(50.56%) were sensitive to amoxycillin, 57 (64.04%) were sensitive to gentamicin, 50 (56.17%) sensitive to ciprofloxacin, 36 (40.44%) were sensitive to erythromycin and cephalexin, 58 (65.16%) were sensitive to cefotaxime, 61 (68.53%) were sensitive to piperacillin / tazobactam, 81 (91.01%) were sensitive to levofloxacin, 82 (92.13%) were sensitive to amikacin and 100 % sensitive to vancomycin.

Cogaulase-negative *Staphylococcus* sp., were 100 % sensitive to amoxycillin, gentamicin, erythromycin, cefotaxime, cephalexin, piperacillin / tazobactam,

levofloxacin, amikacin, vancomycin and 50 % sensitive to ciprofloxacin (Table 2).

Screening for MRSA using Oxacillin disk (1 µg):

All the 89 isolates of *Staphylococcus aureus* were screened for Methicillin resistance using oxacillin disk (1µg), of which 34 (38.21 %) were found to have inhibition zone less than 10mm (Table 3).

Confirmation of MRSA using Cefoxitin disk (30 µg):

All the 89 isolates of *Staphylococcus aureus* were further tested and confirmed for Methicillin resistance using cefoxitin disk (30µg) as in Table 4, of which 36 isolates were identified as MRSA. This accounts for 40.44 % of the total staphylococcal isolates. The MRSA isolates were resistant to Amoxicillin (84.4%), Gentamicin (47.3%), Ciprofloxacin (41.7%), Erythromycin (48.4%), Cefotaxime (66.7%), Cephalexin (75%), Piperacillin / Tazobactam (72.3%), Amikacin (5.6%) and Levofloxacin (2.8%), but not resistant to Vancomycin (Table 5).

4. Discussion

Staphylococcus aureus is the most common wound pathogen. The control of wound infections became more challenging due to widespread bacterial resistance to antibiotics and to greater incidence of infections caused by MRSA. The clinical microbiological laboratory has the task of monitoring MRSA prevalence in pus culture and update on the antibiotic susceptibility of the recent strains, thereby playing a vital role in the treatment of wound infections to prevent development of complications.

This is because in developing countries like India, despite application of strict aseptic precautions, vigorous antibiotic prophylaxis and meticulous surgical techniques, wound infection is still a challenge to the surgeon no matter how skilful one is. In the present study out of 289 specimens, 164 isolates were identified (56.74%), of which 11 (42.30 %) isolates were *Staphylococcus aureus*. Similar, results were observed in the study by Jonathan Isibor *et al* [13], the predominant bacterial isolate in SSI was *Staphylococcus aureus* - (35%). The result remained similar is another study by Eveline Geubbels *et al* [11]. Whereas in a study by Jyoti Sonawane [12] *et al*, the predominant isolate in SSI was also *Staphylococcus aureus* (29.26 %).

In Shittu *et al.* and Brook *et al* [14,15] studies as well, *Staphylococcus aureus* was the predominant microbe isolated from surgical site infections, 22.22 % and 26.54 % respectively. Data from the national nosocomial infections surveillance system [16] also revealed that the most common incisional SSI pathogens are *Staphylococcus aureus*, *Enterococcus* sp., Enterobacteriaceae family and *Pseudomonas aeruginosa*.

Table 1: Age and sex distribution of cases (N = 289)

Age in years	Male	Female	Total
0-10	24	16	40
11-20	17	27	44
21-30	32	57	89
31-40	35	24	59
41-50	9	5	14
51-60	14	13	27
61-70	8	4	12
71-80	4	-	4
Total	143 (49.48%)	146 (50.52%)	289

Table 2: Sensitivity pattern of *Staphylococcus aureus*

Antibiotics	<i>Staphylococcus aureus</i> N=89 (Percentage)
Amoxicillin	45 (50.56)
Gentamicin	57 (64.04)
Ciprofloxacin	50 (56.17)
Erythromycin	36 (40.44)
Cefotaxime	58 (65.16)
Cephalexin	36 (40.44)
Piperacillin / tazobactam	61 (68.53)
Levofloxacin	81 (91.01)
Amikacin	82 (92.13)
Vancomycin	89 (100%)

Table 3: Detection of MRSA by oxacillin screen agar test

Zone (mm)	No of Isolates	Percentage (%)
>14 (MSSA)	55	61.79
<10 (MRSA)	34	38.21

Table 4: Confirmation of MRSA by cefoxitin disk test

Zone(mm)	No. of Isolates	Percentage (%)
>20 (MSSA)	53	59.55
<19 (MRSA)	36	40.44

Table 5: Resistance pattern of MRSA isolates to antibiotics

Antibiotics	MRSA isolates (n=36) percentage (%)
Amoxicillin	84.4
Gentamicin	47.3
Ciprofloxacin	41.7
Erythromycin	48.4
Cefotaxime	66.7
Cephalexin	75
Piperacillin/ tazobactam	72.3
Levofloxacin	2.8
Amikacin	5.6
Vancomycin	0

The aerobic isolates of burn wound in the present study included *Staphylococcus aureus* 63 (56.75%), *Kebsiella pneumoniae* 29 (26.12%), *Pseudomonas aeruginosa* 13 (11.71%), *Enterococci* 2 (1.8%), Coagulase-negative *Staphylococcus* sp. 2 (1.8%), *Escherichia coli* 1 (0.9%) and *Acinetobacter* (0.9%). In concordance with our study, Misra *et al* [16] also reported *Staphylococcus aureus* (60%) as the most common pathogen isolated; and so was the case in reports by Revathi *et al* [17] and S.Vidhani *et al* [18]. The aerobic isolates of traumatic wound in the present study was also predominated by *Staphylococcus aureus* 2 (40%), which was similar to the results obtained in studies by Akinjogunla *et al* [20], Shittu *et al* [14], and Brook and Frazier [19]. Similar results to the antibiotic sensitivity pattern in the present study was observed in the study by Sarita Yadav *et al* [21], Misra *et al* [16], and Shilpa Arora *et al* [22] In contrast to the observations in this study, Fantahun Biadlegne *et al* [23] reported that the sensitivity of *Staphylococcus aureus* to

erythromycin and gentamicin; Sanjay Dhar *et al* [24] to amikacin and ciprofloxacin; Jonathan Osariemen Isibor *et al* [13] to ciprofloxacin, gentamicin, cephalexin and erythromycin.

As MRSA is a major nosocomial pathogen causing significant morbidity and mortality [25] in hospitals/institutions, these transmitted via healthcare workers from infected or colonized patients to other patients [26] The percentage of MRSA isolated in our study was 40.44 % and similar results were observed in other studies like that of Arti Kapil *et al* [27], Shilpa Arora *et al* [30], Vidhani *et al* [28] and Sarita Yadav *et al* [29]. Of these, methicillin resistance was documented as 60.6 % constituted *Staphylococcus aureus* isolates, which was an alarmingly high prevalence of MRSA observed until-to-date.

In this study, the spectrum of antimicrobial resistance amongst the isolated MRSA was potent against ciprofloxacin (90 %). Qureshi *et al* [31] also reported the same with a score of as high as 98.9 %. Pulimood [17] observed only 8% resistance of MRSA to gentamicin as against to 44 % in our study and 97.8 % in a study by Qureshi [32]. *In toto*, we obtained a higher percentage of multidrug resistant MRSA isolates. Majumder *et al* [33] from Assam reported 23.2% of MRSA isolates as multidrug resistant; and similarly, Anupurba *et al* [34] from Uttar Pradesh also reported a higher percentage of multidrug resistant MRSA. Vidhani *et al* [28] from Delhi reported even a higher percentage of multidrug resistant MRSA. These variations might be because of several factors like efficacy of infection control practices, healthcare facilities and antibiotic usage that vary from hospital to hospital.

The most effective way to prevent MRSA infections is by doing continuous surveillance of antibiotic resistance profiles of local *Staphylococcus aureus* isolates to formulate antibiotic policies and effective infection control practices [35].

Conclusion

The predominant isolate was found to be the Gram-positive bacteria, *Staphylococcus aureus* - 89 (54.26%), of which 36 (40.44%) were found to be MRSA. These isolates were susceptible to vancomycin (100%), levofloxacin (97.2%) and amikacin (94.4%).

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