

The prevalence of the methicillin resistant staphylococcus Aureus in the postoperative wound infection in a South Indian hospital

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Prevalence of MRSA

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Abstract

Introduction: Objectives of the present study was to find the prevalence of methicillin resistant staphylococcus aureus at a South Indian hospital. **Methods:** Hundred clinically suspected cases of infected postoperative wounds were subjected to culture and sensitivity. All the S.aureus isolates were screened for MRSA. **Results:** Prevalence of MRSA was 62.5%. All the MRSA strains showed multidrug resistance except to Vancomycin and Cloxacillin. **Conclusion:** Judicious use of antibiotics, strict asepsis and proper hygiene should be strictly applied to reduce postoperative wound infections. The determination of prevalence and antimicrobial profile of MRSA will help the clinician while treating.

Keywords: MRSA; S.aureus; E.coli; Pseudomonas spp; Antimicrobial susceptibility; Multidrug resistance.

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Introduction

Staphylococcus aureus has been reported as a major cause of community and hospital acquired infections [1-3]. The organism has a differential ability to spread and cause outbreaks in hospitals [4]. Ever since its first isolation in 1961, Methicillin resistant Staphylococcus aureus (MRSA) has emerged as one of the commonest causes of hospital acquired infection (HAI) and continues to remain an important factor contributing to failure of management. Most of the isolates of MRSA have acquired resistance to commonly available antibiotics in the market. Several reports have appeared in medical literature regarding the prevalence and incidence in hospitals across the globe including India. Infections due to MRSA are associated with increased

morbidity and mortality in hospitalized patients. It also has the potential to cause sudden outbreaks in hospitals. Cost of treatment is another major problem faced by patients in the developing countries [5].

Despite the advances made in asepsis, antimicrobial drugs, sterilization and operation techniques, postoperative wound infections continues to be a major problem in all the branches of surgery in the hospitals and are responsible for the increasing cost, morbidity and mortality related to surgical operations.

Recent increase of methicillin resistant and multiple drug resistant strains at large hospitals has started to pose great difficulty in selecting antimicrobial agents [6]. Isolation of the organism and antimicrobial profile helps in the prompt management of postoperative

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wound infection. Microbiology plays very important role in diagnosis of infection and guiding the physician regarding accurate antimicrobial therapy. Hence, this study was undertaken to isolate and determine the antibiotic susceptibility pattern of MRSA.

Materials and Methods

The present study was performed at the microbiology department of our institution. The present study included a total of 100 cases of postoperative wound infection. Among them 54 were from men and 46 were from women. The age group of the patients ranged between 3- 84 years. Most of the patients were in the age group of 16 through 30 years (45%), 25% was 31 through 45 years and 46-60 years (16%) in that order. The cases were from the teaching hospital of our institution which is located in South India.

The wound infections other than the postoperative cases and the cases involving endoscopic procedures, incision and drainage of abscess, urethral dilations, slough excision and split skin graft were excluded from the present study. The patient should have a sutured wound in order to be considered in the present investigation.

The samples were collected from the depth of the wound with strict aseptic precautions with the help of dry sterile cotton swab sticks for bacteriological examination [6]. Two culture swabs from the each sample were obtained, one for the direct smear study and the other for aerobic culture which was immediately sent to the laboratory for investigation.

On day 1, direct microscopic examination was done by gram stain to look for pus cells and the bacteria. The first swab was used for making smear by rolling the swab stick on a clean glass slide, which was heat fixed. Gram stained smear was examined under the microscope and the bacteria were segregated into cocci and bacilli, gram positive or gram negative.

This report was then correlated with the growth in the culture plates after 18-24 hours. The second swab was inoculated on sheep blood agar and MacConkey agar as

well as nutrient broth and incubated at 37°C for 18-24 hours under the aerobic conditions. The culture media were procured from Hi- Media laboratory and prepared as per manufacturer's recommendation as well as inputs from Mackie and McCartney [7].

On day 2, identification of the growth was performed and the morphology of the colonies on the blood agar and McConkey agar were studied. Smear from the colonies were prepared and stained with gram stain and segregated into gram positive and negative after the microscopic examination. The bacteria were further microbiologically segregated by using the relevant biochemical and physiological tests [8,9].

The antibiotic susceptibility was studied by using the Kirby-Bauer disc diffusion method. Three to five isolated colonies of similar morphology were inoculated and the incubation was done for 2-8 hours, until the turbidity of the broth is matched. The antibiotic discs were placed on the agar plate within 15 minutes of inoculation by using a sterile needle and pressed firmly against the plate. The plates were inverted and incubated for 18-24 hours at 37°C.

On the day 3, the final identification of the bacteria was made taking into account of the various biochemical and physiological tests. Antibiotic susceptibility pattern were reported by measuring the zone of inhibition with a millimeter scale.

The antibiotic disc was reported as susceptible, intermediate and resistant, based on the criteria provided by NCCLS [10]. The catalase test was done to differentiate Staphylococcus (catalase positive) from Streptococcus and Enterococcus (both are catalase negative).

Enterococcus was identified presumptively by inoculating into 6.5% NaCl broth (salt tolerance) and observing the turbidity. If there was no turbidity, it was considered as Streptococcus. Enterococcus was further identified by heat tolerance (which is surviving at 60°C for 30 minutes). The identification of the gram negative bacilli was also performed. Oxidase positive isolates,

which were suspected to be *Pseudomonas* spp, were further identified by positive Citrate utilization test. Oxidase negative isolates were presumptively identified as *Acinetobacter* and *Enterobacteriaceae* members. *Acinetobacter* was identified following an oxidative reaction or inert reaction when inoculated into O/F medium (Oxidative/ Fermentative medium). The *Enterobacteriaceae* was differentiated by lactose into

lactose fermenters and non-lactosefermenters. Lactose fermenters were subjected to motility and IMViC test and production of H₂S was observed on T.S.I agar. The non-lactose fermenters were inoculated into urease medium. *Proteus* spp were presumptively identified by Urea hydrolysis and further confirmed by Phenyl alanine deaminase positive test.

Results

In the 100 cases of the present study, about 111 bacteria were observed accounting for 1.1 bacteria per case as an average. All these 111 isolates were aerobes and facultative anaerobes. The Gram stain of all direct smears correlated well with the growth on culture. Of these 111 isolates, 36 (32.4%) were Gram positive cocci and 75 (67.6%) were Gram negative bacilli. *E. coli* was isolated most frequently (24.3%) followed by *S. aureus* (21.6%), *Pseudomonas* spp (20.7%), *Klebsiella* spp (13.5%) and Coagulase negative *Staphylococci* (7.2%).

The patterns of bacteria isolated in the present study group are represented in Figure 1. Out of the 100 clinical samples, 85 samples (85%) yielded growth of single bacterium (monomicrobial/pure growth), 13 samples (13%) yielded growth of more than one bacterium (polymicrobial/mixture) and 2 samples (2%) yielded no growth. *Staphylococcus Aureus* was the most common bacteria (91.7%) isolated in pure culture, which is followed by *Pseudomonas* sp (82.6%).

In mixed growth the common bacteria isolated were *E. coli* (33.3%) and *Klebsiella* spp (33.3%). Coagulase negative *Staphylococci* (100%) and *Acinetobacter* (100%) were isolated exclusively in pure culture.

Enterococcus spp and *Proteus* spp were isolated equally in both pure growth and mixtures, while *Citrobacters* spp and *Enterobacters* spp both were isolated only in mixed cultures (50% each). The bacterial isolates in pure growth and mixtures in post-operative wound infection of the present study are represented in Table 1.

Table-1: Showing the distribution of the bacterial isolates (n=111) in pure growth and mixtures in postoperative wound infection.

Bacteria (n=111)	Pure (n1=85)	Mixture (n2=26)
<i>E. coli</i> (n=27)	18 (66.7%)	9 (33.3%)
<i>S. aureus</i> (n=24)	22 (91.7%)	2 (8.3%)
<i>Pseudomonas</i> spp (n=23)	19 (82.6%)	4 (17.4%)
<i>Klebsiella</i> spp (n=15)	10 (66.7%)	5 (33.3%)
*CONS (n=8)	8 (100%)	Nil
<i>Enterococcus</i> spp (n=4)	2 (50%)	2 (50%)
<i>Acinetobacter</i> spp (n=4)	4 (100%)	Nil
<i>Proteus</i> spp (n=4)	2 (50%)	2 (50%)
<i>Citrobacter</i> spp (n=1)	Nil	1 (100%)
<i>Enterobacter</i> spp (n=1)	Nil	1 (100%)

*CONS-Coagulase negative *Staphylococci*

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Out of the 100 clinical samples taken, 49 were from the abdomen, 45 were from limbs, 5 were from groin and 1 was from the neck region.

The frequency of bacteria of postoperative wound infection in relation to site of operation is represented in Table 2. Out of 111 isolates obtained from postoperative wound infection, 58 isolates were found to be from the abdominal surgery (52.2%) followed by 47 isolates from limb surgery (42.3%). *S. aureus* was resistant to penicillin in 95.8% isolates, ciprofloxacin (91.7%), cephalexin (79.2%) and erythromycin (79.2%).

Susceptibility of *S. aureus* was 100% to Vancomycin followed by Cloxacillin (79.2%) and Amikacin (50%). Among Enterococcus spp, resistance to Penicillin was 100% followed by Cloxacillin (75%). All of them exhibited the susceptibility to Vancomycin.

The susceptibility pattern of gram positive cocci has been represented in Table 3. Susceptibility of *E. coli* was more to Amikacin (63%) and Chloramphenicol (59.3%), but resistance was highest to Ampicillin (96.3%) followed by Cotrimoxazole (85.2%), Sparfloxacin (81.5%), Ciprofloxacin (77.8%), Gentamicin (77.8%), Cephotaxime (77.8%) and Ceftazidime (74.1%).

Klebsiella spp showed greater resistance to almost all the antibiotics. The resistance was 100% to Ampicillin followed by Cotrimoxazole (93.3%), Gentamicin (86.7%), Sparfloxacin (86.7%), Ciprofloxacin (80%), Cephotaxime (73.3%), Ceftazidime (73.3%), Chloramphenicol (66.7%) and Amikacin (66.7%).

The susceptibility patterns of gram negative bacilli are given in table nos. 4 & 5. The *Pseudomonas* spp showed maximum susceptibility to Polymyxin B (87%) followed by Amikacin (56.5%), Ceftazidime (52.2%), Piperacilin (52.2%) and Carbenicillin (39.1%). The susceptibility pattern of *Pseudomonas* spp. is given in table no 6.

Table-2: bacteriology of the postoperative wound infection in relation to site of operation.

Bacteria	Abdomen (n=58, 52.25%)	Limbs (n=47, 42.34%)	Groin (n=5, 4.5%)	Neck (n=1, 0.9%)
<i>E. coli</i> (n = 27)	22	3	2	-
<i>S. aureus</i> (n = 24)	9	13	2	-
<i>Pseudomonas</i> sp (n = 23)	11	11	-	1
<i>Klebsiella</i> sp (n = 15)	5	10	-	-
Coagulase negative Staphylococci (n=8)	3	4	1	-
Enterococcus sp (n = 4)	2	2	-	-
<i>Acinetobacter</i> sp (n = 4)	2	2	-	-
<i>Proteus</i> spp (n = 4)	2	2	-	-
<i>Citrobacter</i> sp (n = 1)	1	-	-	-
<i>Enterobacter</i> sp (n = 1)	1	-	-	-

Table-3: Susceptibility pattern of gram positive cocci in postoperative wound infection.

Susceptibility	S. Aureus (n=24)		*CONS (n=8)		Enterococcus spp. (n=4)	
	S	R	S	R	S	R
penicillin	1 (4.3%)	23 (95.7%)	2 (25%)	6 (75%)	0 (0%)	4 (100%)
cloxacillin	19 (79.2%)	5 (20.8%)	4 (50%)	4 (50%)	3 (75%)	1 (25%)
erythromycin	5 (20.8%)	19 (79.2%)	3 (37.5%)	5 (62.5%)	2 (50%)	2 (50%)
cephalexin	5 (20.8%)	19 (79.2%)	1 (12.5%)	7 (87.5%)	2 (50%)	2 (50%)
amikacin	12 (50%)	12 (50%)	5 (62.5%)	3 (37.5%)	2 (50%)	2 (50%)
gentamicin	7 (29.2%)	17 (70.8%)	2 (25%)	6 (75%)	1 (25%)	3 (75%)
ciprofloxacin	2 (8.3%)	22 (91.7%)	2 (25%)	6 (75%)	2 (50%)	2 (50%)
cotrimoxazole	7 (29.2%)	17 (70.8%)	0 (0%)	8 (100%)	2 (50%)	2 (50%)
vancomycin	24 (100%)	0 (0%)	8 (100%)	0 (0%)	4 (100%)	0 (0%)

*CONS-Coagulase negative Staphylococci; S-Susceptible; R-Resistant

Table-4: Susceptibility patterns of gram negative bacilli (E. coli, Klebsiella sp. and Proteus sp.) in postoperative wound infection.

Susceptibility	E. coli (n=27)		Klebsiella spp. (n=15)		Proteus spp. (n=4)	
	S	R	S	R	S	R
Ampicillin	1 (3.7%)	26 (96.3%)	0 (0%)	15 (100%)	0 (0%)	4 (100%)
Cefotaxime	6 (22.2%)	21 (77.8%)	4 (26.7%)	11 (73.3%)	0 (0%)	4 (100%)
Ceftazidime	7 (25.9%)	20 (74.1%)	4 (26.7%)	11 (73.3%)	2 (50%)	2 (50%)
Amikacin	17 (63%)	10 (37%)	5 (33.3%)	10 (66.7%)	2 (50%)	2 (50%)
Gentamicin	6 (22.2%)	21 (77.8%)	2 (13.3%)	13 (86.7%)	1 (25%)	3 (75%)
Ciprofloxacin	6 (22.2%)	21 (77.8%)	3 (20%)	12 (80%)	1 (25%)	3 (75%)
Sparfloxacin	5 (18.5%)	22 (81.5%)	2 (13.3%)	13 (86.7%)	1 (25%)	3 (75%)
Cotrimoxazole	4 (14.8%)	23 (85.2%)	1 (6.7%)	14 (93.3%)	0 (0%)	4 (100%)
Chloramphenicol	16 (59.3%)	11 (40.7%)	5 (33.3%)	10 (66.7%)	1 (25%)	3 (75%)

S-Susceptible; R-Resistant

Table-5: Susceptibility patterns of Acinetobacter sp., Citrobacter sp. and Enterobacter sp.(gram negative bacilli) in postoperative wound infection.

Susceptibility	Acinetobacter sp. (n=4)		Citrobacter sp. (n=1)		Enterobacter sp. (n=1)	
	S	R	S	R	S	R
Ampicillin	0 (0%)	4 (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)
Cefotaxime	2 (50%)	2 (50%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Ceftazidime	2 (50%)	2 (50%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)
Amikacin	3 (75%)	1 (25%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)
Gentamicin	2 (50%)	2 (50%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Ciprofloxacin	1 (25%)	3 (75%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Sparfloxacin	1 (25%)	3 (75%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Cotrimoxazole	1 (25%)	3 (75%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Chloramphenicol	3 (75%)	1 (25%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)

S-Susceptible; R-Resistant

Table- 6: Susceptibility pattern of Pseudomonas spp. in postoperative wound infection.

Susceptibility	Pseudomonas spp. (n=23)	
	S	R
Cefotaxime	3 (13%)	20 (87%)
Ceftazidime	12 (52.2%)	11 (47.8%)
Amikacin	13 (56.5%)	10 (43.5%)
Gentamicin	8 (34.8%)	15 (65.2%)
Ciprofloxacin	2 (8.7%)	21 (91.3%)
Sparfloxacin	20 (87%)	3 (13%)
polymyxin B	9 (39.1%)	14 (60.9%)
Carbenicillin	12 (52.2%)	11 (47.8%)

S-Susceptible; R-Resistant

Discussion

There are several predisposing causes to the emergence of postoperative infected wounds. The patient characteristics which favor the postoperative wound infections include coincident remote site infections or colonization, use of systemic steroids, diabetes mellitus,

and history of cigarette smoking, obesity and old age. The poor nutrition, transfusion of certain blood products before and after the surgery, preoperative hospitalization can also add into it. The environmental factors can prevent phagocytic cells from functioning

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efficiently by lowering tissue oxygen tension (PO_2). The lowered PO_2 inhibits the phagocytosis and will enhance the growth of anaerobic microorganisms [11]. For the most nosocomial wound infections, the endogenous flora of the patient which is present in the body surface and the viscera may become the source of infection [12]. The operating surgeon can decrease the chances of postoperative wound infection by using the drains appropriately, avoiding excessive cautery and not performing intestinal anastomoses if there is any possibility of ischemia.

Perhaps *Streptococcus pyogenes* was the most important cause of the hospital acquired infection earlier is now hardly ever encountered as it is highly susceptible to the antibiotics. Tetanus spores can survive in the dust for a very long time. Hospital acquired tetanus is usually a result of faulty sterilization techniques or other lapses in asepsis [13]. It has been reported that the balance between the host immunity and bacterial virulence has been altered due to the use of higher antibacterial agents [14].

In the present study, the gram negative bacillary infections (67.6%) had a high prevalence than those due to gram positive (32.4%) in the postoperative wound infections. This predominance of gram negative bacteria in the postoperative wound infection in the present investigation is in conformity with the findings of Agarwal et al. [15] and Anvikar et al. [3]. *E. coli* was the commonly isolated strain in the present investigation, accounting for 24.3% of all the isolates.

The present study observed that 22 out of 27 isolates of *E. coli* were from the postoperative wound infection following an abdominal surgery. *S. Aureus* was the next accounting for 21.6% of all the isolates. Among them 24 isolates of *S. Aureus* were isolated from the limb surgery. Agarwal et al. [15] described the predominance of *E. coli* (31.31%) followed by the *S. Aureus* (29.29%) in the postoperative wound infections. The *Pseudomonas* species was accounting for 20.7% of all the isolates. This is similar to Anvikar et al., [3] in their study the isolation of *Pseudomonas* spp. was 25%. But the isolation of *Klebsiella* spp. in the present study

(13.5%) is lesser compared to Anvikar et al. [3] which showed *Klebsiella* spp. (28.8%) as the emerging hospital acquired pathogen. Amikacin, cloxacillin and vancomycin were the most effective antimicrobial agents against the gram positive cocci, in the present investigation. The higher susceptibility of *S. aureus* to the cloxacillin was 79.2% in the present study, which is similar to that of Kowli et al., [16] where it was 76%.

The rapid emergence of *S. aureus* resistant to variety of antibiotics is of a great concern. The resistance to Penicillin is 95.8% followed by Ciprofloxacin (91.7%), Cephalexin (79.2%), Erythromycin (79.2%) and Gentamicin (70.8%). This may be due to the increasing use of beta-lactam antibiotics and Gentamicin. Lack of antibiotic policy is one more probable contributing factor.

Amikacin showed maximum efficacy against *E. coli* (63%) followed by Chloramphenicol (59.3%), while resistance to 3rd generation cephalosporin ranged from 74.1% to 77.8%. The values of the present study are similar with the observations of Arya M et al. [17]. These findings of the present study are similar to that of Anvikar et al. [3]. Against *Pseudomonas* spp., Polymyxin B showed maximum sensitivity of 87% followed by Amikacin (56.5%). These data are lesser compared to that of Kumar AP et al. [18] in which sensitivity to Polymyxin B was 100%. The present study observed the less effectiveness of the Fluoroquinolones.

The resistance to Sparfloxacin and Ciprofloxacin were 91.3% and 65.2% respectively. Resistance to Cephalexin was 87%. These observations of resistance pattern to many antibiotics are similar to the findings of Arya M et al [17].

Conclusion

The postoperative wound infection is the commonest nosocomial infection only after the urinary tract infection. The present microbiological study has determined the bacteriology (aerobes and facultative anaerobes) of postoperative wound infection. The most

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common bacteria isolated in pure culture were *S. aureus* followed by *Pseudomonas* spp. The majority of *E. coli* isolates were from infections following abdominal surgery. The gram positive cocci showed uniform susceptibility to Vancomycin. Cloxacillin was the second best drug effective against gram positive cocci. *S. aureus* displayed maximum resistance against Penicillin, Ciprofloxacin and Erythromycin. *E. coli* showed maximum susceptibility to Amikacin and Chloramphenicol. *Klebsiella* spp. displayed maximum resistance to all the antibiotics, including Aminoglycosides, Fluoroquinolones and third generation Cephalosporins. *Pseudomonas* spp. exhibited maximum susceptibility to Polymyxin B and displayed maximum resistance to Sparfloxacin and Cephalexin.

We suggest that it is necessary to implement urgent measures for restriction of nosocomial infections. Judicious use of antibiotics, strict asepsis and proper hygiene should be applied. We believe that the data of the present study may provide useful guidelines for choosing the effective therapy against the isolates from postoperative infected wound. It is advisable to scrutinize the postoperative infected wound in each and every hospital to evolve the control strategies.

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