Gram Positive Cocci Causing Surgical Site Infection: Identification and Antibiotic Susceptibility Pattern

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Abstract:
Introduction: Surgical site infections are the third most commonly reported nosocomial infections. They have been responsible for the increasing cost, morbidity and mortality related to surgical operations. Surgical site infection rate has varied from a low of 2.5% to a high of 41.9%. Staphylococcus aureus is frequently isolated from post surgical wound infections, which may serve as nidus for the development of systemic infections.

Objectives: The objective of this study was to isolate and identify various gram positive cocci from surgical site infected cases and determine their antimicrobial susceptibility pattern.

Materials and Methods: A total of 53 culture positive isolates of gram positive cocci were obtained from infected surgical sites. All isolates were identified as per standard procedures. Antimicrobial susceptibility testing of all isolates was done by Kirby Bauer disc diffusion method as per CLSI guidelines.

Results: Most common gram positive cocci isolated was Staphylococcus spp(47) followed by Enterococcus spp(6). Out of 47 Staphylococcus isolates 34 were S.aureus (26 MRSA) and 13 Coagulase negative staphylococci (10 MRCONS). Most of the isolates were resistant to ampicillin (96.22%) and cephalexin (87.23%). Out of 6 Enterococcus isolates only 2(33.33%) were sensitive to high strength gentamicin. Sensitivity to vancomycin, doxycycline, pristinomycin and linezolid was also done.

Conclusion: As a result of indiscriminate use of antimicrobials, the spread of antimicrobial resistance is now a global problem. A regular surveillance of hospital acquired infections and formulation of antibiotic policy may be helpful in control of antimicrobial resistance.

Keywords- Surgical site infection, Methicillin resistant Staphylococcus aureus, Inducible clindamycin resistance, D-test

I. Introduction
Infection is encountered by all surgeons: by the nature of their craft, they invariably impair the first line of host defenses — the cutaneous or mucosal barrier. The entrance of microbes into host tissues is the initial requirement for infection. For most of surgical history, death from infection was common, although it was not until the end of the nineteenth century that the bacterial cause of surgical infection was appreciated.¹

Surgical site infection (SSI) is defined as an infection occurring within 30 or 90 days after a surgical operation (or within 1 year if an implant is left in place after procedure) and affecting either incision or deep tissues at the operation site. These infections may be superficial or deep incisional infections or infections involving organ or body space.²

Surgical site infection rate has varied from a low of 2.5% to a high of 41.9%.³ Incidence of SSI in India reported to vary from 3.6% to 22.5%.² Sources of SSI can include the patient’s own normal flora or organisms present in the hospital environment. The common organisms encountered in post-operative wound infections are Staphylococcus aureus, Coagulase-negative Staphylococci, Enterococci, Proteus, Pseudomonas, Escherichia coli and Klebsiella species. In the case of wound infections following appendectomy or other lower bowel surgery, indigenous flora of the lower gastrointestinal tract like Escherichia coli are involved.⁴

Staphylococcus aureus is frequently isolated from post surgical wound infections, which may serve as nidus for development of systemic infections.

II. Material And Methods
This study was conducted in Department of Microbiology of KVG Medical College and Hospital, Sullia.

Specimen collection
Pus specimen was collected from all infected surgical sites under aseptic precautions from the depth of the wound by using 2 sterile swabs.

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Processing of samples
The specimens were brought to the laboratory within 2 h of collection and further processing was done immediately. First swab was used to make a smear on a clean, grease free slide. The second swab was immediately inoculated onto 5% sheep blood agar and MacConkey agar. The culture plates were incubated at 37°C for 18 to 24 hrs. All the biochemical tests were carried out using standard media (Hi Media laboratories Ltd, Mumbai). Antimicrobial susceptibility testing of all isolates was done by Kirby Bauer disc diffusion method as per CLSI guidelines.6

Detection of MRSA
All S. aureus strains were tested for methicillin resistance by Kirby Bauer’s disc diffusion method using cefoxitin (30μg) disc. An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and ≥ 22 mm was considered as methicillin sensitive.

Detection of inducible clindamycin resistance
‘D – TEST’
Mueller Hinton Agar plate was inoculated with staphylococcal bacterial suspension having turbidity equivalent to 0.5 McFarland’s turbidity standard. Erythromycin (15 μg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 μg) disk. Following overnight incubation at 37°C, flattening of zone (D shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance.

Interpretation
MS phenotypes: Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm). Inducible MLSB phenotypes (iMLSb): Staphylococcal isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc. Constitutive MLSB phenotypes (cMLSb): Staphylococcal isolates resistant to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm).

Statistical Analysis
All statistical analysis was performed using SPSS 11.5 version software. The association between different variables was tested using non-parametric tests.

III. Results
Out of a total 119 isolates, 53(44.54%) were Gram positive organisms out of which Staphylococcus aureus (28.57%) was the predominant isolate followed by coagulase negative staphylococci (10.92%) and Enterococcus sp.(5.05%).
Out of 53 Gram positive isolates, 34 were S.aureus and all were sensitive to linezolid(LZ) and vancomycin(VA), 30(88.24%) were sensitive to chloramphenicol(C) and clindamycin(CD), 26(76.47%) were sensitive to erythromycin(E), amikacin(AK), gentamicin(GEN) and pristinomycin(RP), 25(73.53%) were sensitive to tetracycline(TE), 15(44.12%) were sensitive to ciprofloxacin(CIP), 5(14.71%) were sensitive to cefalexin(CN). Only 1(2.95%) isolate was sensitive to ampicillin(AMP). (Fig. 1)

![Fig. 1: Antibiotic sensitivity pattern of Staphylococcus aureus isolates (34)](image-url)
Out of 13 coagulase negative staphylococci (CONS), all were sensitive to linezolid and vancomycin. 11(84.61%) were sensitive to pristinomycin, 10(76.92%) were sensitive to amikacin, 9(69.23%) were sensitive to gentamicin and tetracycline. 8(61.54%) were sensitive to clindamycin, 7(53.84%) were sensitive to ciprofloxacin, 6(46.15%) were sensitive to erythromycin, 1(7.69%) was sensitive to ampicillin and cefalexin. (Fig. 2)

Fig. 2: Antibiotic sensitivity pattern of Coagulase negative staphylococci (CONS) isolates (13)

Out of 6 Enterococcus sp., all were sensitive to linezolid and vancomycin. 5(83.33%) were sensitive to chloramphenicol, 4(66.66%) were sensitive to tetracycline, 3(50%) were sensitive to pristinomycin, 2(33.33%) were sensitive high strength gentamicin(HLG). 1(16.66%) was sensitive to erythromycin, amikacin and azithromycin(AZM). All 6(100%) isolates were resistant to ampicillin. (Fig. 3)

Fig. 3: Antibiotic sensitivity pattern of Enterococcus isolates (6)

In our study, 26(76.47%) of S.aureus were found to be MRSA and 10(76.92%) of CONS were found to be MRCONS, by Cefoxitin disc diffusion test. (Fig. 4 & Fig. 5)
Out of 26 MRSA isolates, all isolates were sensitive to vancomycin and linezolid, 23(88.46%) were sensitive to clindamycin, 22(84.61%) were sensitive to chloramphenicol, 20(76.92%) were sensitive to erythromycin, 19(73.08%) were sensitive to amikacin and pristinomycin, 18(69.23%) were sensitive to gentamicin and tetracycline and 11(42.31%) were sensitive to ciprofloxacin. All MRSA isolates were resistant to cepahexin and ampicillin.
Out of 8 MSSA isolates, 7(87.5%) were sensitive to clindamycin, amikacin, pristinomycin and tetracycline, 6(75%) were sensitive to erythromycin, 5(62.5%) were sensitive to cefalexin, 4(50%) were sensitive to ciprofloxacin and 1(12.5%) isolate was sensitive to ampicillin. All MSSA isolates were sensitive to gentamicin, chloramphenicol, vancomycin and linezolid.

In present study, out of 34 S.aureus, 8(23.53%) were resistant to erythromycin. 4(11.76%) were constitutive MLSB resistant, 2(5.88%) were inducible MLSB resistant (inducible clindamycin resistant) and 2(5.88%) belonged to MS phenotype. 26(76.47%) were susceptible to both erythromycin and clindamycin. (Fig. 6)

IV. Discussion

Surgical site infections are the 3rd most commonly reported Nosocomial infections and thus account for approximately a quarter of all nosocomial infections.\textsuperscript{7} SSI are recognized as a common surgical complication. Potential complications include tissue destruction, failure or prolongation of proper wound healing, incisional hernias, occasional bacteremia, recurrent pains, disfiguring and disabling scars. SSI also results in substantial morbidity, prolonged hospital stays and increased direct patient costs.\textsuperscript{7} SSI continues to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis.\textsuperscript{8,9}

S.aureus was the predominant bacterial species (28.57%) isolated, followed by P.aeruginosa (26%), Coagulase negative staphylococci (10.92%), K.pneumoniae (8.4%), E.coli(7.56%), Enterococcus spp (5.05%), C.freundii (5.05%), K.oxytoca (4.2%), Acinetobacter spp (2.52%) and C.koseri (1.68%). Similar findings were observed in several studies reported from India.\textsuperscript{10,11,12}
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S. aureus, the predominant isolate of our study exhibited almost total resistance (97.05%) to ampicillin. Most of other studies\textsuperscript{2,11,12} reported over 80% resistance to ampicillin. S. aureus also exhibited over 50% resistance to cephalaxin (85.29%) and ciprofloxacin (55.88%). Latter is high compared to previous reports.\textsuperscript{2,12}

Other antibiotics for which S. aureus exhibited resistance were erythromycin (23.53% vs 31.15% to 80%\textsuperscript{2,11,12}), clindamycin (11.76% vs 14.75% to 40.54%)\textsuperscript{11,14}, amikacin (23.53% vs 9% to 45%)\textsuperscript{11,13,15,16}, gentamicin (23.53% vs 11% to 66.6%)\textsuperscript{11,13,14,15,16}. However, all isolates were sensitive to linezolid which corroborates earlier reports.\textsuperscript{2,12} Unlike previous studies which have reported 3.28% to 18.10% resistance to vancomycin\textsuperscript{34,58} our S. aureus isolates were 100% susceptible to vancomycin.

In our study 76.47% of the isolates were MRSA’s which is in concurrence with other findings.\textsuperscript{13} All the MRSA were sensitive to both linezolid and vancomycin. While various other workers reported 100% vancomycin sensitivity of MRSA’s\textsuperscript{2,8,12}, Malik et al\textsuperscript{11} from lucknow and Reddy et al\textsuperscript{13} reported 3.28% & 18.10% vancomycin resistance respectively. All coagulase negative staphylococci (CONS) were sensitive to linezolid and vancomycin. Other antibiotics to which CONS exhibited resistance were ampicillin (92.30% vs 70% & 75%)\textsuperscript{11,14}, cephalexin (92.30%) ciprofloxacin (46.15% vs 75%)\textsuperscript{14}, erythromycin (53.84% vs 20%)\textsuperscript{17}, clindamycin (38.46% vs 50%)\textsuperscript{14}, amikacin (23.07%), gentamicin (30.77% vs 75% & 30%)\textsuperscript{14,17}, pristinomycin (15.38%), tetracycline (30.77% vs 50% & 20%)\textsuperscript{14,17} and chloramphenicol (46.15% vs 25% & 70%)\textsuperscript{14,17}.

Enterococci are intrinsically resistant to cephalosporins, penicillinase resistant penicillins and monobactams and exhibit low level resistance to many aminoglycosides, are intermediate susceptibility or resistant to fluoroquinolones. They are inhibited but not killed by them.\textsuperscript{18} All Enterococcus spp. isolated in our study were sensitive to linezolid and vancomycin; however vancomycin resistant enterococci are on the increase in USA\textsuperscript{18}. All 6(100%) isolates were resistant to ampicillin. 83.33% were resistant to erythromycin, amikacin and azithromycin. 66.66% were resistant to high strength gentamicin(120mcg), 50% were resistant to pristinomycin and 33.33% were resistant to tetracycline. Various other studies reported with regard to resistant patterns of Enterococcus spp.\textsuperscript{2,11,14}

Methicillin resistance among strains of S. aureus

In our study, 26(76.47%) of S. aureus were found to be MRSA. It was similar to incidence reported by Maksimovic et al\textsuperscript{19} and Reddy et al\textsuperscript{13}. Results of cefoxitin disc diffusion test is in concordance with the PCR for mcaA gene, and thus the cefoxitin disc diffusion method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA in resource constraint settings.\textsuperscript{20} Protective measures for health care workers against MRSA include contact isolation of the patient, using protective gown, gloves, mask and goggles and most importantly cleaning hands with alcoholic solution at glove removal and between patients. These measures are also of paramount importance to prevent the transmission of MRSA from patient to patient.\textsuperscript{21}

Antibiotic sensitivity of MRSA : In our study, all MRSA isolates were sensitive to vancomycin and linezolid. It was similar to findings of Jain et al\textsuperscript{2} from pune. Naik et al\textsuperscript{7}, Wassef et al\textsuperscript{8}, Mawalla et al\textsuperscript{7} reported 100% sensitivity of MRSA to vancomycin. Malik et al\textsuperscript{11} from lucknow reported 55.7% of MRSA and 3.28% VRSA but all were sensitive to linezolid.

Inducible Clindamycin resistance in S. aureus

The increasing frequency of staphylococcal infections among patients and changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin therapy to treat such infections. In our study, out of 34 S. aureus isolates, 26(76.47%) were sensitive to erythromycin and 30(88.23%) to clindamycin, 2(5.88%) were found to exhibit inducible clindamycin resistance, 4(11.76%) exhibited constitutive MLSB resistance and 2(5.88%) were MS phenotype and was found to be statistically not significant (p<0.05). Various other studies have reported different incidences.\textsuperscript{23,24,25,26}

However, widespread use of MLSB antibiotics has led to an increase in number of staphylococcal strains acquiring resistance to MLSB antibiotics. The most common mechanism for such resistance is target site modification mediated by erm genes which can be expressed constitutively (constitutive MLSB phenotype) or inducibly (inducible MLSB phenotype).\textsuperscript{23}

V. Conclusion

The most common causative agent of SSI is S. aureus. No vancomycin resistance is seen among species of Staphylococcus and Enterococcus. Methicillin resistance is a common problem in S. aureus which can be routinely detected by cefoxitin disc test. The MRSA strains in this study were most sensitive to linezolid and vancomycin followed by clindamycin. The presence of inducible clindamycin resistance among S. aureus strains indicates the importance of detection of such strains by D test to avoid treatment failure with clindamycin.

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