Bacterial Profile of Post-Operative Wound Infections and Evaluation of Povidone-Iodine

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Abstract: Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity. Post-operative wound infections are major global problem in the field of surgery leading to many complications leading to increased morbidity and mortality. A total 75 pus samples were collected from post-operative wound infections of which 63/75 (84%) bacterial isolates were obtained. Among the isolates, 29/63 (46%) were Gram positive and 34/63 (54%) were Gram negative organisms. The percentage prevalence of the bacterial isolates was as follows- *Staphylococcus aureus* 18/*63 (29%), Pseudomonas aeruginosa* 14/63 (22%), Coagulase negative staphylococci (CoNS) 11/63 (17*%), Klebsiella pneumoniae* 8/63 (13%), *Proteus vulgaris* 7/63 (11%) and *E.coli* 5/63 (8%). Staphylococcal isolates showed 100% sensitivity to vancomycin and linezolid. 5/18 (28%) of *S.aureus* and 4/11 (36%) CoNS were found to be methicillin resistant. All the gram negative isolates exhibited 94% sensitivity towards imipenem. Antibacterial activity of Povidone-Iodine (PVP-I) was evaluated. The MIC of PVP-I ranged from 1:4 to 1:64.

Keywords: Post-operative wound infections, Antibiotic resistance, MRSA, Povidone-Iodine

1. INTRODUCTION

Surgical wound infections are those infections which are confined to the incisions and involving structures adjacent to the wounds that were exposed during operation [1]. Hospital acquired surgical site infection (SSI) is one of the major health problems throughout the world and is a serious complication affecting hospitalized patients [2]. Among hospital acquired infections, SSI accounts for 14-16% of the inpatient infections [3]. SSI is dangerous condition with a heavy burden on the patient has been associated with an increased morbidity, mortality and health care cost that have huge economic impact [4].

Post-operative wound infection: is defined as an infection in the tissues of the incision and operative area that can commonly occurs between the fifth and 30th days after surgery. Surgical infections which are acquired in the hospitals are recognized to be associated with an extended length of hospital stay, pain, discomfort and sometimes prolonged or permanent disability. Surgical site infections (SSIs) are common complications that follow all types of operative procedures. These infections are usually caused by the exogenous and endogenous microorganisms that enter the operative wound during the course of the surgery [5]. The incidence of the infected surgical wounds may be influenced by factors such as pre-operative care, the theatre environment, post-operative care and the type of surgery.

Microorganisms can get access into a wound either direct contact and poor hand washing techniques of health care practitioners during pre and post operative phases of patient care are considered to be major factors [6]. The risk of developing a surgical wound infection is largely determined by three factors: the load, type of microbial contamination of the wound and host susceptibility [7]. Cleansing is a vital component of wound management. Iodine is a highly effective topical antimicrobial that has been used clinically in the treatment of wounds for more than 170 years. It has a broad spectrum of antimicrobial activity with efficacy against bacteria, mycobacteria, fungi, protozoa and viruses and can be used to treat both acute and chronic wounds [8]. Therefore, the present study was taken up to screen for the presence of aerobic bacteria from post-operative wounds, to detect antibiotic susceptibility pattern and to evaluate the antibacterial activity of povidone-iodine.

2. MATERIALS AND METHODS

2.1) Sample collection:

A surgical wound with pus discharge, wounds with serous or seropurulent discharge and negative cultures, but with signs of sepsis present concurrently (warmth, erythema, induration and pain) and the physician diagnosis was considered as surgical site infection [9]. Pus samples were collected from each patient with the help of two sterile cotton swabs under aseptic precautions, of which one was used for smear preparation and the other was used for culture.

2.2) Sample processing:

The first wound swab was used to make Gram stain smears and the second swab was inoculated onto blood agar, MacConkey agar and mannitol-salt agar. The plates were incubated at 37°C for 24–48 hours. Identification of Gram positive bacteria was done using catalase test, coagulase test and DNase test as per the standard protocols. Gram-negative bacteria were identified based on colony morphology on blood agar and MacConkey agar, followed by standard biochemical methods [10].

2.3) Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was carried out by Kirby Bauer disc diffusion method for the following antibiotics- (in μg/disc) [11]

Gram positive bacteria were tested against amikacin (30), gentamicin (10), netilmicin (30), tetracycline (30), erythromycin (15), linezolid (30) and vancomycin (30). Gram negative bacteria were tested against amikacin (30), gentamicin (10), tetracycline (30), chloramphenicol (30) and ciprofloxacin (5). Screening for methicillin resistance was done by cefoxitin disc diffusion method as per CLSI guidelines.

2.4) Evaluation of antibacterial activity of topical antiseptic- Povidone-Iodine (PVP-I)

Standard suspension of the isolates was made and the turbidity was matched to Mac Farland Standard 0.5. A series of doubling dilutions of the antiseptic ranging from 1:2 to 1:256 was made. 1ml Muller Hinton broth (MHB) was taken in tubes from 1 to 8. 1ml of the antiseptic solution was added in the first tube and 1 ml was transferred from the first tube to the second tube and consecutively 1ml of the contents in the second tube was transfer to the third tube. Doubling dilutions were continued in this manner till tube number 8. 0.1ml of the culture suspension was added in each tube and it was incubated at 37°C for 24hrs. Antiseptic solution was not added to the control tube. The highest dilution showing no growth was considered to be the Minimal Inhibitory Concentration (MIC).

2.5) Determination of Minimum Bactericidal Concentration (MBC)

The concentration of the test compound that completely killed the organism was taken as MBC. Samples were taken from the dilution tube and were spot inoculated on Muller Hinton agar plates and incubated at 37ºC for 24 hrs. The lowest concentration that showed no growth on plates was recorded as MBC.

3. RESULTS

A total of 75 pus samples were collected from post-operative wound infections of which 63/75 (84%) bacterial isolates were obtained. Among the isolates, 29/63 (46%) were Gram positive and 34/63 (54%) were Gram negative organisms. The percentage prevalence of the bacterial isolates was as follows- *Staphylococcus aureus* 18/*63* (29%), *Pseudomonas aeruginosa* 14/63 (22%), Coagulase negative staphylococci (CoNS) 11/63 (17%), *Klebsiella pneumoniae* 8/63 (13%), *Proteus vulgaris* 7/63 (11%) and *E.coli* 5/63 (8%). The most predominant gram positive organism was *Staphylococcus aureus* and among the gram negative organism the most predominant was *Pseudomonas aeruginosa.* (Figure 1 & 2)

Figure 1: Prevalence of Gram positive and Gram negative organisms from post-operative wounds.

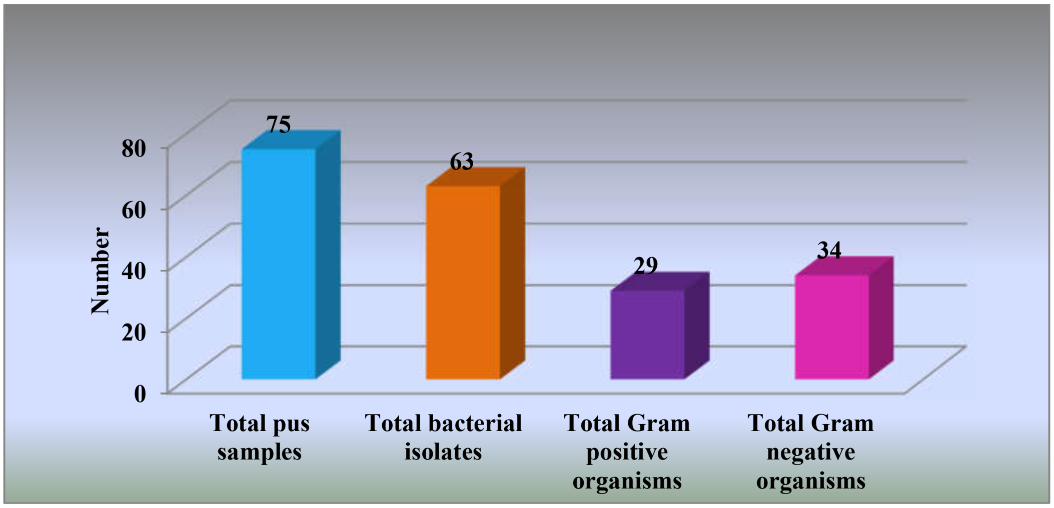
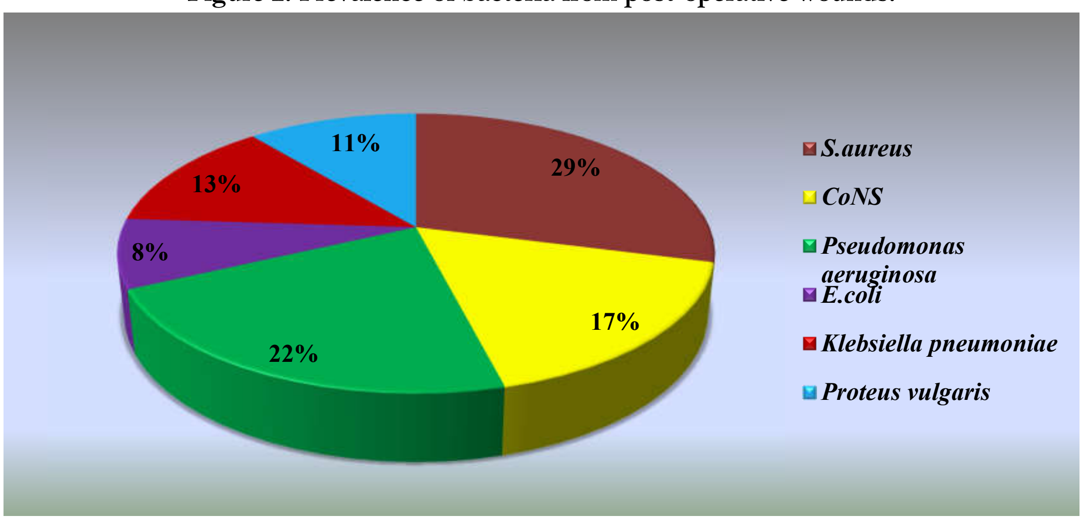


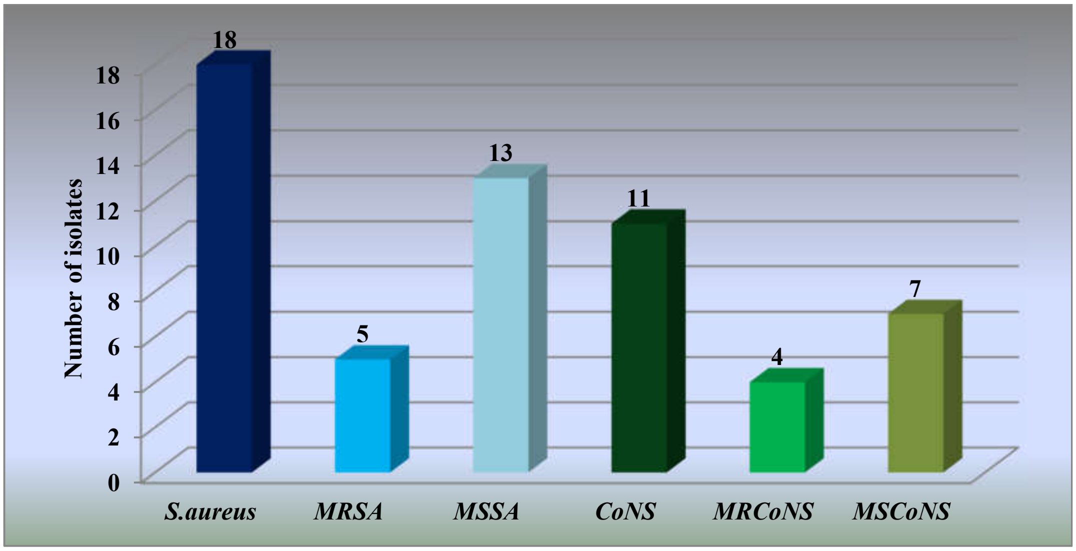
Figure 2: Prevalence of bacteria from post-operative wounds.



3.1) Antibiotic Sensitivity Testing:

S.aureus showed highest resistance towards netilmicin (71%) followed by amikacin (66%), erythromycin (61%), tetracyclin (58%) and gentamicin (28%). CoNS showed highest resistance towards amikacin (78%) followed by erythromycin (70%), netilmicin (63%), tetracycline (60%) and gentamicin (41%). *S.aureus* and CONS showed 100% sensitivity towards linezolid and vancomycin. Antibiotic resistance was found high among CoNS isolates compared to *S.aureus*. Methicillin resistance among staphylococcal isolates was done using cefoxitin disc diffusion method as per the CLSI guideline. 5/18 (28%) of *S.aureus* and 4/11 (36%) CoNS were found to be methicillin resistant. (Figure 3)

Figure 3: Prevalence of methicillin resistance among Staphylococcal isolates.



All the gram negative isolates exhibited 94% sensitivity towards imipenem. *Pseudomonas aeruginosa* showed highest resistance towards amikacin (84%). *E.coli* showed highest resistance towards chloramphenicol (80%) followed by tetracycline (76%) and amikacin (61%). *Klebsiella pneumoniae* showed highest resistance towards ciprofloxacin (70%) followed by chloramphenicol (66%) and amikacin (59%). *Proteus vulgaris* exhibited high resistance towards tetracycline (78%) followed by chloramphenicol (63%).

3.2) Evaluation of topical antiseptic- Povidone-Iodine (PVP-I)

The MIC was found to be same as MBC. MIC of PVP-I ranged from 1:4 to 1:64. The MIC of the staphylococcal isolates ranged between 1:4 to 1:32. The following was the MIC observed among the gram negative organisms- 1:2 to 1:16 for *Pseudomonas aeruginosa*, 1:4 to 1:32 for *E.coli*, 1:8 to 1:32 for *Klebsiella pneumoniae* and 1:4 to 1:64 for *Proteus vulgaris.*

4. DISCUSSION

Post-operative wound infections are a major problem in the field of surgery over the decades. The surveillance of nosocomial infections with an emphasis on antimicrobial audit will reduce the risk of postoperative wound infections and mortality [12]. Data from the past several years show an increasing resistance for drugs that were considered as the first line of treatment for post-operative wound infections this should be replaced with newer antibiotics [13].

In our study, a total of 75 pus samples were collected from post-operative wound infections of which 63/75 (84%) bacterial isolates were obtained. Wound infections rate in this present study was 84%. This was found to be in consistent with gowswani, 2011[14]. However, our finding was lower than reports from South Ethiopia 92% [15] and West Ethiopia 96.3% [16]. This difference in prevalence of post-operative infection may be due to variation in common nosocomial pathogens inhabitant, difference in policy of infection control and prevention between countries and hospitals.

In our study, 29/63 (46%) were Gram positive and 34/63 (54%) were Gram negative organisms. The percentage prevalence of the bacterial isolates was as follows- *Staphylococcus aureus* 18/*63* (29%), *Pseudomonas aeruginosa* 14/63 (22%), Coagulase negative staphylococci (CoNS) 11/63 (17%), *Klebsiella pneumoniae* 8/63 (13%), Proteus *vulgaris* 7/63 (11%) and *E.coli* 5/63 (8%). The most predominant gram positive organism was *Staphylococcus aureus.* This was found to be in agreement with that of Mahmood (2010) [17] and among the gram negative organism the most predominant was *Pseudomonas aeruginosa* which was similar to other study done by Verma *et al*. (2012) [18] and Anbumani *et al.*(2006)[19].

Antimicrobial resistance has been a problem in the field of surgery, as advances in control of infections have not completely eradicated this problem [20]. The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant pathogens contributing to morbidity and mortality. The antibiotics resistant pathogens are acquired either from hospital environment or from the skins of infected patients [21]. Hospital acquired infections are further complicated by an increasing prevalence of multidrug resistant organisms like MRSA, methicillin resistant coagulase negative *Staphylococci* (MRCoNS) and vancomycin resistant *Entrococci* (VRE) spp [22].

In the present study*, S.aureus* showed highest resistance towards netilmicin (71%) followed by amikacin (66%), erythromycin (61%), tetracyclin (58%) and gentamicin (28%). CoNS showed highest resistance towards amikacin (78%) followed by erythromycin (70%), netilmicin (63%), tetracycline (60%) and gentamicin (41%). *S.aureus* and CONS showed 100% sensitivity towards linezolid and vancomycin. Antibiotic resistance was found high among CoNS isolates compared to *S.aureus*. A study by Weigelt *et al.* (2010) [23] in USA, found an incidence of 20.6% MRSA in SSIs. Still higher incidences of 45% and 58.2% MRSA have been documented by Eagye *et al. (*2009) [24] and Keith *et al.* (2009) [25]. Our study showed, 5/18 (28%) of *S.aureus* and 4/11 (36%) CoNS isolates resistant to methicillin.

All the gram negative isolates exhibited 94% sensitivity towards imipenem. *Pseudomonas aeruginosa* showed highest resistance towards amikacin (84%). *E.coli* showed highest resistance towards chloramphenicol (80%) followed by tetracycline (76%) and amikacin (61%). *Klebsiella pneumoniae* showed highest resistance towards ciprofloxacin (70%) followed by chloramphenicol (66%) and amikacin (59%). *Proteus vulgaris* exhibited high resistance towards tetracycline (78%) followed by chloramphenicol (63%). Majority of the gram negative isolates were sensitive to imipenem and this was found to be in agreement with Ranjan *et al.,* 2010[26]. Postoperative wound infection in any hospital depends on the hospital environment and use of antibiotics. Antibiotic resistance can be controlled by appropriate antimicrobial prescription, prudent infection control, new treatment alternatives and continued surveillance.

The antimicrobial properties of iodine were first demonstrated in 1882 by Davaine [27]. In the First World War, iodine was found by Alexander Fleming to reduce the incidence of gas gangrene in the wounds of soldiers when compared to carbolic acid [28]. Since the mid-19th century, iodine-based preparations have an important role in the prevention of surgical site infections. Povidone iodine preparations are commonly used as an antiseptic to decolonise the patient's skin before surgery and are also used by surgeons and health care workers as a skin cleanser and antiseptic in pre-operative hand scrubs.

In the present study, the antibacterial activity of PVP-I was evaluated and the MIC of PVP-I ranged from 1:4 to 1:64. The MIC of the staphylococcal isolates ranged from 1:4 to 1:32. The following was the MIC observed among the gram negative organisms- 1:2 to 1:16 for *Pseudomonas aeruginosa*, 1:4 to 1:32 for *E.coli*, 1:8 to 1:32 for *Klebsiella pneumoniae* and 1:4 to 1:64 for *Proteus vulgaris.*

5. CONCLUSION

Post-operative wound infections were found to be high 84%. 28% of MRSA and 36% MRCoNS were obtained in this study. Both the gram positive and gram negative organisms showed multidrug resistance leaving clinicians with few choices of drugs for the treatment of post-surgical wound infected patients. Indiscriminate use of antibiotics should be prevented and continued surveillance should be done to prevent further development of bacterial drug resistance. An effective national & state level antibiotic policy along with infection control measures and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management. PVP-I was found to be effective in inhibiting the growth of multidrug resistant organisms. Therefore, it can be used as an antiseptic as a skin cleanser and in hand scrubs.

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