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Prevalence of Methicillin Resistant *Staphylococcus aureus* Post Operative Wound Infections in a Trust Hospital

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A B S T R A C T

Post-operative wound infection is an important cause of morbidity and cost burden for the patients. Surveillance of these data is an important foundation of effective infection control programs. To determine the prevalence of MRSA among *Staphylococcus aureus* isolates. A prospective study done in a trust hospital in 2015 for about a year. A total number of 86 isolates were obtained from 120 wound swabs. The screening and confirmation of MRSA production was done by Cefoxitin disc diffusion method among the *S.aureus* isolates. The predominant pathogen was *Staphylococcus aureus* (50%) followed by *Klebsiella spp* (19.7%) and *Escherichia coli* (12.7%). Out of 43 *S. aureus* isolates 16 (37.2%) were detected as MRSA. The drugs highly effective for GPC were Vancomycin (100%) & Gentamicin (91.1%) and for GNB Imipenem (100%) and Piperacillin / Tazobactum (97.5%). This study concludes that *Staphylococcus aureus* is the most common pathogen in post operative wound infections with increasing tendency towards MRSA. Since the *S.aureus* constitutes the common skin and nasal flora, stringent monitoring of aseptic surgical and health care procedures, pathogen surveillance and effective anti microbial sensitivity screening are essential to avoid the poor outcome of surgical treatment. By adhering to the strict infection control measures and rational antibiotic policies, we can prevent the emergence and dissemination of MRSA in our community.

Introduction

Post operative wound infections (POWI) or surgical site infections (SSI) are the most common Procedure and Health care associated nosocomial infections (14 %-16%) (Centers for Disease Control and Prevention, 2007). SSIs are identified in surgical ward patients mostly associated with Cardiac, Abdominal, Orthopedics and Neurosurgery procedures (Ibtesam *et al.*, 2010). Postoperative wound infections can be caused by two major sources: exogenous and endogenous bacteria (Amrita *et al.*, 2010). The most common predisposing factors for these infections are prolonged hospital stay, increased duration of surgery, extensive handling of tissues during procedures, impaired immune status of the patient, nutritional deficiency. These factors will lead to increased morbidity and mortality in postoperative patients. Micro-organisms responsible for wound infection depends on the surgical site, patient's systemic host defenses, local wound conditions, microbial burden and antimicrobial use in the hospital. The outcome of antimicrobial therapy, both prophylactically and therapeutically can only be defined when these factors are under control. These infection rates are an established measure of quality of clinical care and provision of reliable surveillance data is the foundation for effective infection control programs (Khaleid *et al*., 2010).

Antimicrobial-resistant pathogens that cause healthcare-associated infections pose an increasing challenge to hospitals, both in the clinical treatment of patients and in the prevention of the cross-transmission of this problematic pathogens (Khaleid *et al*., 2010). Since the regular surveillance could play a significant role in the early recognition & intervention for better management, the study was designed to evaluate the prevalence of bacteriological profile in surgical site infections.

The main of this study includes, to detect the bacterial profile, to evaluate their antimicrobial sensitivity pattern in post operative wound infections and to identify the MRSA among *Staphylococcus aureus* isolates.

Materials and Methods

A prospective study was conducted during the period from December 2014- November 2015 in a trust hospital, shevapet, Salem, after obtaining informed consent from patients admitted in post operative wards with complaints of wound discharge and pain in the operated site. The samples were collected from total no of 120 patients, two samples (swabs) from each were collected and transported to the Microbiology laboratory immediately and processed as per standard guidelines.

Bacterial isolation procedure

The first swab was used for direct Gram's staining and the smear was observed for pus cells & bacteria under oil immersion (100x). The second swab was inoculated immediately into the following media for bacterial isolation namely, Nutrient agar, MacConkey agar and Blood agar. Streak culture method was followed for inoculation, and the plates were incubated at 37°C for 24 hours. After incubation, the pure isolates were observed and identified by their colony morphology which were then confirmed & speciated as per standard biochemical reactions (Washington *et al*., 2006).

Antimicrobial sensitivity testing was done for all bacterial isolates as per Clinical and Laboratory Standards Institute (CLSI) guidelines by Disc Diffusion method. The Gram positive cocci were tested against the panel of antibiotic discs like Ampicillin (10 µg), Erythromycin (15 µg), Amox/clav (20/10 µg), Cotrimoxazole (23.75/ 1.25 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg) and Vancomycin (30 µg). Along with the above discs for screening & confirmation of Methicillin resistance Cefoxitin (30 µg) discs were used[7] and Quality control strains, MRSA ATCC 43300 and Methicillin-sensitive *S. aureus* (MSSA) ATCC 25923, were used as positive and negative controls respectively (Clinical and Laboratory Standards Institute, 2013; Bhadravathi *et al*., 2014).

The Gram negative bacilli were tested against the panel of antibiotic discs namely, Cotrimoxazole (23.75/1.25µg), Amikacin (30 µg) Gentamicin (10 µg), Ciprofloxacin (5 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Amox/clav (20/10 µg), Imipenem (10 µg) and Piperacillin / Tazobactum (100/10µg). The results were recorded and interpreted as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI).

Results and Discussion

Out of total population (120), 73 male (60.8%) and 47females (39.1%) were affected by post operative wound infection. From total samples, 86 were positive for culture which includes 52 male (60.4%) and 34 females (39.5%). The samples collected from General surgery and OG departments showed culture positivity in 61(70.9%) and 25(29%) samples respectively.

Among the 86 culture positive specimens, 45 Gram Positive Cocci (52.3%) and 41Gram Negative Bacilli (47.6%) were isolated. Among the isolates, *Staphylococcus aureus (*50%) was the predominant isolate, followed by Klebsiella *spp* (19.7%), *Escherichia coli* (12.7%), *Proteus spp* (8.1%), *Pseudomonas aeruginosa* (6.9%) and the least is *CONS* (2.3%) [Chart -1].

The Antibiotic sensitivity pattern of Gram positive cocci showed sensitivity to Ampicillin (33.3%), Erythromycin (28.8%), Amox/clav (55.5%), Cotrimoxazole (68.8%), Gentamicin (91.1%), and Ciprofloxacin (75.5%) and Vancomycin (100%). Among 43 *Staph aureus* isolates 16(37.2%) were identified and confirmed phenotypically as MRSA by Cefoxitin disc diffusion method. The results with zone inhibition diameter for cefoxitin (30 µg per disc) ≤ 21 mm indicated resistance to Methicillin [Table -1].

The antibiotic sensitivity pattern of Gram Negative Bacilli showed sensitivity to Cotrimoxazole (34.1%), Amikacin (87.8%), Gentamicin (85.3%), Amox/clav (51.2%), Ciprofloxacin (65.8%), Cefotaxime (90.2%), Ceftazidime (46.3%), Imipenem (100 %) and Piperacillin /Tazobactum (97.5%) [Table - 2].

The incidence of post operative wound infections is increasing in alarming rate and has been a problem in the field of surgery for a long time. Most of the organisms occur as saprophytes and colonise the skin, nasopharynx and gastro-intestinal tract with little potential for causing disease because they induce first line defense mechanisms inside the body. Skin barrier is disrupted by surgical procedures, diseases, nutritional deficiency and other factors that affect the first line defenses.

The present study showed that male patients (60.4%) were affected more than females (39.5%), and the most affected age group was >50 years of age. which is similar to the study by Amrita *et al.*, (male 72% and females 28%) and Adegoke *et al.*, This may be due to the increased prevalence of other illnesses like diabetes, malnutrition etc and increased chances of injury for male patients by occupation, and they are more prone to acute abdomen problems than females

In this present study S.aureus (50%) was the predominant isolate in postoperative wound infection. [Table 1] This is supported by various studies like Amrita *et al* study which showed it to be 63 %, other studies by Khaleid *et al.*, showed 42.6%, Arti *et al.*, as 32%, and Adegoke *et al.*, *as* 25% (Afrough *et al*., 2012; Sarita yadav *et al*., 2010).

The present study showed, the range of Gram negative isolates include *Klebsiella spp* (19.7 %), Escherichia *coli* (12.7%), *Proteus spp* (8.1%) and *Pseudomonas aeruginosa* (6.6%). This result is supported by Amrita *et al's* study showing the Gram negative isolates in post operative wound infections namely *Escherichia coli* 9.5%, *Pseudomonas* spp 5 %, *Klebsiella spp* 3.5% and *Proteus spp* 3.5%. Khaleid M. *et al* study showed that *Klebsiella spp*, *P. aeruginosa* and *E. coli* were detected in the frequency of 19.2%, 10.6% & 4.3% respectively and also in Maida Sisirak *et al.*, study *Escherichia coli* 8.7%, *Pseudomonas* spp 8.9 %, *Klebsiella spp* 3.5% and *Proteus spp* 6.5% were isolated in surgical site infections.

Antimicrobial sensitivity of Gram positive cocci showed highest sensitivity for Vancomycin 100%. The lowest sensitivity pattern was towards Ampicillin 33.3% and

Erythromycin 28.8% [Table 2]. This report is similar to Amrita *et al.,* and Adegoke *et al.,* studies which showed sensitivity to Vancomycin 100%, Ampicillin 1.8% &15%, and Gentamicin 98% & 20% respectively.

In the present study, sensitivity pattern of Gram Negative Bacilli showed highest sensitivity towards Imipenem 100%, Piperacillin/ Tazobactum 97.5%, and other third generation Cephalosporins 60-90% and Amino glycoside 86% & Quinolones 60-70% respectively. The lowest sensitivity was towards Cotrimoxazole 34.1% [Table 3]. This is similar to the study by Amrita *et al.,* which showed sensitivity to Aminoglycosides, Quinolones and Cephalosporins in the range of 40- 60%.

In this study, 20 MRSA strains (46.5%) were isolated out of 43 *Staph aureus* isolates [Table 2]. This report is similar to other studies by Khaleid *et al.,* (65%), Sarita *et al.,* (60.6%), Bhutia *et al.,* (42.1%) and Pillai *et al.,* (53.4%) with MRSA isolates.

In conclusion, the Centre for Disease Control and Prevention (CDC) in Atlanta, USA, estimates that 2.7% of surgical procedures are complicated by infections. Surgical site infections (SSI) represent 16% of all hospital-acquired infections, making them the third most frequent type of nosocomial infections in developed countries. These infections increase the mortality and morbidity, prolong hospitalization and have adverse impact on clinical outcome (Kirkland *et al.,* 1999). It is very useful to explore the causative agents of these infections to demonstrate the magnitude of the problem.

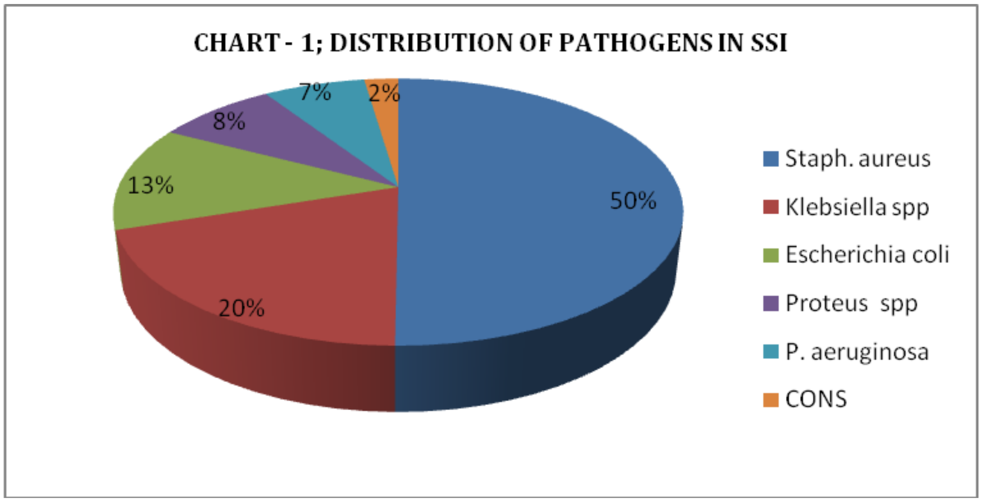
Table.1 Antimicrobial sensitivity pattern for GPC (number and percentage)

S.No	Name of the Drugs	*Staph. aureus* (N=43)	CONS(N=2)	Total no of Isolates (N=45)
1.	Ampicillin (A)	15 (34.8%)	0 (0%)	15 (33.3%)
2.	Erythromycin (E)	13 (30.2%)	0 (0%)	13 (28.8%)
3.	Amox/clav (AMC)	24 (55.8%)	1(50%)	25 (55.5%)
4.	Cotrimoxazole (CO)	30 (69.7%)	1(50%)	31 (68.8%)
5.	Gentamicin (G)	39 (90.6%)	2(100%)	41 (91.1%)
6.	Ciprofloxacin(CF)	31 (72%)	2 (100%)	34 (75.5%)
7.	Vancomycin (V)	43 (100%)	2 (100%)	45 (100%)
8.	Cefoxitin (CX)	16 (37.2%)	2 (100%)	18 (40%)

Table.2 Antimicrobial sensitivity pattern for GNB (number and percentage)

S.No	Name of the Drugs	*Klebsiella* spp (n= 17)	*E.coli* (n-11)	*Proteus* spp (n=7)	*P. aeruginosa* (n= 6)	Total no of isolates (n-41)
1	Cotrimoxazole (CO)	6 (35.2%)	5 (45.4%)	3 (42.8%)	0(0%)	14(34.1%)
2	Amikacin (AK)	16 (94.1%)	10 (90.9%)	5 (71.4%)	5 (83.3%)	36 (87.8%)
3	Gentamicin (G)	15 (88.2%)	10 (90.9%)	5 (71.4%)	5 (83.3%)	35 (85.3%)
4	Ciprofloxacin (CF)	12(70.5%)	8 (72.7%)	4 (57.1%)	3 (50%)	27 (65.8%)
5	Cefotaxime (CE)	15 (88.2%)	10 (90.9%)	5 (71.4%)	2 (33.3%)	37 (90.2%)
6	Ceftazidime(CAZ)	10 (58.8%)	5 (45.4%)	2 (28.5%)	2 (33.3%)	19 (46.3%)
7	Imipenem(IMP)	17 (100%)	11 (100%)	7 (100%)	6 (100%)	41 (100%)
8	Pip / Taz (PIT)	17 (100%)	10 (90.9%)	7(100%)	6 (100%)	40 (97.5%)
9	Amox/Clav (AMC)	9 (52.9%)	8 (72.7%)	3 (42.8%)	1(16.6%)	21(51.2%)

Chart .1



In this study among total 120 samples, 86 were culture positive. Out of them (60.4%) were male and 44(39.5%) were females. Predominant isolate among total organisms 43 were *S.aureus*. Among the *S.aureus* isolates, 20 (46.5%) MRSA were detected by Cefoxitin disc diffusion method. All MRSA isolates were sensitive to Vancomycin.

The study showed *Staphylococcus aureus* as the major pathogen in post operative wound infections and increasing incidence of MRSA in wound infection. Since the patients colonized with MRSA have an increased risk for developing infection compared with non-colonized patients and it can be easily transmitted to inpatients through health workers and patient attenders. Therefore, rapid and accurate identification of multi-drug resistant Staphylococcus aureus is crucial and essential both for initiation of appropriate antimicrobial therapies and for effective infection control strategies to limit the spread of MRSA. In spite of the fact that there are many new rapid and less costly methodologies, the traditional susceptibility testing, such as disk diffusion method with cefoxitin, is available in every bacteriological laboratory and this method can be performed easily to detect MRSA (Mohammed Reza *et al.*, Gargi Dangre *et al.*, & Shadi *et al.*,). It also remains the most recommended methods of CLSI. The present study showed that *S. aureus* isolates were also resistant to other common & routinely used antibiotics like Erythromycin and Cotrimoxazole. A general overview of the antibiogram of all the bacterial isolates indicated that Gram positive bacteria exhibited a greater level of antimicrobial sensitivity to Vancomycin (100%) and Gram negative bacteria to Imipenem (100%) & Piperacillin/ Tazobactum (97.5%) So it is the need of the hour to evaluate the infection control measures such as the proper pathogen surveillance, antimicrobial sensitivity pattern and enforcement of hand washing by health workers and these may help in arresting the spread of MRSA and other resistant pathogens in hospital settings. An antibiotic policy and the monitoring of sensitivity patterns for pathogens may also help in decreasing the prevalence of MRSA and antibiotic resistance, thereby preventing the morbidity and mortality in postoperative patients.

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