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INCIDENCE OF SURGICAL SITE INFECTIONS, THEIR ETIOLOGY, ASSOCIATED ANTIMICROBIAL USE AND ANTIMICROBIAL RESISTANCE IN A TERTIARY CARE CENTRE IN NORTHERN INDIA.

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ABSTRACT

Introduction: Surgical site infections are third most commonly reported nosocomial infection. Reported surgical site infection rate in India has varied from a low of 2.5% to a high of 41.9% over a period of decades. **Material and Methods:** This study was done on patients who underwent major operative procedures between 21 Jan to 26 August 2011. Total 2536 cases were operated, of these 228 (8.99%) cases developed SSI. Ceftriaxone, Amikacin and Metronidazole were most commonly used antimicrobial agents during preoperative and postoperative periods. Wound status was assessed according to Southampton Scoring. Samples were collected by proper aseptic techniques. Quality of specimen was assessed by Q-score. Culture and sensitivity was done as per standard microbiological procedures. ESBL, AmpC, MBL, MRSA, HLAR were detected. **Results:** Of 228 cases, 60% cases were contaminated, 3.6% dirty and 36.4% clean wounds respectively. 72.7% cases had superficial SSI while 34.5% had deep SSI. According to Southampton scoring, 56.4% were classified under grade IV (purulent discharge along wound), 34.5% cases grade V (wound dehiscence), 7.2% grade III (prolonged serous discharge) while 2% were grade II (tender localised swelling). Polymicrobial etiology was observed in 17 and monobacterial in 211 cases. The main pathogens being gram-positive bacteria (51.7%). *S. aureus* (46.5%) being the most common organism followed by *E. coli* (32.0%), *Citrobacter spp* (9.6%). Among patients falling under Southampton grade IV & V, *Citrobacter sp* (30.0%) dominated followed by *E. coli* (18.1%), *S. aureus* (16.3%). Among these 30% were ESBL and 64.7% were AmpC producers while 39.6% were MRSA strains. No MBLs were detected. **Conclusion:** Coryneforms exhibited maximum resistance with majority sensitive to only vancomycin. Maximum drug resistance was observed in Grades IV and V SSI.

KEYWORDS: Multidrug resistance, surgical site infection, southampton scoring.

INTRODUCTION

Surgical site infections are third most commonly reported nosocomial infection & they account for approximately a quarter of all nosocomial infections.[1] They have been responsible for increasing cost, morbidity and mortality related to surgical operations and continues to be a major problem even in hospitals with most modern facilities & standard protocols of preoperative preparation and antibiotic prophylaxis.[2] Reported surgical site infection rate in India has varied from a low of 2.5% to a high of 41.9% over a period of decades.[3,4] In 1992, the Centre for Disease Control and Prevention (CDC)'s National Nosocomial Infections Surveillance (NNIS) system modified the definition of surgical wound infection slightly and changed the name to surgical site infection.[5] The increasing number of immunocompromised patients and increased use of indwelling devices, as well as widespread use of antimicrobial agents in hospital settings, particularly in intensive care units (ICUs), contributes to antimicrobial resistance among pathogens causing nosocomial infections.[6,7]

Organisms associated with SSIs vary with the type of procedure and the anatomic location of the operation. In clean surgical procedures, *Staphylococcus aureus* is the usual cause of infection.[4] In other categories of surgical procedures, including clean-contaminated, contaminated, and dirty, the polymicrobial aerobic and anaerobic flora closely resembling the normal endogenous microflora of the surgically resected organ are the most frequently isolated pathogens.[8]

Antimicrobial-resistant pathogens that cause healthcare-associated infections (HAIs) pose an ongoing and increasing challenge to hospitals, both in the clinical treatment of patients and in the prevention of the cross-transmission of these problematic pathogens.[9] These pathogens include methicillin-resistant *Staphylococcus aureus*(MRSA), vancomycin-resistant *Enterococcus*

species, extended-spectrum β-lactamase– producing *Escherichia coli* and *Klebsiella*species, and fluoroquinolone- or carbapenem- resistant Enterobacteriaceae or *Pseudomonas aeruginosa*. The scenario worsens in cases of metallo-beta-lactamase production where the drugs of last resort—the carbapenems—are rendered inactive.[10]

This study was conducted in Department of Microbiology, Jawaharlal Nehru Medical College and Hospital with the objective to determine the pathogens associated with surgical site infections (SSIs), analyse patterns of antimicrobial usage and drug resistance in post-operative patients admitted in departments of Orthopedics, Surgery and Obstetrics & Gynaecology.

MATERIAL AND METHODS

Study Group

The study group consisted of consecutive patients who underwent major operative procedures over a period of 8 months from Jan to August 2011. Three main operative departments were included in the study. Postoperative patients included in this study were those admitted in orthopedic, surgery and obstetrics and gynaecology wards.

A total of 2536 cases were operated over this period (998 orthopedic ward, 1252 surgical ward, 286 Obstetrics &gynaecology ward. Out of these 228 (8.99%) cases developed SSI.

History & Clinical evaluation of Wound

Detailed history was elicited from each patient developing SSI. Details that were recorded include fever, pain, any systemic symptoms, type of operation, drain used, antibiotic prophylaxis, preoperative hospital stay and total stay. History of antibiotic intake during pre and post operative period was also taken. Patients were followed up till discharge from hospital.

Wound was inspected at the time of first dressing, 4th and 9th day of surgery. Wound status was assessed according to Southampton Scoring.[11] Wounds were further categorized as superficial and deep & whether they were clean, contaminated and dirty.

Sample collection

Samples were collected by proper aseptic techniques. Two swabs were collected per patient. Samples were also collected in sterile syringes where ever possible and sent to microbiology laboratory without delay or kept in refrigerator if some delay was occurred.

Processing of samples

Direct microscopy was done by gram staining. Quality of specimen was assessed by Q-score.[12] Culture was done on 5% sheep blood agar, Mac conky agar and nutrient broth. Identification was done as per standards.[13]

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed by disc diffusion method by the Kirby Bauer technique according to CLSI guidelines on Mueller Hinton agar.[14] Gram positive isolates were tested against Amikacin (30 µg), Gentamicin (10µg), Levofloxacin (5 µg), Sparfloxacin (5 µg), Erythromycin (15µg), Vancomycin (30µg), Oxacillin (1µg), Tobramycin (10µg), Clindamycin (2µg), Amoxycillin (30µg).

Gram negative isolates were tested against, Amikacin (30 µg), Gentamicin (10µg), Levofloxacin (5 µg), Sparfloxacin (5 µg), Ceftriaxone (30 µg), Cefoperazone (75 µg), Cefoperazone-sulbactam (75 µg, 1:1), Cefixime (5 µg) Ceftriaxone-salbactam (30/15µg), Piperacillin (100 µg), Piperacillin-tazobactum (100:10 µg), Cefotaxime (30 µg) and Tobramycin (10 µg), Cefoxitin (µg), Imipenem (10µg). All discs were obtained from HiMedia, India.

Detection of extended spectrum beta lactamases

ESBL detection was done by DDST using cefoperazone (CP) and cefoperazone-sulbactam (CPS) combination.[10]

Detection of inducible and derepressed Amp Cbeta lactamase

Isolates resistant to ceftriaxone, cefixime, cefoperazone and cefoperazonesulbactam, and cefoxitin were tested for AmpC production. Organism sensitive to cefoxitin and resistant to cefoperazone and cefoperazone combination were considered to be Amp C producers.[14]

Detection of Metallo-beta-lactamases

If the zone of Imipenem was reduced to 16-20 mm or less or heaping occurred, we tested the isolate for MBL production. Hodge test and Double Disc synergy test using EDTA were used for detection of MBL. The method was as described by *Lee et al.* [15]

Screening for Methicillin resistance using Oxacillin disc test

Test was performed on Muller Hilton agar with 4% NaCl using Oxacillin 1µg disc. Any decrease in sensitivity zone was considered as resistant.

HLAR Resistance

In case of *Enterococcus*, HLAR was detected using High content gentamycin (120µg) and streptomycin (300µg).

RESULTS

A total of 2536 cases were operated over period of 8 months (998 orthopedic ward, 1252 surgical ward, 286 Obstetrics & gynaecology ward). Out of these 228 (8.99%) cases developed SSI (72 from orthopedic ward, 99 from surgical ward, 57 from Obstetrics &gynaecology ward) as seen in Table 1. Among the orthopedic and surgery patients who developed SSI, 12(16.6%) and 21(21.2%) were females while 60(83.3%) and 78(78.7%) were males respectively.

Of the 228 patients who developed SSI, 137(60%) cases had contaminated, 8(3.6%) dirty and 83(36.4%) clean wounds respectively. 150(65.5%) cases had superficial SSI while 78(34.5%) had deep SSI. 50% had fever. Ceftriaxone, Amikacin and Metronidazole were most commonly used antimicrobial agents during preoperative and postoperative periods.

According to Southampton scoring, 56.4% were classified under grade IV (purulent discharge along wound), 34.5% cases grade V (wound dehiscence), 7.2% grade III (prolonged serous discharge) while 2% were grade II (tender localised swelling) as shown in Table 2. Monobacterial etiology was observed in 211 and polymicrobial in 17 cases. Gram-positive bacteria dominated with 118(51.7%) cases. *S.aureus* (46.5%) was the most common pathogen followed by *E.coli* (32.0%), *Citrobacter spp* (9.6%) as shown in Table 2.

Gram negative etiology 126(61%) was observed in majority of patients falling under Southampton grade IV & V with *Citrobacter sp* 63(30.0%) dominating followed by *E.coli* 38(18.1%), *P.aeruginosa* 19(9.0%) and others 19(9.0%). Gram positive etiology was observed in 81(40%) cases with *S.aureus* 34(16.3%), *Coryneform spp* 34(16.3%) being seen in equal number.

Among the Enterobacteriaceae, susceptibility profile of *E.coli* to aminoglycosides, fluoroquinolones, cephalosporins, and cephalosporin + β-lactamase inhibitor combination was 35.1%, 51.35%, 14.81% and 53.4% respectively while that of *Citrobacter species* was 37.9%, 36.35%, 23.87% and 45.5% respectively. *P.aeruginosa* was more resistant with 25.36%, 16.6%, 10.76% and 33.3% sensitivity demonstrated against aminoglycosides, fluoroquinolones, cephalosporins, and cephalosporin + b-lactamase inhibitor combination respectively (Fig 1) *Staphylococcus aureus* was most susceptible to erythromycin (70.7%), azithromycin (63.2%), and sparfloxacin (54.7%). *Coryneforms* and *Enterococcus* were resistant to all antibiotics used except vancomycin (Fig 2).

Among gram negative bacteria 30% were ESBL and 64.7% were AmpC producers. No MBLs were detected with all isolates being sensitive to imipenem. *Klebsiella spp* and *Acinetobacter spp* showed maximum resistance with all strains being AmpC producers. Among the gram positive isolates 39.6% strains of *S.aureus* were methicillin resistant No HLAR was detected in *Enterococcus spp*. (Table 4). Maximum drug resistance was observed in Grades IV and V SSI. Among which 84(66%) were Amp C, 36(28%) were ESBL producers, 14(41.1%) were MRSA strains.

Table 1: Incidence of SSI in different operative wards.

Name of ward	No. of cases operated(n=2536)	Incidence of SSI (n=228)
Orthopaedics	998	72 (31.5%)
Surgery	1252	99 (43.4%)
Obs&gynae	286	57 (25%)

Table 2: Distribution of SSI according to Southamptons grading.

Southampton's grade	No. of cases	Polymicrobialetiology	Monomicrobialetiology
Grade I (mild bruising)	0	-	-
Grade II (erythema, tenderness, localised swelling)	4 (2%)	-	4
Grade III (clear/ hemoserous discharge)	17 (7.2%)	-	17
Grade IV (purulent discharge)	128 (56.4%)	9	119
Grade V(deep/severe wound infection)	79 (34.5%)	8	71

Table 3: Etiology in SSI and resistance pattern of different isolates.

Organism	Incidence Number (%)	No. of resistant strains	Type of resistance
S.aureus	106 (46.5%)	42 (39.6%)	MRSA
E.coli	73 (32%)	25 (34.2%)	ESBL
		34 (46.5%)	Amp C
Citrobactersp	22 (9.6%)	4 (18.18%)	ESBL
		12 (54.5%)	Amp C
P.aeruginosa	21 (9%)	5 (23.8%)	ESBL
		13 (61.9%)	Amp C
Coryneformsp	9 (3.9%)	9 (100%)	-
Enterococcus sp	3 (1.3%)	0 (0%)	HLAR
Klebsiellasp	3 (1.3%)	3 (100%)	Amp C
Acinetobactersp	3 (1.3%)	3 (100%)	Amp C

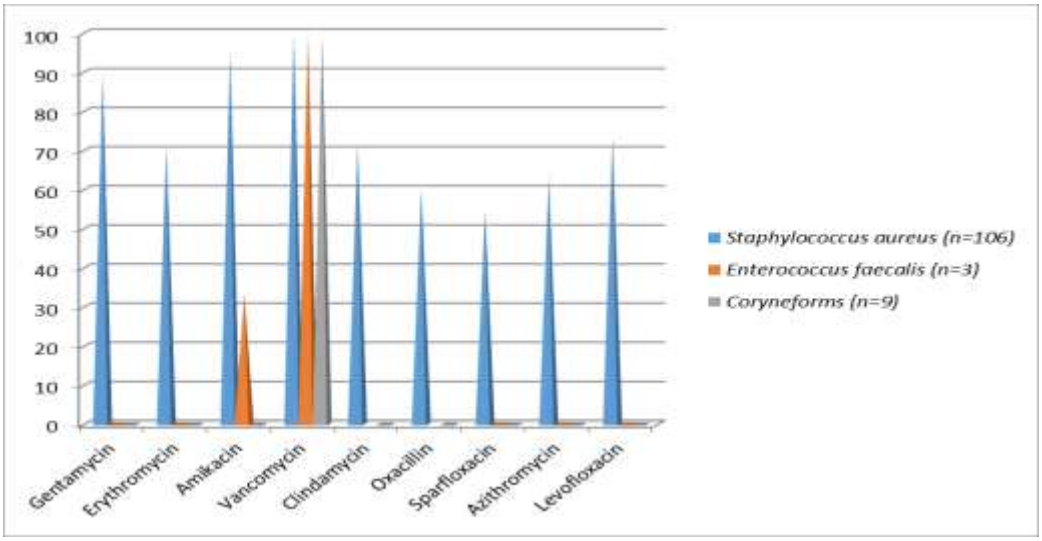

Fig1: Sensitivity pattern of Gram positive isolates.

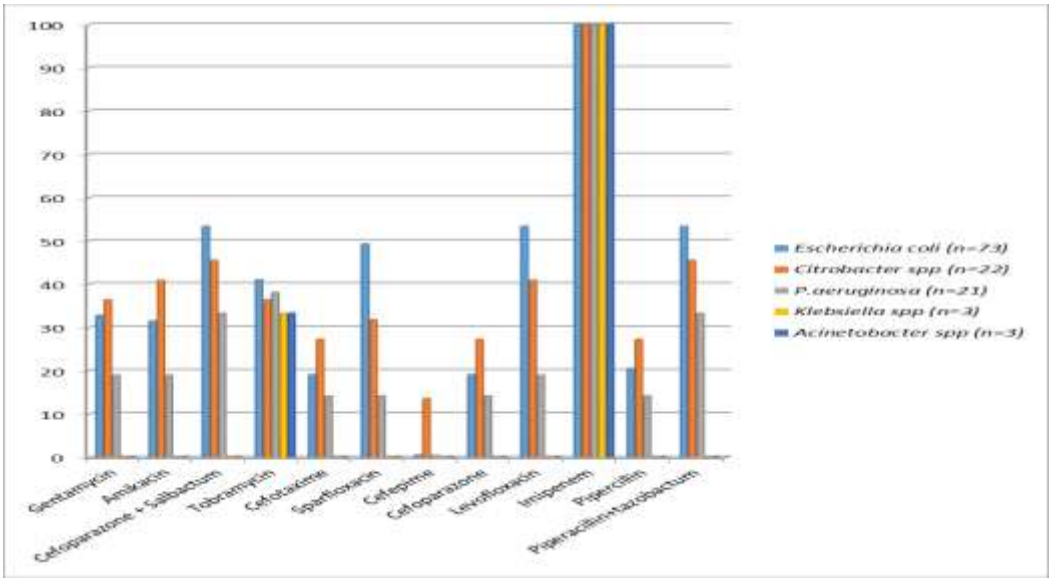

Fig2: Sensitivity pattern of Gram negative isolates.

DISCUSSION

Risk of nosocomial SSIs in developing countries is influenced by characteristics of the patient, the healthcare staff and the hospital. In theory, reducing risk is relatively simple and inexpensive, especially when compared to the cost of the infections themselves, but in practice it requires commitment at all levels of the healthcare system. For most SSIs, the source of the pathogen is usually endogenous and comes from the patient's skin, mucous membranes or bowel and rarely from another infected site in the body. Exogenous sources of SSI pathogens are occasionally responsible. These include organisms from members of the surgical team (e.g., hands, nose or other body parts), contaminated surfaces in the operating room, air, and contaminated instruments used in the surgery. In addition, treatment failures with broad spectrum cephalosporins is an increasing problem in developing countries.

In our study, 228 (8.9%) cases developed SSI, this finding is consistent with findings of Lilani et al.[4] Other studies have reported quite a high incidence of SSI at 18.9 % and 17.6%.[16,17] In our study, incidence of SSI was highest in surgery ward (43.4%) followed by orthopaedics (31.5%) and obs&gynae (25%) wards. One reason for high rates of SSI in surgery was high patient turn over. Incidence of SSI (31.5%) in orthopaedics ward was much higher as compared to previously published data.[18]

In our study, maximum number of cases in surgery and orthopaedic wards were males. This finding is almost double of that reported by Sahra et al.[19] One reason for this could be that majority of cases presenting at our centre complained of physical trauma and accidents.

Out of 228 cases of SSI majority were contaminated wounds (60%), 36.4% were clean wounds. This finding is quite high as compared to the findings of Razavi et al.

who reported the incidence of clean and contaminated wounds to be 13.6% and 45.8% respectively.[17] In our study, 72.7% were superficial wounds while 34.5% were deep wounds. Raka et al (2007) reported slightly different incidence of superficial (55.5%) and deep (40.7%) wounds.[20]

Majority of our cases (90.9%) fell under Grade IV (56.4%) and V (34.5%) of Southampton scoring. Least were Grade II and none was Grade I. There is no published data till date that compared the relative incidence of different grades. Sahra et al (2011) used Southampton's scoring but they took equal number of cases in each grade and did not report the relative incidence.[19]

Polymicrobial aetiology was observed in 17 (7.4%) cases in which Gram negative bacteria were the main pathogens. This is low as compared to the findings of other studies.[18]

The main pathogens being gram-positive bacteria (51.7%). Most common isolate was *S.aureus* (46.5%) followed by *E.coli* (32.0%), *Citrobacter spp* (9.6%). Suchitra et al (2009) also reported Gram positive to be the main pathogen with *S. aureus* being most common isolate (33.3%).[21] Raka et al (2007) reported *E.coli* (36.4%) as the main pathogen in SSI followed by *S.aureus* (14.4%).[20] Other studies reported *P.aeruginosa* to be the most common pathogen [18] while in our study incidence of *P.aeruginosa* was only 9.0%.
Among patients falling under Southampton grade IV & V, *Citrobactersp* (30.0%) dominated followed by *E.coli* (18.1%), *S.aureus* (16.3%), *Coryneformspp* (16.3%), *P.aeruginosa* (9.0%) and others (9.0%). Maximum drug resistance was also observed in Grades IV and V SSI. There is no study for such comparison.

Among the Enterobacteriaceae, average susceptibilities of *E.coli* to aminoglycosides, fluoroquinolones, cephalosporins, and cephalosporin + β-lactamase inhibitor combination were 35.1%, 51.35%, 14.81% and 53.4% respectively while that of *Citrobacter species* were 37.9%, 36.35%, 23.87% and 45.5% respectively. *Klebsiella species* were the most resistant pathogens sensitive only to imipenem. *P.aeruginosa* was found to be more resistant with average susceptibilities of 25.36%, 16.6%, 10.76% and 33.3% against aminoglycosides, fluoroquinolones, cephalosporins, and cephalosporin + β-lactamase inhibitor combination respectively. All the gram negative isolates were uniformly sensitivity to Imipenem. Patzer et al (2008) had reported imipenem resistance in 2.4% isolates.[22] Around 30% gram negative isolates were ESBL producers and 64.7% were AmpC producers. No MBLs were detected. Singh et al (2012) had reported 27% ESBL production among the nosocomial isolates, however, in their study only 18% isolates were Amp C producers.[23] On the basis of our findings we conclude that gram negative etiology with MDR pathogens is common in contaminated wounds.

Among the gram positive isolates 39.6% strains of *S.aureus* were methicillin resistant which was quite high as compared to 14% incidence of MRSA in another study.[21] In our study, resistance to Clindamycin was 28.4% as compared to 22% reported by Suchitra et al.although resistance to fluroquinolone was 51% which is same as that in our study (54.7%) but we used newer fluoroquinolone.[21] Resistance to amikacin was low (4.8%) and for gentamycin (10.4%) in our study. In this study amikacin and vancomycin were better therapeutic options for *S.aureus* infection.

No HLAR and VRE were detected in *Enterococcus species* while Suchitra et al reported 1.4% incidence of VRE.[21] This could be due to more number of isolate of *E.feacalis* (33.3%) while in our study this was only 1.3%. *Coryneforms* and *Enterococcus* were resistant to all antibiotics used except vancomycin.

CONCLUSION

SSI caused considerable morbidity among surgical patients. Appropriate active surveillance and infection control measures should be introduced during preoperative, intra-operative, and postoperative care to reduce infection rates. Irrational use of antibiotics should be stopped. Our ability to successfully treat infections due to these increasingly resistant organisms demands a multifactorial approach combining continued research and development of novel classes of antibacterial agents, more prudent use of existing agents and an increased emphasis on more effective infection control measures.

REFERENCES

1. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection. *Infect control hospepidemiol*., 1999; 20: 247-78.
2. Yalcin AN, Bakir M, Bakici Z, Dolmetas I, Sabir N. Postoperative wound infections.*J hosp Infect.*,1995; 29: 305-9.
3. Anvikar AR, Deshmukh AB, Karyakarte RP, Damle AS, Patwardhan NS, Malik AK, et al. A One Year Prospective Study of 3280 Surgical Wounds. *Indian J Med Microbiol.*, 1999; 17(3): 129 - 32.
4. Lilani SP, Jangale N, Chowdhary A, Daver GB. Surgical site infection in clean and clean-contaminated cases. *Indian J Med Microbiology.* 2005; 23(4): 249-52.
5. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections.*Am J Infect Control*., 1992; 20(5): 271–274.
6. Fridkin SK, Steward CD, Edwards JR, Pryor ER, McGowan JE Jr, Archibald LK, et al. Surveillance of antimicrobial use and antimicrobial resistance in United States hospitals: project ICARE

phase 2. Project Intensive Care Antimicrobial Resistance Epidemiology (ICARE) hospitals. *Clin Infect Dis.*, 1999; 29: 245–52.
7. Valles J, Leon C, Alvarez-Lerma F. Nosocomial Bacteremia in Critically Ill Patients: A Multicenter Study Evaluating Epidemiology and Prognosis.*Clin Infect Dis.*, 1997; 24: 387–95.
8. Nichols RL. Prevention of infection in high risk gastrointestinal surgery. *Am J Med.*, 1984; 76: 111-9.
9. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended spectrum β-lactamase production in Enterobacteriaceae bacteraemia: A systemic review &meta analysis. *J AntimicrobChemother*, 2007; 60: 913–920.
10. Rizvi M, Fatima N, Rashid M, Shukla I, Malik A, Usman A, Siddiqui S. Extended spectrum AmpC and metallo-beta-lactamases in Serratia and Citrobacter spp. in a disc approximation assay. *Infect Developing Countries*, 2009; 3(3): 177-186.
11. Wilson AP, Treasure T, Sturridge MF, Gruneberg RN. A scoring method (ASEPSIS) for postoperative wound infections for use in clinical trials of antibiotic prophylaxis. *Lancet.*,1986; 1: 311-313.
12. Matkoski C, Sharp SE, Kiska DL. Evaluation of the Q score and Q234 systems for cost-effective and clinically relevant interpretation of wound cultures. *J ClinMicrobio*, 2006; 44: 1869-1872.
13. Baron EJ, Finegold SM. Bailey and Scott's diagnostic microbiology. 8th edition, The CV Mosby Co., St. Louis. 1990; p. 212-222.
14. Clinical and laboratory Standards Institute 2003. Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement: Approved standards M100-S18. Clinical and Laboratory Standards Institute, Baltimore, USA. 2008.
15. Lee KY, Chong HB, Shin YA, Yong KD, Yum JH. Modified Hodge test and EDTA disc synergy tests to screen metallo beta lactamase producing strains of Pseudomonas and Acinetobacter species. *ClinMicrobiol Infect*, 2001; 7: 88-91.
16. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect control and hospepi*, 2008; 29: 11-13.
17. Razavi SM, Ibrahimpoor M, Kashani AS, Jafarian A. Abdominal surgical site infections: incidence and risk factors at an Iranian teaching hospital. *BMC Surgery*, 2005; 5: 2-4.
18. Le TA, Sohn AH, Nguyen PT, Vo TC, Vo VN, Tran Nguyen TH, Ewald B, Dibley M.Reduction in surgical site infections in neurosurgical patients associated with a bedside hand hygiene program in Vietnam. *Infection Control hosp epidemiology*, 2006; 27(8): 855-62.
19. Sahra ZA, AlaaEldeen SM, Abd-Elwahb M, Ahmed AM. Impact of Educational Program among Open Heart Surgery Patients on Minimizing the Incidence of Post Operative Infections. *Journal of American Science*, 2011; 7(6).
20. Raka L, Krasniqi A, Hoxha F, Musa R, Mulliqi G, Krasniqi S, Kurti A, Dervishaj A, Nuhiu B,Kelmendi B, Limani D, Tolaj I. Surgical site infections in abdominal surgical ward at Kosova teaching hospital. *J Infect Developing Countries*, 2007; 1(3): 337-341.
21. Suchitra JB, Lakshmidevi N. Surgical site infections: Assessing risk factors, outcomes and antimicrobial sensitivity patterns.*African Journal of Microbiology Research*, 2009; 3(4):175-179.
22. Patzer JA, Dzierz˙anowska D, Turner PJ. Trends in antimicrobial susceptibility of Gram-negative isolates from a paediatric intensive care unit in Warsaw: results from the MYSTIC programme (1997–2007). *Journal of Antimicrobial Chemotherapy*, 2008; 62: 369–375.
Singh RKM, Pal NK, Banerjee M, Sarkar S, Sen Gupta M. Surveillance on Extended Spectrum β-lactamase and AmpC β-lactamase producing gram negative isolates from nosocomial infections. *Archives of clinical microbiology*., 2012; 3: 3:1.