Original Article

BACTERIAL ISOLATES FROM SURGICAL SITE INFECTION AND THEIR PATTERN OF ANTIBIOTICS SENSITIVITY

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ABSTRACT

Background: *Surgical site infection (SSI) is a widespread complication after surgery, leading to significant morbidity and mortality in patients. Improper use of antibiotics leads to an increasing frequency of SSI and the emergence of resistant bacterial strains.*

Objective: *To determine most common pathogens involved in SSI and to investigate their antibiotic susceptibility/ sensitivity profile.*

Study design : *A Qualitative Study*

Duration and place of study : *Mardan Medical Complex (MMC) in Mardan, Pakistan, from December 2013 to October 2014*

Material and Methods: *Pus specimens were obtained from the patients having SSI at Mardan Medical Complex (MMC) in Mardan, Pakistan, from December 2013 to October 2014 and were processed for microbial analysis at the Department of Pathology at Bacha Khan Medical College (BKMC) in Mardan. The specimens were inoculated on both MacConkey and 5% blood agar, and bacterial colonies were identified by gram stain, physical appearance, and biochemical tests. Furthermore, the antibiotic susceptibility test was done using the modified Kirby-Bauer disc diffusion method.*

Results: *Pathogenic organisms were Staphylococcus aureus (36.44%), followed by Escherichia coli (25.23%), Pseudomonas aeruginosa (13.88%), Klebsiella spp. (6.5%), Enterococcus spp. (6.5%), Acinetobacter spp. (3.7%), Proteus spp. (2.8%), Coagulase-negative staphylococci (2.8%), and other miscellaneous gram-negative rods (0.93%). About 65.7% of S. aureus were methiciline-resistant S. aureus (MRSA), while 83.3% of Klebsiella spp. and 53.8% of E. coli were Extended Spectrum Beta Lactamases (ESBL). S. aureus and Enterococcus spp. were both highly susceptible to amikacin, vancomycin, linezolid, doxycycline, and chloramphenicol. S. aureus was highly resistant to Erythromycin (65%), Penicillin (96%), Ciprofloxacin (51%), and Amoxicillin/Clavulanic Acid (65%). All the GNR, including P. aeruginosa, were highly susceptible to Imipenem, Amikacin, Tazobactam/Pipracillin, and Cefoperazone+Sulbactam. E. coli, Klebsiella spp., and other GNR were highly resistant to ampicillin, ceftriaxone, amoxicillin/clavulanic acid, cotrimoxazole, and ciprofloxacin. P. aeruginosa was also highly susceptible to Meropenem and Cefepime but showed moderate resistance to Ciprofloxacin, Ceftazidime, and Polymyxin.*

Conclusion: *As compared to Ceftriaxone, Ciprofloxacin, and Amoxicillin/Clavulanic Acid, Tazobactam/Pipracillin, Cefoperazone+Sulbactam, and Doxycycline showed high efficiency, and cross-resistance was found in MRSA, Ciprofloxacin, and other non-beta lactam antibiotics.*

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Date Received: Jan-20-2020
Date Accepted: Feb-25-2020

Citations: Kalim Ullah, Imtiaz Ahmad, `, F. J., & Mohtasimbillah. (2020). BACTERIAL ISOLATES FROM SURGICAL SITE INFECTION AND THEIR PATTERN OF ANTIBIOTICS SENSITIVITY: Original Article. Journal of Bacha Khan Medical College, 1(02), Page No. 33–40. https://doi.org/10.69830/jbkmc.v1i02.67

INTRODUCTION

Surgical site infection (SSI) is an infection that occurs within 30 days after surgery (if no implant is left in place) or within 1 year if an implant is left in place after the operation. According to the Centers for Disease Control and Prevention (CDC), SSI is divided into three categories: superficial, deep incisional, and organ or body space infection (Horan et al., 1992).

Post-operative surgical site infection (SSI) is considered the third most common nosocomial infection in admitted hospital patients (Mangram et al., 1999). Post-operative SSIs are caused by bacteria that enter the surroundings, which may either be normal flora of the skin, nasopharynx, and other tracts of the body or may be hospital-acquired bacteria like Pseudomonas aeruginosa, Klebsiella pneumoniae, and Methicillin-resistant Staphylococcus aureus (MRSA) (Cross et al., 1983). Although modern aseptic and sterilization techniques are being applied during surgeries, surgical site infection is still one of the major complications, which leads to a high degree of antibiotic resistance in pathogenic bacteria (Thomas, 1981). Regular culture and sensitivity analysis are required to provide targeted treatment and reduce the economic burden of antibiotics, morbidity, and motility ratios in post-operative patients. They may also help physicians and clinicians predict the possible pathogens of SSI and their susceptibility patterns.

Here we are reporting the findings of our study carried out at various surgical units of Mardan Medical Complex (MMC) in Mardan, Pakistan, from December 2013 to October 2014. The aim of this study was to determine the most common pathogens involved in SSI and to investigate their antibiotic susceptibility and sensitivity profiles.

METHODS

Collection of specimens: After obtaining the informed consent of each patient, the specimens (n = 136) were collected aseptically during dressing from the purulent discharge of the infected surgical site through a sterile swab before cleaning the wound with an antiseptic solution under the supervision of a trained practitioner. The demographic and clinical information of the patients, including type of surgery, history of antibiotics used before and after the surgery, length of postoperative stay in the hospital, and history of associated diseases like diabetes mellitus, etc., were recorded on a special questionnaire.

Processing of the specimens

The specimens were processed for clinical and diagnostic examination at the Department of Pathology, Bacha Khan Medical College (BKMC), Mardan, Pakistan. The study samples were inoculated on both differential and enriched media (MacConkey and 5% blood agar) to determine the physical characteristics of the bacterial colonies. After inoculation, the plates were incubated at 37 oC for 24-48 hours aerobically.

Identification of pathogenic bacteria

All the pathogenic bacteria were identified by their colony morphology, Gram staining behavior, hemolysis on blood agar, enzymatic activity, and physical appearance on the differential media and biochemical tests. The biochemical tests used were the oxidase test, urease test, Simmon citrate, Indole test, triple sugar iron (TSI), catalase, and DNase test. The bacterial isolates with ambiguous features were retested with the Analytical Profile Index (API 10S) under the guidelines of the manufacturer's instructions (Biomerieux, France).

Antibiotic susceptibility/sensitivity testing

The antibiotic susceptibility test was performed by the modified Kirby-Bauer disk diffusion method (M07-A9. CLSI, 2012). For inoculum preparation, at least three morphologically identical colonies from the agar plate were transferred to a test tube with 1.5 ml of sterile peptone broth. The tubes were incubated for up to two hours at 37°C in order to achieve log phase growth. After incubation with peptone broth, a sterile swab was dipped into the suspension and streaked evenly on the nutrient plate for equal distribution of the inoculum. While for staphylococci, the inoculum was prepared by the direct colony suspension method (M100-S17, CLSI). Both peptone and direct suspension were adjusted to a turbidity equivalent to 0.5 McFarland barium sulfate standard (M02-03, Section 8.1. CLSI 10Th) on the front white paper with a black line.

After achieving uniform streaking, the commercially available antibiotic disk (OxoidTM) was applied to the nutrient plates of both test and control strains. The diameter of the zone of inhibition was measured to the nearest millimeter by using a ruler or caliper. Similarly, the susceptibility and resistivity of specific bacterial pathogens to each drug were determined using the published guidelines of the Clinical and Laboratory Standards Institute (CLSI). All the Gram-positive Cocci (GPC) were tested against Amikacin (30µg), Vancomycin (30µg), Choloramphenical, Erythromycin, Doxycycline, and Clindamycin, Ciprofloxacin, Penicillin, Gentamicin, and Cotrimoxazole. Methicillin-resistant Staphylococcus aureus (MRSA) was detected by a disc diffusion test using a cefoxitin (30 µg) disc (Broekema et al., 2009), and clindamycin resistance in S. aureus was determined by a D-test (Fiebelkorn et al., 2003). All oxidase-negative Gram-negative rods (GNR) were tested against Amoxycillin/Clavulanic, Amikacin, Gentamicin, Ciprofloxacin, Cephradine, Ceftriaxone, Imipenem, Cefoperazone+Sulbactam, and Tazobactam/Pipracillin. The ESBL production was detected by the double disc diffusion method on nutrient agar by placing the amoxycillin/clavulanic at the center of the plate and the cefotaxime (30µg) and ceftazidime (30µg) at 20mm apart from each side of the amoxycillin/clavulanic. Oxidase-positive GNR (Pseudomonas spp.) was tested against Tazobactam/pipracillin., ciprofloxacin, ceftazidime (30 µg), Cefoperazone+ Sulbactam, Polymoxine B, Imipenem, and Meropenem.

RESULTS

A total 136 patients (mean age 33.5±14.1) having SSI were included in this study from December, 2013 to October, 2014. Out of the total, 66.2% were male (n=90) and 33.8% were female (n=46) with their age ranged from 15-85 years. The study samples were collected from hospitalized patients of different wards, including 41.9% (n=57) from general surgery ward, 40.4% (n=55) from orthopedic ward and 17.6% (n=24) from gynecology ward of Mardan Medical Complex (MMC) Mardan, Pakistan. The most common type of surgery done in the orthopedic ward was open reduction and internal fixation (ORIF) (36.7%) (see Table 3.1 for clinical information) . While in general surgery ward laparotomy and appendectomy were the common procedures and the cesarean section and vaginal hysterectomy were found the most common procedures in gynecology ward. It was noted that the post-operative hospital stay of the patients in orthopedic ward was more than 10 days while in general surgery and gynecology ward it was 3 to 7 days. We found that 92% of patients had used metronidazole, ciprofloxacin and third generation cephalosporins for prophylaxis. Whereas, amoxicillin/clavulanic acid, ciprofloxacin and tazobactam/ piperacillin were commonly used after surgery.

DISCUSSION

Total 102 different bacterial isolates were obtained from this study having different susceptibility pattern. Majority of these isolates were resistant to

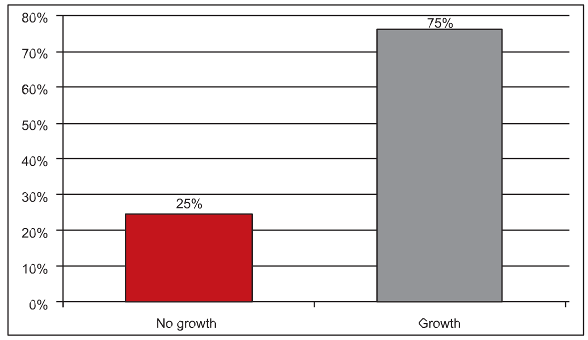

Figure 3.1: Percentage of positive-growth vs no growth specimens

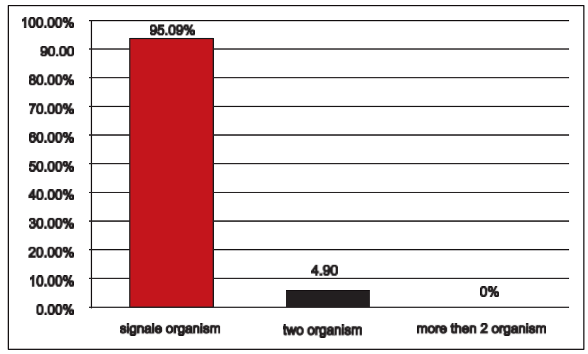

Figure 3.2: Number of bacterial isolate per specimen

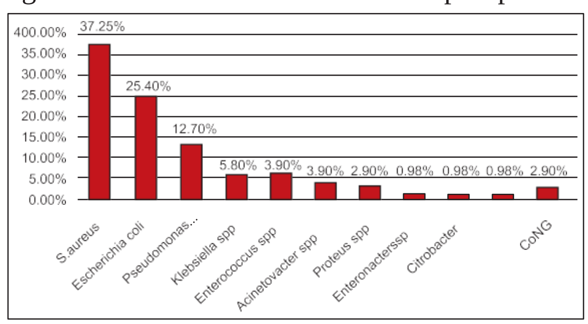

Figure 3.3: Percentage of different bacterial isolates from SSI

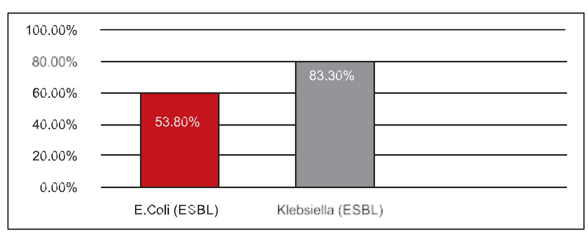

Figure 3.4a: Percentage of ESBL in E. coli and Klebsiella Spp

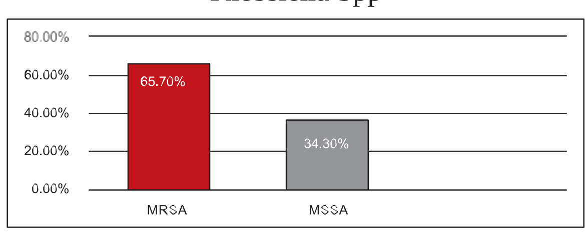

Figure 3.4b: Percentage of MRSA and MSSA

Table 3.1: Clinical and demographic features of the patients

Variables	Total No /Percentage
Age (years)	
Range	15-85
Mean	33.5 ± 14.1
Median	29.5
Gender	
Female	46 (33.8%)
Male	90 (66.2%)
Surgery Department	
Gynecology ward	24 (17.6%)
Orthopedic ward	55 (40.4%)
General surgery ward	57 (41.9%)
Type of operation	
Laparotomy	16 (11.6 %)
Open reduction & Internal fixation (ORIF)	50 (36.7%)
Appendectomy	16 (11.6%)
Cesarean section / vaginal hysterectomy	24 (17.6%)
Others*	27 (19.9%)

*Surgical debridement: Surgery for Firearms injuries and Herniorrhaphy

Table 3.2: Species and frequency of bacterial isolate from SSI

Bacterial isolate	Frequency (n=102)	%
S.aureus	38	37.25
Pseudomonas aeruginosa	13	12.7
E.coli	26	25.4
Klebsilla spp	6	5.8
Enterococcus spp	6	5.8
Acinetobacter spp.	4	3.9
Proteus spp	3	2.9
Enterobacter spp	1	0.98
Citrobacter Spp	1	0.98
Morganella morganii	1	0.98
CoNS*	3	2.9
Total	102	100.0

*Coagulase-negative staphylococcus

commonly used antibiotic. Through various biochemical analysis 11 different types of bacteria were identified (Table 3.2; Figure 3.3).These pathogenic bacteria identified from the SSI included Staphylococcus aureus (37.25%), Escherichia coli (25.40%), Pseudomonas aeruginosa (12.70%), Klebsiella Spp (5.8%),

Table 3.3a: Antibiotic sensitivity pattern of Enterobacteriaceae

Antibiotics	Result	E.coli	Klebsiella spp	*Other GNR
	S	20%	0	1%
Ceftriaxone	R	80%	100 %	99%
	S	28%	0	33.33%
Amoxicillin/ Clavulanic acid	R	72%	100%	66.66%
	S	34.8 %	20%	55.55%
Ciprofloxacin	R	65.2%	80%	55.55%
	S	96%	90%	80%
Imipenem	R	4%	10%	20%
	S	100	100%	90%
Amikacin	R	0	0	10%
	S	92%	100	80%
Tazobactam/ pipracillin	R	8%	0	20%
	S	88%	100%	80%
Cefoperazone+ Sulbactam	R	22%	0	20%
Cotrimoxazole	S	15%	—	—
	R	85%	—	—
	S	10.1%	0	33.33%
Ampicillin	R	90.9%	100%	77.77%

*Other GNR= Acinetobacter spp, Proteus spp, Enterobacter spp, Citrobacter & Morganella spp. S=sensitive, R= Resistance

Table 3.3b: Antibiotic sensitivity pattern of Pseudomonas aeruginosa

Antibiotics	Result	Pseudomonas aeruginosa
	S	53.9%
Ciprofloxacin	R	46.1%
	S	92.3%
Imipenem	R	7.7%
	S	100%
Amikacin	R	0
	S	69.23%
Tazobactam/ pipracillin	R	30.7%
	S	92.3%
Cefoperazone+ Sulbactam	R	7.7%
	S	100
Meropenem	R	0
	S	88.8%
Cefepime	R	11.2%
Ceftazidime	S	53.9%
	R	46.1%
Polymyxin B	S	50%
	R	50%

R=resistant, S=sensitive

Table 3.4: Antibiotic susceptibility of gram positive bacterial isolates

Antibiotic	Result	Staphylo coccus aureus	Entero-coccus spp
Cefoxitin	S	34.3%	—
	R	65.7%	—
Erythromycin	S	35%	—
	R	65%	—
Doxycycline	S	96%	75%
	R	4%	25%
Clindamycin	S	66.67%	60%
	R	33.33%	40%
Ciprofloxacin	S	49%	33.34%
	R	51%	66.66%
Penicillin	S	4%	—
	R	96%	—
Cotrimoxazole	S	50%	—
	R	50%	—
Amikacin	S	89.5%	100%
	R	10.5%	0%
Vancomycin	S	100%	100%
	R	0%	0%
Linezolid	S	99%	100%
	R	1%	0%
Gentamicin	S	77.42%	—
	R	22.58%	—
Chloramphenicol	S	96%	75%
	R	4%	25%
Amoxycillin Clavu-lanic acid	S		83.33%
	R		16.67%

R =resistant, S=sensitive

Table 3.5: Comparison between antibiotic susceptibility pattern of MRSA and MSSA

Antibiotic	Result	MSSA	MRSA
Erythromy-cin	S	7 (70%)	4 (21.4%)
	R	3 (30%)	15 (78.9%)
Doxycycline	S	9 (90%)	19 (100%)
	R	1 (19%)	0 (0%)
Clindamycin	S	7(88.8%)	11(50%)
	R	2 (22.2%)	11 (50%)
Ciprofloxacin	S	5 (62.5%)	11 (50%)
	R	3 (37.5%)	11 (50%)
Cotri-moxazole	S	5 (46%)	10 (50 %)
	R	6 (54)%	10 (50%)
Amikacin	S	13 (100%)	19 (80%)
	R	0 (0%)	5 (20%)
Gentamicin	S	13 (100%)	11 (57.9%)
	R	0 (0%)	8 (42.1%)
Chloram-phenicol	S	13 (100%)	21(100%)
	R	0 (0%)	0 (100%)

MSSA= Methicillin-Sensitive Staphylococcus Aureus,
MRSA= Methicillin-resistant Staphylococcus aureus

Enterococcus spp (5.8%), Acinetobacter spp (0.98%), Coagulase-negative staphylococci (2.90%), Citrobacter spp and Enterobacter spp (0.98%). Out of the total 37.25% Staphylococcus aureus about 65.7 % were methicillin resistant Staphylococcus aureus (MRSA). Similarly, 83.3% of Klebsiella Spp and 53.8 % Escherichia coli were Extended Spectrum Beta Lactamases (ESBL) (see Figure 3.4a). Staphylococcus aureus was the predominate species followed by Escherichia coli (25.40%), Pseudomonas aeruginosa (12.70%) in the present study. Similarly, previous studies have also shown that Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli were the three most common pathogens of SSI (Mahmood, 2000; Le Thi Anh et al., 2006; Adegoke et al., 2010; Azene et al., 2011; Verma, 2012). Beside these three dominant species, the bacterial isolates having moderate existence were Klebsiella spp, Enterococcus spp, Acinetobacter spp, Proteus spp and Coagulase-negative staphylococci. These findings are in agreement with the previous study by Dessalegn et al., (2014). The third category of bacterial isolates identified in this study was miscellaneous gram negative rods which include Morganella Morganii, Citrobacter spp and Enterobacter spp. These species had very low percentage almost less than 3.5%. Similar findings were previously reported which can support the results of our study (Mahmood, 2000; Shaikh et al., 2003). Some of these studies demonstrated that Pseudomonas aeruginosa and other gram negative rod are the most prevalent bacteria in SSI but majority of the studies have shown that Staphylococcus aureus is more common cause of this infection. These variations may be due to high prevalence of MRSA in some study sets and may be due to the climatic variation i.e. temperature and relative humidity.

The variation in frequency of different bacterial isolates also depends upon the respective surgical units from where the study subjects have been ascertained. Previous studies have reported Staphylococcus aureus being the most frequently identified pathogen from orthopedics surgical specimens (Bercion et al., 2007) and Escherichia coli in general surgery (viscus surgery) specimens (Giacometti et al., 2000). Majority of our study sample set were taken from orthopedics and general surgery units and thus the most dominant bacterial isolates were Staphylococcus aureus and Escherichia coli, which may satisfy the findings of previous studies (Table 3.1).

In the specimens taken general surgery unit the frequency of E. coli was higher as compare to other pathogens. In general surgery unit, the main surgeries were laparotomy and appendectomy in which gastrointestinal tract is opened thus increasing the chance of of E. coli infection. But in case of specimens taken from orthopedic surgical unit Escherichia coli was second dominant pathogen (next to S. aureus) A recent study from the same region reported the incidence of Escherichia coli as the second dominant pathogen (next to S. aureus) in the SSI of implants surgery of orthopaedic unit (Salman et al., 2014).

In our study about 65.7% Staphylococcus aureus isolate were MRSA. This high incidence of MRSA in SSI is in agreement with previous studies (Mahmood, 2000; Shagufta et al., 2005) (Khorvash et al., 2008). This high incidence of MRSA in SSI may be subjects to lack expertise in following MRSA control protocol.

The antimicrobial susceptibility and resistant pattern determined in this study demonstrated that majority of Staphylococcus aureus isolates were resistant to penicillin, erythromycin, ciprofloxacin and cotrimoxazole (Table 3.4). Previously, penicillin, erythromycin and cotrimoxazole were found resistant but Ciprofloxacin have shown activity against S. aureus (Mahmood, 2000; Shaikh et al., 2003). These studies have reported that Staphylococcus aureus was more susceptible ciprofloxacin (69.7%) but according to our findings Staphylococcus aureus was resistant to ciprofloxacin (51%). These variation may be due to the increase use of ciprofloxacin for antimicrobial prophylaxis in our study sets. Furthermore, we found that Staphylococcus aureus was highly susceptible to amikacin, chloramphenicol, vancomycin, clindamycin, gentamicin and doxycycline. Similar data has been reported previously which is in agreement with our findings for amikacin, chloramphenicol, vancomycin, clindamycin and gentamicin but disagree in case of doxycycline. These studies also shown high resistance of Staphylococcus aureus to doxycycline but we identified that S. aureus was highly susceptible to doxycycline (Mahmood, 2000; Shaikh et al., 2003). These variation may be possible due to insufficient use of doxycycline against Staphylococcus aureus in MMC.

As compare to MSSA, a cross resistance of MRSA to other antibiotics like ciprofloxacin, erythromycin, clindamycin, gentamicin was found in this study (Figure 3. 6). Similar pattern of resistance was previously published by Japoni et al., (2010). MSSA were more resistant to doxycycline as compare to MRSA, these variation probably due to the insufficient use of doxycycline against MRSA in this community as a result it become susceptible to doxycycline.

In the present study it was investigated that Escherichia coli, Klebsiella Spp and other GNR like Citrobacter spp, Enterobacter spp, Acinetobacter spp and Proteus spp were highly susceptible to amikacin (90-100%), cefoperazone+ sulbactam (80-92%), imipenem (80-96%) and tazobactam/ pipracillin (70-100 %) but were highly resistant to ceftriaxone, amoxicillin/clavulanic acid, cotrimoxazole, ampicillin and ciprofloxacin (Table 3.3a; Figure 3.5b). A study conducted by Shaikh et al, (2003) has presented the same results for all above given antibiotics except ceftriaxone and ciprofloxacin. Shaikh et al study had demonstrated that Escherichia coli, Klebsiella Spp and other GNR were highly susceptible to Ceftriaxone and Ciprofloxacin but according to the investigation of current study Escherichia coli, Klebsiella Spp and other GNR were highly resistant to ciprofloxacin and ceftriaxone (Table 3.3a; Figure 3.5b). These variations can probably be due to improper use of ceftriaxone and ciprofloxacin in this community for antimicrobial prophylaxis. Furthermore, it may also be due to the ESBL Cross-resistance to ciprofloxacin (Afunwa et al., 2011).

In the current study Enterococcus spp were highly susceptible to erythromycin, clindamycin, doxycycline, amikacin, vancomycin, linezolid but showed high resistant to ciprofloxacin (Table 3.4). These findings were in some degree of agreement with previous finding (Lakshmidevi, 2009) but high degree of incensement was observed in resistance pattern of ciprofloxacin. The Pseudomonas aeruginosa were found to have high susceptibility to meropenem, imipenem, cefoperazone+ sulbactam and cefepime but show moderate resistant to ciprofloxacin, and ceftazidime (Table 3.3b). Our findings for these antibiotics were found in agreement with previous finding (Mahmood, 2000) but show slightly variation in case of ciprofloxacin. According to another study Pseudomonas aeruginosa were highly susceptible to ciprofloxacin (Shaikh et al., 2003) but our finding demonstrated that it was moderately resistant to ciprofloxacin (Table 3.3b).

CONCLUSION AND RECOMMENDATIONS

Strictly aseptic technique is required to minimized the chance of SSI especially MRSA control protocol is necessary because majority of isolates were MRSA. Every surgical unit should develop percentile list of possible pathogens along the susceptibility profile because it will help to minimize the morbidity and motility rate of post-operative patients. Majority of antibiotic that is used for prophylaxis is now became highly resistant to all possible pathogens so a suitable protocol for the proper use of antibiotics should be adopted in each unit. As compare to ceftriaxone, ciprofloxacin and amoxicillin/clavulanic acid the tazobactam/ pipracillin, cefoperazone+ sulbactam, doxycycline and linezolid will showed high efficiency if use prophylactically. Regular culture and sensitivity is necessary to provide targeted treatment and reduce economic burden of antibiotics, morbidity and motility ratio of post-operative patient. Our study will guide the physicians and clinicians to predict possible pathogen of SSI and their susceptibility pattern. The regular culture and sensitivity technique should be adopted for all patients to provide targeted treatments and to reduce morbidity and mortality ratio of post-operative patients.

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Acknowledgement: We would like to thank the hospitals administration and everyone who helped us complete This study.

Disclaimer: Nil

Conflict of Interest: There is no conflict of interest.

Funding Disclosure: Nil

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