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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES CAUSING SURGICAL SITE INFECTION

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ABSTRACT

Background and objectives: Surgical site infections (SSI) constitute about one fourth of all nosocomial infections. Hence this study was carried out to isolate and identify the bacteria causing surgical site infections and to determine their antibiotic sensitivity pattern.

Methods: A total of 204 clinically diagnosed cases of surgical site infections were taken for the study. Antibiotic sensitivity pattern of the isolates was determined. *Isolates of Staphylococcus aureus were screened for methicillin resistance by cefoxitin disc diffusion test and were confirmed by minimum inhibitory concentration (MIC) determination using oxacillin E-test. Extended spectrum beta lactamase (ESBL) production was tested in Escherichia coli and Klebsiella pneumoniae.*

Results: The most frequent isolate in our study was *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*. Most of the Gram negative organisms were sensitive to piperacillin/tazobactam, ceftazidime and amikacin and all of them were sensitive to imipenem. Gram positive organisms were found to be more sensitive to gentamicin, clindamycin and linezolid. Sensitivity to vancomycin was seen in all the Gram positive isolates. 12 (22.2%) strains of *Staphylococcus aureus* were methicillin resistant by cefoxitin disc diffusion test and by oxacillin E-test. ESBL production was seen among 25% and 22.22% isolates of *Klebsiella pneumoniae* and *Escherichia coli* respectively.

Interpretation and conclusion: Common organisms causing surgical site infections show resistance to various antibiotics, a major problem being the occurrence of *methicillin resistant Staphylococcus aureus* (MRSA) and ESBL producing *Escherichia coli and Klebsiella pneumoniae*. Hence it is important to test for the presence of these organisms and treat appropriately.

Key words: *Extended spectrum beta lactamase - methicillin resistant Staphylococcus aureus* - surgical site infection

INTRODUCTION

Surgical site infections (SSI) constitute about one fourth of all nosocomial infections and has been documented for at least 4000-5000 years. They are an important cause of morbidity following various surgeries and also account for additional hospital costs. Most nosocomial surgical site infections (60-80%) occur in the incision, but some involve deep soft tissue or adjacent sites [1,2].

The Centers for Disease Control and prevention (CDC) has proposed specific criteria for the diagnosis of surgical site infection. This splits surgical site infections into three groups- superficial incisional, deep incisional and organ space infections, depending on the site and the extent of infection [3].

Sources of surgical site infections can include the patient's own normal flora or organisms present in the hospital environment, and in the case of wound infections following appendicectomy or other lower bowel surgery, indigenous flora of the lower gastrointestinal tract are involved like *Escherichia coli* [4]. This study was aimed to identify the various pathogens causing surgical site infections and their antibiotic sensitivity and resistance pattern including *methicillin resistant Staphylococcus aureus (MRSA)* and *extended spectrum beta lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae in our hospital.*

MATERIALS AND METHODS

A total of 204 clinically diagnosed cases of surgical site infections admitted in various surgical wards in our

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hospital were taken for the study. CDC criteria was used to define the type of surgical wound (class I, II, III, IV) [5]. The samples were collected with aseptic precautions using sterile cotton swabs and were processed immediately in the laboratory using standard microbiological methods[6,7]. Anaerobic bacteria were not included in the present study. *Isolates of Staphylococcus aureus were screened for methicillin resistance by cefoxitin disc diffusion test and were confirmed by MIC detection using oxacillin E-test*[7-9]. *MRSA strains* which were erythromycin resistant were subjected to double disc diffusion test (D-test) to detect inducible macrolide-lincosamide-streptogramin B (MLS_B) resistance [10]. *Isolates of Escherichia coli and Klebsiella pneumoniae were initially screened for ESBL production using* ceftazidime (30µg) and cefotaxime (30µg) discs and were confirmed with ceftazidime (30µg) plus ceftazidime-clavulanic acid (30/10µg) and cefotaxime (30µg) plus cefotaxime-clavulanic acid (30/10µg) discs *using* Clinical and Laboratory Standards Institute *(CLSI) guidelines* [7]. *Isolates of Pseudomonas aeruginosa were screened for* metallo-beta-lactamase production using imipenem disc diffusion test [11]. The association between different variables was tested using non-parametric tests. A P (predictive) value of <0.05 was considered as significant association between the variables tested.

RESULTS

In the present study, maximum number of SSI patients were in the age group of 21-30 years. 116 (56.87%) cases belonged to class I wound , 74 (36.27%) to class II, 8 (3.92%) to class III and 6 (2.94%) cases belonged to class IV wounds. 166 (81.38%) samples yielded growth on culture and 38 (18.62%) samples yielded no growth. Among the culture positive samples, 153 (92.16%) samples yielded a single organism on culture and 13 (7.84%) samples yielded 2 organisms. Gram negative bacteria were isolated from 60.33% of the cases and Gram positive bacteria accounted for 39.67% of the isolates. The most common bacteria isolated was *Staphylococcus aureus* (30.17%) followed by *Pseudomonas aeruginosa* (22.34%), *Klebsiella pneumoniae* (15.66%), *Escherichia coli* (15.08%) and *Staphylococcus epidermidis* (8.38%). Other bacteria isolated were *Proteus mirabilis* (4.48%), *Klebsiella oxytoca* (2.23%), *Enterococcus species* (1.11%) and *Acinetobacter species* (0.55%).

The Gram negative organisms were most sensitive to imipenem (100%), followed by piperacillin/tazobactam (99.07%), ceftazidime (69.44%) and amikacin (65.74%). The least sensitive antibiotic against Gram negative organisms was ampicillin (0.92%). The Gram positive organisms were most sensitive to vancomycin (100%), followed by clindamycin (83.09%), linezolid (74.64%) and gentamicin (67.60%) (Table. 1).

Among the 54 strains of *Staphylococcus aureus*, 12 (22.2%) strains were methicillin resistant by cefoxitin disc diffusion test and by oxacillin E-test. The number of methicillin resistant isolates having MICs of >256, 192, 8, 6 and 4 µg/ml were 4, 1, 2, 2 and 3 respectively by E-test (Fig. 1). Out of 12 strains of MRSA, 7 (58.33%) were resistant to erythromycin. 2 (16.67%) were constitutive MLS_B resistant, 3 (25%) were inducible MLS_B resistant (inducible clindamycin resistant) and 2 (16.67%) belonged to MS phenotype. 5 (41.66%) strains were susceptible to both erythromycin and clindamycin. 88.09% and 69.04% strains of methicillin sensitive *Staphylococcus aureus* (MSSA) were sensitive to clindamycin and cotrimoxazole respectively, while it was 58.3% and 25% with MRSA strains showing a significant difference (P value of 0.05 for clindamycin and 0.02 for cotrimoxazole). ESBL production was seen among 7 (25%) and 6 (22.22%) isolates of *Klebsiella pneumoniae* and *Escherichia coli* respectively. Imipenem resistant strains of *Pseudomonas aeruginosa was not seen in our study.*

DISCUSSION

In our study 18.62% samples yielded no growth, which correlates with a study in Mumbai, in which 82.36% cases of SSI were culture positive and 17.64% were culture negative [12]. Culture negativity may be due to antibiotic therapy prior to culture of material from an apparently infected site [13]. We observed 7.84% of the samples yielded mixed organisms. In various studies mixed organisms were found to be ranging from 7.14% to 55.86% [12,14,15].

Table no. 1 – Antibiotic susceptibility pattern of organisms

Antibiotics	Gram negative organisms						Gram positive organisms		
	Pseudomonas aeruginosa Total no. 40	Klebsiella pneumoniae Total no. 28	Escherichia coli Total no. 27	Proteus mirabilis Total no. 8	Klebsiella oxytoca Total no. 4	Acinetobacter species Total no. 1	Staphylococcus aureus Total no. 54	Staphylococcus epidermidis Total no. 15	Enterococcus species Total no. 2
Ampicilin (%)	0(0)	0(0)	0(0)	1(12.5)	0(0)	0(0)	-	-	-
Gentamicin (%)	15(37.5)	19(67.8)	15(55.5)	5(62.5)	3((75)	1(100)	38(70.3)	10(66.6)	0(0)
Amikacin (%)	20(50)	22(78.5)	20(74)	5(62.5)	3(75)	1(100)	-	-	-
Ofloxacin (%)	17(42.5)	17(60.7)	12(44.4)	4(50)	1(25)	0(0)	-	-	-
Ciprofloxacin (%)	15(37.5)	14(50)	10(37)	4(50)	1(25)	0(0)	-	-	-
Tetracycline (%)	7(17.5)	7(25)	6(22.2)	5(62.5)	2(50)	0(0)	31(57.4)	7(46.6)	0(0)
Chloramphenicol (%)	14(35)	11(39.2)	11(40.7)	3(37.5)	3(75)	1(100)	21(31.8)	7(46.6)	0(0)
Cotrimoxazole (%)	8(20)	17(60.7)	15(55.5)	3(37.5)	2(50)	0(0)	32(59.2)	10(66.6)	1(50)
Cephalothin (%)	5(12.5)	10(35.7)	12(44.4)	5(62.5)	0(0)	0(0)	-	-	-
Amoxyclav (%)	13(32.5)	16(57.1)	14(51.8)	6(75)	3(75)	0(0)	-	-	-
Cefepime (%)	9(22.5)	18(64.2)	19(70.3)	5(62.5)	3(75)	0(0)	-	-	-
Ceftazidime (%)	23(57.5)	21((75)	21(77.7)	5(62.5)	4(100)	1(100)	-	-	-
Cefotaxime (%)	9(22.5)	17(60.7)	21(77.7)	4(50)	3(75)	0(0)	-	-	-
Piperacillin/ tazobactam (%)	39(97.5)	28(100)	27(100)	8(100)	4(100)	1(100)	-	-	-
Imipenem (%)	40(100)	28(100)	27(100)	8(100)	4(100)	1(100)	-	-	-
Penicillin (%)	-	-	-	-	-	-	3(5.5)	0(0)	0(0)
Clindamycin (%)	-	-	-	-	-	-	44(78.5)	13(86.6)	2(100)
Linezolid (%)	-	-	-	-	-	-	38(70.3)	14(93.3)	1(50)
Erythromycin (%)	-	-	-	-	-	-	32(59.2)	9(60)	1(50)
Vancomycin (%)	-	-	-	-	-	-	54(100)	15(100)	2(100)

The organisms most frequently involved in SSI change from time to time and from place to place and also their sensitivity to various antibiotics. Our study correlates with other studies, in which the *most common organism causing SSI was Staphylococcus aureus followed by Pseudomonas aeruginosa* [15,16]*. Other organisms have also been implicated as the most common organisms causing SSI in different studies* [17,18]. Most of the Gram negative organisms were sensitive to piperacillin/tazobactam, ceftazidime and amikacin and all of them were sensitive to imipenem. Gram positive organisms were found to be more sensitive to gentamicin, clindamycin and linezolid. Sensitivity to vancomycin was seen in all the Gram positive isolates. The least sensitive antibiotic against Gram positive organisms was penicillin. A high percentage of penicillin resistance among strains of *Staphylococcus aureus* (91%) and *Enterococcus faecalis* (100%) causing SSI was observed in another study [19]. Out of 54 isolates of *Staphylococcus aureus*, 12 (22.22%) were found to be MRSA strains. 25% of MRSA strains were inducible clindamycin resistant. The use of D test is important to detect the presence of inducible clindamycin resistance and will help us in determining

Table no. 2 – Antibiotic susceptibility pattern of Klebsiella pneumoniae and Escherichia coli

Anti biotics	Klebsiella pneumoniae Total no. 28			Escherichia coli Total no. 27		
	ESBL Total no. 7	Non ESBL Total no. 21	P value	ESBL Total no. 6	Non ESBL Total no. 21	P value
Ampicillin (%)	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
Gentamicin (%)	3 (42.8)	16 (76.1)	0.24 N S	3 (50)	12 (57.1)	0.88 NS
Amikacin (%)	4 (57.1)	18 (85.7)	0.29 NS	4 (66.6)	16 (76.1)	0.95 NS
Ofloxacin (%)	3 (42.8)	14 (66.6)	0.5 NS	2 (33.3)	10 (47.6)	0.88 NS
Ciprofloxacin (%)	2 (28.5)	12 (57.1)	0.38 NS	0 (0)	10 (47.6)	0.03 Sig
Tetracycline (%)	0 (0)	7 (33.3)	0.21 NS	0 (0)	6 (28.5)	0.35 NS
Chloramphenicol (%)	2 (28.5)	9 (42.8)	0.82 NS	2 (33.3)	9 (42.8)	0.96 NS
Cotrimoxazole (%)	3 (42.8)	14 (66.6)	0.5 NS	2 (33.3)	13 (61.9)	0.44 NS
Cephalothin (%)	0 (0)	10 (47.6)	0.02 Sig	0 (0)	12 (57.1)	0.04 Sig
Amoxyclav (%)	0 (0)	16 (76.1)	0.002 HS	0 (0)	14 (66.6)	0.015 Sig
Cefepime (%)	0 (0)	18 (85.7)	0.002 HS	0 (0)	19 (90.4)	0.0001 HS
Ceftazidime (%)	0 (0)	21 (100)	0.001 HS	0 (0)	21 (100)	0.0001 HS
Cefotaxime (%)	0 (0)	17 (80.95)	0.008 HS	0 (0)	21 (100)	0.00 01 HS
Piperacillin/ tazobactam (%)	7 (100)	21 (100)	NA	6 (100)	21 (100)	NA
Imipenem (%)	7 (100)	21 (100)	NA	6 (100)	21 (100)	NA

HS- Highly Significant; Sig- Significant; NS- Not Significant; NA- Not Applicable

the true sensitivity to clindamycin. Vancomycin was found to be the most sensitive antibiotic against methicillin resistant *Staphylococcus aureus* followed by linezolid and clindamycin.

There was a significant difference for sensitivity to clindamycin and cotrimoxazole between MRSA and methicillin sensitive *Staphylococcus aureus* (MSSA) strains. Microorganisms responsible for surgical site infections like *Klebsiella pneumoniae* and *Escherichia coli* have the ability to produce ESBL resulting in limiting of therapeutic option. Among ESBL producing isolates imipenem and piperacillin/tazobactam were found to be the most sensitive antibiotics followed by amikacin, gentamicin and ofloxacin, and there was a significant difference for sensitivity to cephalosporins between ESBL producing and non ESBL producing *Klebsiella pneumoniae* and *Escherichia coli* isolates (Table. 2). The presence of metallo beta lactamase producing *Pseudomonas aeruginosa was not detected in our study and warrants further studies to detect their presence in this hospital.*

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