Bacteriological Profile and Antimicrobial Susceptibility Patterns of Bacteria Isolated from Surgical Site Infections of Patients Attending a Tertiary Care Hospital

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ABSTRACT

Introduction: Surgical site infections (SSIs) are one of the major causes of morbidity and mortality in developing countries despite recent advances in aseptic techniques. The SSIs due to emerging multidrug resistance (MDR) bacteria isolates are considered as grave threats to the public health worldwide. Each hospital has specific type of microbial flora from which infection initiates. In such condition microorganism shows unique type of antibiotic susceptibility and resistance pattern. In this study we report on the microbiological spectrum of SSIs and the antimicrobial susceptibility pattern with a view to provide guide to the clinicians for making rational decision over the choice of antibiotics in the management of surgical site infection.

Material and Methods: This cross-sectional study was carried out in a tertiary care hospital and the 560 samples were collected, processed in the microbiology laboratory and evaluated for the study.

Results: Out of 376 growth positive samples, Gram Negative Bacilli (GNB) were isolated from 271 (72%) samples and Gram Positive Cocci (GPC) were isolated from 105 (28%) samples. Among the isolated GNB, only 5 to 10% were sensitive to third generation Cephalosporins. Sensitivity to Fluroquinolones (Ciprofloxacin and Levofloxacin) was also low (Only 5% to 10% were sensitive). Only 3 to 15% of the GNB were found sensitive to Co-amoxiclav and 6.5% to 23% were sensitive to Ampicillin-Sulbactam. About 60% of the Pseudomonas aeruginosa and about 40% of the Klebsiella spp and Acinetobacter baumanii were found resistant to Meropenem.

Conclusion: Gram Negative Bacilli predominated over Gram Positive Cocci in surgical site infections in our set up. Very low sensitivity to 3rd generation Cephalosporins, Ampicillin, Co-amoxiclav and Fluroquinolones was noted among GNB isolates and an increasing pattern of resistance to Aminoglycosides and Carbapenems was also noted among MDR Pseudomonas aeruginosa and Acinetobacter baumanii isolates. More than 50% of the MDR Acinetobacter spp. were was found sensitive to Ampicillin- Sulbactam and Minocycline.

Keywords: Surgical Site Infection (SSI), Multi-drug Resistance (MDR), Gram Negative Bacilli (GNB), Sensitive, Resistance

INTRODUCTION

Surgical site infections (SSIs) are one of the major causes of morbidity and mortality in developing countries despite recent advances in aseptic techniques. Globally, surgical site infection rates have been reported in a range from 2.5 to 41.9 percent.[1,2,3,4,5,6] The incidence of these infections has estimated to be 15.45% and 11.32% by the Centre for Disease Control and Prevention (CDC) USA and the UK Nosocomial Infection Surveillance respectively.[7] SSI leads to serious consequences, including increased costs due to its treatment and increased length of hospital stay.[8,9]

The emergence of antibiotic resistance and its rapid spread among pathogenic bacteria isolates are considered as grave threats to the public health worldwide. During the last few decades, multidrug-resistant Gram negative bacterial strains such as Acinetobacter baumannii, E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Gram-positive Methicillin-Resistant Staphylococcus auras (MRSA) were increasingly associated with pus infections under hospital settings due to extensive overuse of antibiotics [10–12]. Rapid emergence of multidrug-resistant bacteria poses a serious threat to public health globally due to the limited treatment options and lukewarm discovery of new classes of antibiotics.[12,13]

Each hospital has specific type of microbial flora from which infection initiates. In such condition microorganism shows unique type of antibiotic susceptibility and resistance pattern. When such type of epidemiological data is available, a surgeon can interpret and treat in an empirical method and can prevent surgical site Infection and antibiotic resistance which may be a serious threat in future.

In this study we report on the microbiological spectrum of post operative wound infections and the antimicrobial susceptibility pattern with a view to provide guide to

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How to cite this article: Prasanta Kumar Panda, Rakesh Kumar, Purnima Raj, Pragya Khusro. Bacteriological profile and antimicrobial susceptibility patterns of bacteria isolated from surgical site infections of patients attending a tertiary care hospital. International Journal of Contemporary Medical Research 2020;7(2):B5-B9.

DOI: http://dx.doi.org/10.21276/ijcmr.2020.7.2.30

the clinicians for making rational decision over the choice of antibiotics in the management of surgical site infection.

MATERIAL AND METHODS

This was a cross sectional study conducted in the Department of Microbiology of LAM Government Medical College and associated KGH hospital, Raigarh, from 1st October 2018 to 30th September 20019. A total of five hundred and sixty specimens obtained from patients who had undergone surgical operations were analysed.

Pus swabs were aseptically obtained from surgical sites. The specimen was collected on sterile cotton swab without contaminating them with skin commensals. The samples were transported to the laboratory soon after being obtained. In the laboratory, the specimens were registered and macroscopically examined for their appearances. The swabs were cultured and smears made on clean slides for Gram staining techniques.

Smears were air-dried, heat fixed and stained by Gram's technique. The stained slides were examined microscopically under oil immersion lens for pus cells and bacterial cells. The specimens were inoculated on both differential and enriched media (MacConkey agar and 5% blood agar respectively). The culture plates were incubated aerobically for 24-48 hours.

Preliminary identification of bacteria was based on colony characteristics of the organisms i.e. haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed from primary cultures for final identification of the isolates.

The inoculum was prepared by picking parts of similar test organisms with a sterile wire loop. This was suspended in sterile peptone water (broth) and incubated up to two hours to allow organisms reach their log-phase in growth. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. A sterile swab was dipped into the suspension of the isolate in peptone water, squeezed free from excess fluid against the side of bottle and then spread over the agar plate.

Susceptibility testing was performed by Kirby-Bauer technique (Bauer *et al.,* 1966). The test organism was uniformly seeded over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic - impregnated paper disk into the agar medium. The isolate was then incubated at 37 for 16-18 hours. Organisms sensitive to the antibiotic were inhibited from growing in a circular zone around the antibiotic impregnated paper disk. A comparison of the inhibition zone diameter that was produced by a control strain was used to interpret the antimicrobial sensitivity. Grades of sensitivity recognised are sensitive, intermediate and resistant by comparison of zone of inhibition as indicated. Antibiotics were tested according to CLSI 2018.

RESULTS

A total number of 560 pus/wound swab samples were received to the microbiology laboratory for culture and sensitivity during 1 year. Out of 560 pus/wound swabs, 376 (67%) specimens were found culture positive for one or more organisms where as 33% had no growth. A single pathogen was isolated in 35% of culture positive samples and 2 agents were isolated from 60% samples; whereas 3 agents were isolated from each of the remaining samples.

Gram Negative Bacilli predominated over the Gram Positive Cocci. Out of 376 growth positive samples, Gram Negative Bacilli were found in 271 (72%) samples and Gram Positive Cocci were isolated from 105 (28%) samples.

Escherichia coli (21%) were the most commonly isolated pathogens, followed by Coagulase Negative Staphylococcus (12.7%), Staphylococcus aureas (11.7%) and Klebsiella spp (10.6%). Acinetobacter spp. (11.7%) and Pseudomonas aeruginosa (9.3%) were also isolated from the large number of specimens. Most of the Pseudomonas aeruginosa were isolated from the specimens received from Orthopaedics wards. Citrobacter spp. (5%), Proteus spp. (6.9%), Enterobacter spp. (6.1%) and Enterococci (3.4%) were also isolated in significant numbers. (Fig.1)

Among the isolated GNB, only 5 to 10% were sensitive to third generation Cephalosporins, whereas they were slightly more sensitive to 4th generation Cephalosporin cefepime (6% to 23%) and 10 to 30% were sensitive to Cefepime plus Tazobactam. Among GNB, all were resistant to 2nd generation cephalosporins Cefuroxime and cefazolin (100%).

Sensitivity to Fluroquinolones (Ciprofloxacin and Levofloxacin) among GNB was also low (Only 5% to 10% were sensitive). 65% of the Proteus spp. were sensitive to Piperacillin-Tazobactam, whereas only 13% of the Klebsiella spp., 16% of the the Pseudomonas aeruginosa, and 15% of the Acinetobacter baumanii. were sensitive to it.

25% to 40% of the GNB were sensitive to Gentamycin, Tobramycin and Amikacin. About 30% of the Pseudomonas aeruginosa were also sensitive to Tobramycin. All the isolated GNB were resistant to Ampicillin. Only 3 to 15% were sensitive to Co-amoxiclav and 6.5 to 23% were sensitive to Ampicillin-Sulbactam except Acinetobacter. About 50% of the Acinetobacter were sensitive to Ampicillin-sulbactam.

Among the GNB about 60 to 88% are sensitive to Meropenem but about 60% of the Pseudomonas and about 40% of the Klebsiella and Acinetobacter were found resistant to it. Sensitivity to Imipenem was slightly less than Meropenem (ranging from 77% for Proteus to about 36% for Klebsiella and Acinetobacter).

All the GNB isolates were sensitive to Tigecycline and Colistin except 10% of the Klebsiella spp., which were resistant to Tigecycline. Acinetobacter showed showed good sensitivity to Minocycline, about 72% of the isolates were sensitive (table-1).

All the isolated S. auras were resistant to Ampicillin

Antibiotics	Klebsiella spp. (Total= 61)	E.coli (Total = 84)	Enterobacter spp. (Total = 23)	Proteus spp. (Total = 26)	Pseudomonas aeruginosa (Total = 35)	Acinetobacter spp. (Total = 44)
Ampicillin	NT	0%	0%	0%	NT	NT
Cefazolin	0%	0%	0%	0%	NT	NT
Gentamicin	27.9%	38%	26%	27%	29.9%	7
Tobramycin	24.6%	42.8%	26%	27%	29.9%	13.6%
Amikacin	24.6%	42.4%	34.8%	37%	34.5%	(20%)
Amoxi-clav	3.3%	2.4%	8.7%	15.4%	NT	NT
Ampicillin-Sulbactam	6.5%	7%	21.7%	23%	NT	50%
Cefoperazone –Saulbactam	10%	21%	13%	30.8%	11.5%	15%
Ceftriaxone	5%	3.5%	8.7%	15.38%	NT	0%
Cefotaxime	5%	3.5%	8.7%	15.38%	NT	0%
Piperacillin + Tazobactam	13%	28.5%	39%	65.3%	16%	15%
Cefuroxime	0%	0%	0	0	NT	NT
Cefepime	13%	17.8%	17%	23%	9.2%	4.5%
Ciprofloxacin	5%	10.7%	13%	15.4%	10.3%	13.6%
Levofloxacin	8%	14.3%	17%	15.4%	15%	15.9%
Cefepime + Tazobactam	26.2%	30%	43%	38.5%	12%	31.8%
Meropenem	57.4%	76.2%	60%	88%	42%	59%
Imipenem	36%	38%	26%	77%	32.2%	36%
trimoxazole	9.8%	10%	17%	30.8%	NT	9%
Ceftazidime	4.9%	9.5%	12%	30.8%	5.7%	4.5%
Colistin	100%	100%	100%	NR	100%	100%
Tigecycline	90%	100%	100%	100%	NT	NT
Minocycline	NT	NT	NT	NT	NT	72.7%

Table-1: Antibiotic susceptibility pattern of gram negative isolates in percentage (% Sensitive)

Antibiotics	Staphylococcus aureus (Total = 44)	CONS (Total = 44)	Enterococcus Spp. (Total =13)
Penicillin	0	0	53.8%
Ampicillin	0	0	61.5%
Amoxy-clav	25%	9%	NT
Erythromycin	13.6%	13.6%	20%
Clindamycin	59%	54.5%	NT
Ciprofloxacin	32%	27%	NT
Gentamicin	82%	70.4%	NT
Co-trimoxazole	20%	19%	NT
Vancomycin	100%	100%	100%
Linezolid	100%	100%	100%
High level Gentamicin (For Enterococci only)	NT	NT	40%

Table-2: Antibiotic Sensitivity Pattern of Gram Positive Bacteria (% sensitive)

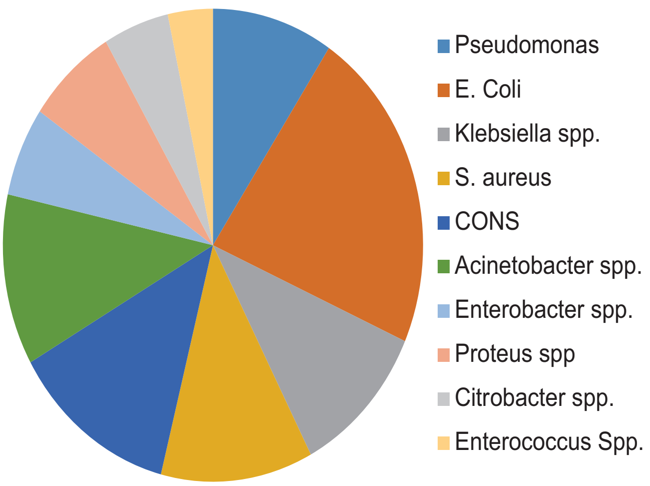

Figure-1: Distribution of organisms in pus/wound swab

(100%) and 9% were found sensitive to Co-trimoxazole, 13% were sensitive to Erythromycin, 59% were sensitive to Clindamycin, 37% were sensitive to Ciprofloxacin and Levofloxacin, 82% to Gentamycin, 100% were sensitive to Vancomycin and Linezolid.

Coagulase Negative Staphylococcus (CONS) were slightly more resistance to all the above antibiotics than S. auras except Vancomycin and Linezolid to whom all the CONS were sensitive.

Among Enterococci, 61.5% were sensitive to Ampicillin and about 50% to Penicillin, 20% to Erythromycin, 40% to high level Gentamycin and 100% were sensitive to Vancomycin and Linezolid. (Table 2)

DISCUSSION

In this study, a large number of wound/pus swab samples, taken from surgical site had shown growth of one or more than one organisms. 67 percent of the pus/wound samples were found culture positive and this finding is similar to jain Khayati et al (2017)[14] but it is much higher than the study by Taye et al 2005[15] Tesfahunegn et al, 2009[16] and Biadglign et al, 2009[17] with culture positivity of 14.8%, 44.1% and 53.0% and it is lower than studies conducted by Jonathan et al., 2008[18] and Adegoke et al., 2010[19] (98.5%- 100%). The high rate of culture positivity in this study may be due to relatively high number of abdominal surgery and lack of awareness about hospital acquired infection- prevention and control among health care givers in our hospital.

In this study, gram negative bacilli predominated gram positive cocci. Out of 560 growth positive samples, Gram-negative bacilli were found in 48.4% samples and Gram-positive cocci were isolated from 18.75% samples. Escherichia coli (21%) were the most commonly isolated pathogen. This trend of Gram Negative Bacilli dominating the gram positive cocci has been observed in Anvikar A L et al.[20], Tripathy B.S et al.[21] and Kamath N et al.[22] also.

Conversely in some previous studies, gram positive organisms, especially S.aureus, has dominated over gram negative bacilli (Lilani SP et al.[23], Rao A S et. al[4] Prabhaker H et al[24]). This difference in the pattern of distribution of bacterial isolates in different setups may be due to diversity of the study population and local antimicrobial use pattern which results in the emergence of pathogens that have the potential to resist antibiotics used currently. Another reason for the predominance of Gram negative organisms may be the fact that most of the infected patients in our study had undergone abdominal surgery and Gram negatives are predominantly reported to be involved in intra-abdominal procedures.

In the present study, a single pathogen was isolated in 35% of culture positive samples and 2 agents were isolated from 60% samples; whereas 3 agents were isolated from each of the remaining samples. According to Nandita pal et al.[25] 23.3% showed single isolates whereas 36.7% showed multiple isolates. Mama et al[26] reported single isolates in 91.6% whereas multiple isolates in 8.4% and also reported that 87.4% samples were culture positive and 12.6% samples did not show any bacterial growth. In the present study, multiple agents were isolated in relatively high number of samples.

Multi Drug Resistance (MDR) is a dreaded problem in our hospital. Our study reveals Enterobacteriaceae showing good sensitivity to meropenem (57% to 88%) and all are sensitive to Tigecycline and colistin. About 25% to 40% of the Enterobacteriaceae were also sensitive to Gentamycin, Tobramycin and Amikacin, but 90 to 95% of the Enterobactriaceae were showing resistance to 3rd generation Cephalosporins like Ceftriaxone, Cefotaxime, Cefoperazone-Sulbactam and Ceftazidime. Sensitivity to 4th generation Cephalosporin like cefepime and Cefepime plus Tazobactam is slightly more (6 to 24%) as compared to 3 rd generation. Very low sensitivity is also noted with Fluroquinolones (5 to 10%). Though, Proteus spp. were showing good sensitivity to Piperacillin-Tazobactam (65%) but only 13% of the Klebsiella spp., 16% of the the Pseudomonas , and 15% of the Acinetbacter were sensitive to it. All the Enterobacteriaceae were resistant to Ampicillin and only 3 to 15% were sensitive to Co-amoxiclav and 6.5 to 23% were sensitive to Ampicillin- Sulbactam. This could be due to the overuse of these drugs and high prevalence of extended spectrum beta lactamases (ESBLs) among these organisms.

Pseudomonas isolates in this study showed very low sensitivity to Cefoperazone, Ceftazidime Cefepime, Pipercillin- Tazobactam and also to Ciprofloxacin and Levofloxacin whereas about 30 to 35% of isolates were sensitivite to Gentamicin, Tobramycin and Amikacin and only 40% were sensitive to Meropenem.

Most of the Acinetobacter isolates in this study were MDR but showed good sensitivity to Ampicillin- Sulbactam (50%), Minocycline (74%) and Meropenem (60%).

Staphylococcus auras showed maximum sensitivity to Linezolid (100%), Vancomycin (100%), Gentamycin (82%) and Clindamycin (100%). All the Enterococci were also sensitive to Linezolid (100%) and Vancomycin (100%) and good numbers of them (40%) were sensitive to high level Gentamycin (HLG).

In many previous studies also, Penicillin, Ampicillin and Co-amoxiclav for both Gram Positive Cocci and Gram Negative Bacilli have shown very poor performance[27,23,4,21,22] and very low sensitivity is also noted with 2nd and 3rd generation Cephalosporins which is similar to our study but one old study of Hyderabad(1975)[4] has observed good sensitivity of Cephalosporins against Gram Negative Bacilli. This is also suggestive of increasing resistance with the passing years due to misuse and overuse of antibiotics.

Sensitivity of Gram positive cocci to Linezolid (100%) and Vancomycin (100%) is similar to our study in Kamath et. al.[22]

CONCLUSION

Gram Negative Bacilli predominated over Gram positive cocci as the leading cause of surgical site infections in our set up. Very low sensitivity is noted with 3rd generation Cephalosporins, Ampicillin, Co-amoxiclav and Fluroquinolones and an increasing pattern of resistance is also noted with Aminoglycosides and carbapenems. Prevalence of MDR pseudomonas and Acinetobacter are increasing. This could be due to overuse of antibiotics and high prevalence of ESBLs among Enterobacteriacae. Prevention and Control of hospital acquired infections and implementation of antimicrobial stewardship programme in every health care set up is the only way forward to decrease the SSIs and to prevent antimicrobial resistance.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 26-12-2019; **Accepted:** 28-01-2020; **Published:** 29-02-2020