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Original Research Article

Antibiotics sensitivity status and antibiogram patterns of aerobic bacterial isolates from surgical site infections

Balvinder Singh Arora[1]*, Santhosh Rajan[1], Ravinder Mohil[2], Neeraj Narayan Mathur[3]

[1]Department of Microbiology, [2]Department of Surgery, [3]Department of Otorhinolaryngology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India

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***Correspondence:**
Dr. Balvinder Singh Arora,
E-mail: dr_arorabalvinder007@yahoo.com

ABSTRACT

Background: Surgical Site Infections (SSIs) are the third most common nosocomial infections. Emergence and spread of drug resistant strains have been found to pose a serious challenge in the management of such infections. There is limited information on the epidemiology of such pathogens. The antibiotic sensitivity patterns of aerobic bacterial isolates from post-operative SSIs show wide variations that lead to difficulties in empirical selection of the right kind of drug for treatment. Properly planned studies about antibiotic sensitivities patterns of such isolates can help in judicious management of SSIs and cause reduction in morbidity and mortality.

Methods: A total of 50 patients diagnosed by the surgeon and fulfilling the case definition of SSI, were studied for bacteriological analysis. All the clinical specimens were cultured and identified applying standard culture techniques. The aerobic bacterial isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer Disc Diffusion method to arrive at the drug sensitivity patterns. Data were entered in MS Excel spread sheet and analysed using SSPP software version 21.00

Results: A total of 32 patterns of sensitivity were observed. For *Esch. coli*, a total of nine patterns were observed. All strains of *Esch. coli* were found sensitive to tigecycline (100%) and colistin (100%). For *Klebsiella spp.* a total of 9 patterns were obtained with TIG-COL being the predominant pattern in 6 cases. For Acinetobacter spp. only colistin was found most effective drug. In case of Pseudomonas aeruginosa, except colistin (100% sensitivity), there were wide variations in sensitivity with imipenem (71%) as next most effective drug. In Proteus spp. - most of the in-use drugs were effective except cephalosporins. Among gram positive organisms, only three strains of Staphylococcus aureus were isolated, and these were MRSA (100%). Two strains of enterococcus were isolated, and these showed sensitivity to linezolid only.

Conclusions: Wide variations in sensitivity status observed in the study are suggestive that antibiotic usage should be tailored to individual needs and proper selection of antibiotics for management of SSIs must be guided by laboratory antibiogram.

Keywords: Antibiogram pattern, Antimicrobial sensitivity, *Escherichia coli*, Gram positive *cocci*, *Klebsiella*, Surgical site infections

INTRODUCTION

Surgical Site Infections (SSIs) are the most common post-operative complications even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis. These are the third commonest nosocomial infections and account for approximately 10-40% of all Health Care Associated (HAI) Infections. Multi and single centered studies show that the majority of organisms causing SSI are gram negative bacilli e.g. *E. coli*, *Klebsiella*, *Pseudomonas* and *Enterobacter spp.* and gram-positive *cocci* e.g. *Staphylococcus aureus*. The problems of antimicrobial management of infecting pathogens in Surgical Site Infections (SSIs) is currently the most important challenge because most SSIs are hospital acquired and the isolates show wide variations in the antimicrobial susceptibility patterns.[1-4] It contributes to substantial morbidity and mortality.[5,6] The present study was undertaken to know the antibiotic sensitivity patterns of infecting isolates in SSIs cases so that the clinicians can be effectively assisted in management of such infections by choosing the right kind of antibiotics.

METHODS

The present study was a hospital based observational study undertaken in the Department of Microbiology and Department of Surgery at Vardhman Mahavir Medical College (VMMC) and Safdarjang hospital, New Delhi over duration of six months from April 2016 to September 2016. Inclusion criteria : The study included a. patients who developed SSI after abdominal surgery - emergency or elective - with purulent drainage from the site of incision; b. at least one of the signs and/or symptoms of fever more than 37^{0}C, c. localized pain or tenderness, or d. abscess formation on direct examination. Exclusion criteria: Patients who had already undergone any bacteriological investigation especially bacterial cultures were excluded from the study. A total of 50 patients diagnosed by the treating surgeon as a case of SSI, fulfilling the case definition, were studied for bacteriological analysis.[1] From each patient two wound swabs were collected from surgical site(s) before antiseptic cleansing of the wound and submitted to Department of Microbiology at the earliest possible but not later than 30 minutes of collection. Gram stain preparations were made from one swab and culture was processed with the other. The wound swabs were inoculated on Blood agar, MacConkey agar (all Oxoid), Nutrient agar, and Mannitol Salt Agar (MSA) and incubated in an aerobic atmosphere at 37^{0}C for 18-24 hours overnight. Observations of the plates were made the following day but extended to 48 hours if there was no bacterial growth in less than 24 hours. Community acquired strains are ruled out as the patients selected were those who developed the infection post operatively after 48 hours of admission. Colonies were counted subjectively by naked eye using hand lens. Colonies counts more than 30 were taken as infection. Plates showing ≤2 types of organism were considered as potential pathogens and studied further including Gram stain and appropriate biochemical tests for final identification.[7] Extended sugar tests for identification, wherever needed, were carried out according to the standard biochemical tests as described by Mackie and McCartney Microbiology, 14th edition, and CDC Guidelines.[8,9] Antimicrobial susceptibility tests were performed on Mueller-Hinton agar medium (Oxoid) by disk-diffusion method as per Clinical and Laboratory Standards Institute directions.[10] All isolated gram-positive and gram-negative aerobic bacteria were tested against ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amoxicillin-clavulanic acid (30 µg), trimethoprim-sulphmethoxazole (25 µg), and ceftriaxone (30 µg). In addition, gram-positive bacteria were tested against penicillin G (10 IU), erythromycin (15 µg), and vancomycin (30 µg). Additional antimicrobial discs available in the department were used to ascertain the sensitivity status of the isolate in question. Reference strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 25923) were included as controls available in the department. Statistical analysis in terms of percentages and frequencies was performed for calculating percent sensitivity status and antibiogram formulations using Statistical Package for the Social Sciences (SPSS) licensed software version 21.00.

RESULTS

A total of fifty pus samples were collected and bacteriological diagnosis could be made in forty-seven cases while in one case only candida albicans was isolated. Two (02) specimens were bacteriologically sterile. In all, ninety four percent (94%) samples had bacterial etiology with a yield rate of 1.02. Age and gender distribution, type of wound and nature of surgery revealed maximum number of patients in age group of 21-30 years (26%) followed by 41-50 years (20%); Seventy eight percent patients were males and twenty two percent females with male to female ratio as 3.5:1. Eighty percent surgeries were of emergency nature (Table 1).

Table 1: Age, gender, type of wound and type of surgery in 50 cases of SSIs.

Age group	No. (%)	Gender distribution	
11-20	9(18%)	Males	Females
21-30	13(26%)	39(78%)	11(22%)
31-40	7(14%)	Types of SSIs	
41-50	10(20%)	Superficial	Deep
51-60	7(14%)	27(54%)	23(46%)
61-70	1(2%)	Nature of Surgery	
Total	50	Emergency	Elective
		42(84%)	08(16%)

Among 49 isolates, the most common was *Escherichia coli* 14(28.5%) closely followed by *Klebsiella spp.* - 13 (26.5%) while *Enterococcus spp.* was the least - 2(4%) (Table 2). Susceptibility testing revealed a total of 9 sensitivity patterns for 14 strains of *Esch. coli* with AK NET PIT CFS ERT MER IMP TIG COL as the predominant pattern in 3 strains (Table 3). For 13 strains of *Klebsiella spp*, a total of 9 patterns were obtained with TIG COL being the predominant pattern in 6 cases (Table 3). For *Esch. coli* and *Klebsiella* together, Tigecycline (100%), colistin (100%), amikacin (40 -50%), netilmicin (40-50%) and meropenem (>40%) were sensitive (Table 2). For *Acinetobacter*, only colistin (100%) and imipenem (approx. 70%) showed sensitivity. For *Pseudomonas*, only colistin (100%) was effective and other antimicrobials revealed wide variations of susceptibility. In case of *Staphylococcus* - all the three strains were MRSA and vancomycin and linezolid showed 100% sensitivity. For *Enterococcus spp.* only linezolid was 100% sensitive.

DISCUSSION

For *Escherichia coli*-tigecycline and colistin were the most effective drugs with hundred percent sensitivity while ciprofloxacin and cefotaxime were not effective at all. Amikacin and netilmicin revealed only fifty percent sensitivity. It was followed by meropenem as nearly forty three percent (42.9%), ertapenem, and cefoperazone+sulbactam as 28.6% each. Piperacillin+tazobactam and imipenem were found to be 14.2% effective (Table 2). In a study by Trojan R et al, cefotaxime and ciprofloxacin were found to be effective in 20% of strains of *E.coli*, whereas Cefoperazone+sulbactam was effective in 34% which is close to 28.6% in this strains.[11]

Amikacin and netilmicin in our study was found effective in about fifty percent strains each while it is seventy five and fifty seven percent respectively by same authors. Meropenem (42.9%) in our study is close to 68% recorded in Trojan et al study and 65% recorded by Mantravadi et al, study.[11,12] It is worthwhile to state that fourteen strain of *Esch. coli* isolated in the study were found to have a total of nine patterns of sensitivity (Table 3).

For *Klebsiella spp*, tigecycline and colistin were found hundred percent effective whereas cefotaxime and ciprofloxacin were the least (0%) (Table 2). Susceptibility status of Klebsiella spp to ciprofloxacin and cefotaxime documented in literature show low sensitivity about 15 to 20 %.[13] For other drugs viz. imipenem, ertapenem, Piperacillin+Tazobactam, Cefoperazone+sulbactam including netilmicin, amikacin and meropenem showed range from 13.3% to 46.6% in our study which is in accordance with other studies done by other Indian research scholars.[14] All *Klebsiella* strains, fifteen in number, were found to have a total of nine patterns of sensitivity - of these TIG COL pattern was most common represented by 6 strains (Table 3).

For *Acinetobacter* - Colistin and imipenem were found as the most effective drugs - hundred percent and seventy two percent respectively. In literature, extremely wide variations from 0% to 100% susceptibility are well documented.[15] For these seven strains, two sensitivity patterns were observed with colistin being most effective (Table 3).

In case of Pseudomonas aeruginosa, except colistin (100% sensitivity), there was wide variation in susceptibility observed with imipenem (71%), netilmicin (57%), and piperacillin+tazobactam (57%) in that order (Table 2). Amikacin, ciprofloxacin, meropenem were least effective drugs (<50%). These findings are in accordance with other studies viz Trojan R et al, showed 50% susceptibility to amikacin, 40% to piperacillin+tazobactam, 30% to netilmicin,80% to imipenem. Mohanty S et al, and Golia et al, also showed similar observation. A total of 5 types of sensitivity pattern were observed.[7,11,15]

Table 2: Sensitivity of *Escherichia coli and Klebsiella; and Pseudomonas aeruginosa.*

Antimicrobial agent	*Escherichia coli* (S%)	*Klebsiella spp.* (S %)	*Pseudomonas aeruginosa*	
			Antimicrobial agent	Sensitive (%)
Cefotaxime	0%	0%	Amikacin	29%
Ciprofloxacin	0%	0%	Ceftazidime	43%
Amikacin	50%	46.6%	Netilmicin	57%
Netilmicin	50%	46.6%	Ciprofloxacin	29%
Piperacillin + Tazobactar	14.2%	20%	Piperacillin+Tazobactam	57%
Cefoperazone + sulbactaı	28.6%	26.6%	Meropenem	43%
Ertapenem	28.6%	13.3%	Imipenem	71%
Meropenem	42.9%	46.6%	Colistin	100%
Tigecycline	100%	100%		
Colistin	100%	100%		

For *Proteus spp.* amikacin, netilmicin, piperacillin+tazobactam, cefoperazone-sulbactam, ertapenem, meropenem, imipenem, tigecycline, and colistin were highly effective except cephalosporins (50%). These findings are in accordance with other studies viz Trojan R et al, Mantravadi HB et al, Praveen kumar et al.[11,12,16] Two types of sensitivity pattern were observed. All these 03 strains were MRSA (100%). A total of 2 types of sensitivity pattern were observed which includes VAN LZ represented by 2 strains and CIP COT ERY CD GEN VAN LZ by 1 strain. For *Staphylococcus* - for only three strains isolated and only two patterns were observed but all the three strains were MRSA. The number of strains isolated is too small to make a definitive opinion. With more strains, at least fifty, isolated over a longer period of time would be appropriate to make any comments on this issue. For Enterococcus, 2 strains were isolated showing 100% sensitivity to linezolid, followed by 50% sensitivity to penicillin, erythromycin and ‘gentamicin, while, interestingly, vancomycin and ampicillin were 100% ineffective (resistant). Here also, like *Staphylococcus*, the number of strains is too small to comment. However, a large Study by Golia S et al, have shown similar findings with penicillin and vancomycin being not effective at all (100% resistant).[15] For ‘gentamicin, Mantravadi et al, have shown Enterococcus being sensitive up to 50%. Praveen kumar et al, study has also shown 50% sensitivity to penicillin and erythromycin. In this study, two types of sensitivity pattern were observed.[12,15,16] A total of 32 sensitivity patterns observed in the study (as shown in table 3) reflect wide variations in susceptibility status. It emphasizes that antibiotic usage should be tailored to individual needs. Proper selection of antibiotic therapy is a critical adjunct to the effective treatment plan. Limitation of the study: The samples size in this study is small but nonetheless it gives an idea about the epidemiological pattern and the types of pathogens' prevalence and their antibiotic sensitivity status in this institution.

Table 3: Sensitivity patterns of forty-nine bacterial pathogens (non-duplicate strains) isolated form 50 cases of SSIs.

Organism	Numbers (%)	Susceptibility pattern (number of strains)	Number of pattern
Escherichia coli	14 (28.5%)	AK NET PIT CFS ERT MER IMP TIG COL (3) AK NET PIT CFS ERT MER TIG COL (1) AK NET CFS ERT MER IMP TIG COL (2) AK NET ERT MER IMP TIG COL (1) AK NET CFS TIG COL (1) AK NET MER TIG COL (1) AK NET TIG COL (2) MER TIG COL (1) TIG COL (2)	9
Klebsiella spp.	15(30.6%)	AK NET PIT CFS ERT MER IMP TIG COL (2) AK NET PIT CFS ERT MER TIG COL (1) AK NET PIT CFS MER CIP TIG COL (1) AK NET MER IMP TIG COL (1) AK NET CFS MER TIG COL (1) AK NET MER TIG COL (1) AK NET TIG COL (1) MER TIG COL (1) TIG COL (6)	9
Acinetobacter spp.	7(14.3%)	NET COL (2) COL (5)	2
Pseudomonas aeruginosa	7(14.3%)	CTZ AK NET PIT CFS MER IMP CIP COL (1) CTZ AK NET CFS COL (1) NET PIT CFS MER IMP CIP COL (1) PIT CFS MER IMP COL (1) NET COL (1) COL (2)	6
Proteus spp.	2(4%)	AK NET PIT CFS ERT MER IMP (1) AK NET PIT CFS ERT MER (1)	2
Staphylococcus aureus	3(6.1%)	CIP COT ERY CD GEN VAN LZ (1) VAN LZ (2)	2
Enterococcus spp.	2(4%)	ERY VAN LZ G-120 (1) LZ (1)	2
Total	49		32

CONCLUSION

Wide variations and large number of sensitivity patterns in only forty-nine strains from fifty cases suggest that the antimicrobial sensitivity testing of each bacterial isolate must be done before modifying the existing or initiating the newer antibiotics in every case of SSI. It will help reduce the morbidity and mortality in the long run.

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