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Original Work

Antimicrobial susceptibility pattern of bacterial isolates from surgical wound infections in Tertiary Care Hospital in Allahabad, India

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ABSTRACT: The aim of present study to analyze the occurrence and in-vitro antimicrobial susceptibility of bacterial pathogens isolated from surgical wound infections. Specimens from a total of 129 patients undergoing either emergency or elective surgery were collected from infected sites or stitch lines and inoculated onto appropriate media. The bacterial cultures were identified utilizing standard microbiological and biochemical methods. Isolates were tested for susceptibility to antimicrobials using the Kirby Bauer disk diffusion method. Statistical analysis was performed using the chi-square test. Of 129 patients investigated (62 emergency and 67 elective surgery cases), bacterial isolates were isolated with almost equal frequency both from emergency and elective surgery cases. Of 108 (83.72%) culture positive samples, 62 (57.41%) were Gram negative, 39 (36.11%) Gram positive, and 7 (6.48%) showed multiple organisms. Of total 115 bacteria isolated (101 single and 7 double organisms culture positive), 33 (28.69%) were *Escherichia coli* and were also the commonest; followed by *Staphylococcus aureus,* 30 (26.09%) cases. *S. aureus* and *Streptococcus* spp. showed maximum susceptibility (100%) to linezolid and vancomycin. Maximum susceptibility of *E. coli* was observed to ciprofloxacin (75.7%), followed by gentamicin (54.5%); of *Klebsiella spp.* to ceftriaxone and gentamicin (66.6% each), of *Proteus spp.* to gentamicin (70%) followed by ciprofloxacin (60%), and of *Pseudomonas aeruginosa* to piperacillin (100%) and tobramycin (71.4%). *E. coli* and *S. aureus* were the most common and *Salmonella spp.* and *Acinetobacter spp.* were the least common organism causing surgical site infections. The definitive therapy included ciprofloxacin and gentamicin for *E. coli*; linezolid and vancomycin for *S. aureus* and *Streptococcus spp;* ceftriaxone and ciprofloxacin for *Klebsiella spp., Citrobacter spp., acinetobacter spp* and *Salmonella spp.*

KEY WORDS: ***Antimicrobial susceptibility pattern; Bacterial isolates; Gram negative; Gram positive organisms***

INTRODUCTION

Bacterial infections at surgical sites and surgical wounds are fairly common despite aseptic measures. Surgical site infections are responsible for delayed wound healing, prolonged stay in hospital, increased cost of therapy and are also important determinants of morbidity and mortality of the patient.

Infections of surgical wounds occur whenever the combination of number and virulence of bacteria in the wounds is sufficiently large to overcome the local host defense mechanisms and establish progressive growth. Essentially, all clean operative wounds contain small numbers of bacteria at the end of the procedure, but only a small number develop infection[1]. The development of surgical

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site infections (SSIs) are related to three factors firstly, the degree of microbial contamination of the wound during surgery; secondly, the duration of the procedure, and thirdly, host factors such as diabetes, malnutrition, obesity, immune suppression, and a number of underlying disease states[2]. Factors leading to higher risk of infection have been described and prophylaxis with antibiotics will definitely curtail/prevent surgical wound infection[3,4].

Although, a large number of antimicrobial agents have been developed more recently, yet development of resistance to large number of antimicrobials is quite alarming. Further, imprudent use of antimicrobial agents promotes growth of resistant micro-organisms and can cause serious toxicity.

Initiation of optimal empirical antibiotic therapy requires knowledge of the most likely infecting micro-organism(s) and their susceptibilities to antimicrobial drugs[5]. Thus for surgeons to decide the appropriate prophylactic and therapeutic antibiotics, there should be data on the spectrum of common pathogens encountered in the surgical unit and their antimicrobial susceptibility data at each hospital setting. The present study was therefore undertaken to explore these relevant objectives.

METHODOLOGY

The study was carried out in 129 patients undergoing either emergency or elective surgery in general surgery wards at Swaroop Rani Nehru Hospital, attached to MLN Medical College, Allahabad during the period January to December 2007. Patients of all ages and both sexes were included in the study. The ethical committee clearance and informed consent from patients were obtained though it was a routine sample for diagnosis.

A personal identification number was allotted to each patient and a detailed history, such as date of admission, date of surgery, name of the surgical procedure relevant clinical features and associated complaints, presence or absence of fever, presence or absence of drain, history of antimicrobial agents used (if any) were recorded. The samples investigated were pus, swab from the stitch line and swab from any other infected sites, which were collected with proper aseptic precautions and transported to the department of microbiology within two hours of collection. For swabs an appropriate transport medium Tryticase Soya Broth (TSB) was used. The specimen collected was divided into three parts: first part was used to make Gram smear as per standard protocol; second part of the sample was plated directly on the blood agar and McConkey agar and incubated at 37°C for 18-24 hours; third part of the sample was inoculated in Brain heart infusion broth and incubated overnight at 37°C.

The colonies grown either on primary plating or after sub-culturing were identified by colony characteristics, gram-staining character, , motility, and a set of biochemical tests including catalase test, coagulase test, methyl red, indole test, Voges-Proskaeur test, urease test, citrate utilization test, nitrate reduction test, triple sugar iron (TSI) test and oxidase test, as per standard protocol[6]. Antibiotic susceptibility testing was performed by the Kirby-Baeur disc diffusion method (Himedia Laboratories Pvt. Ltd., Mumbai), as recommended by National Committee for Clinical Laboratory Standards (NCCLS)[7]. The chi-square test was used for statistical analysis.

RESULTS

This prospective study comprised of 129 patients including 62 emergency cases and 67 elective surgery cases. The majority of samples were collected from patients who underwent abdominal surgery - 104 (80.62%) cases, followed by surgery of extremities - 14 (10.85%) and head and neck - 11 (8.53%) cases.

Among 129 specimens, 108 (83.72%) were culture positive and 21 (16.28%) were sterile samples. M:F ratio was 1.92:1 in culture positive cases. Out of 62 cases of emergency surgery, 54 (87.09%) were culture positive and only 8 (12.90%) were culture negative. Among 67 cases of elective surgery, 54 (80.59%) were culture positive, while 13 (19.40%) were culture negative. The chi-square test revealed insignificant ($\chi 2$ = p > 0.58, DF = 1, p=0.4470053) difference between type of cases (emergency/elective) and culture.

Maximum culture positive, 48/129 (37.21%) cases were in the age group of 21-40 years, followed by 0-20 years 31 (24.03%) cases and minimum 8 (6.20%) cases were in the age group of 61-80 years. The chi-square test revealed insignificant ($\chi 2$ = 4.29, DF=3, p=0.23146525). Of 129 admitted cases, fever was present in 65 cases, and absent in 64 cases. Among 65 febrile cases, 60 cases were culture positive. In 64 afebrile cases 48 were culture positive. The association between fever and culture positivity was found to be significant ($\chi 2$=5.87, DF=1, p=0.0153585). Of 108 culture positive cases, 62 (57.41%) were Gram-negative, 39 (36.11%) were Gram-positive, and 7 (6.48%) cases were considered contaminated, hence these were rejected.

Out of a total 115 bacterial isolates (101 single isolate culture positive and 7 mixed isolates (2 isolates) culture positive), *E. coli* was most frequently isolated [33 (28.69%)], followed by *S. aureus* [30 (26.09%)]. *Salmonella spp. non-typhoidal* was isolated in only one case, and that too in elective surgery case (**Table 1**).

It may be stated that an intermediate sensitive organism upon judicious exposure to higher concentration of an antimicrobial agent may become sensitive; hence intermediate sensitive organisms were considered as sensitive to that antimicrobial agent for the purpose of the present study.[8,9] Though opposite views have also been expressed.

Table 1: Distribution of bacterial isolates in the samples

S.No	Micro-organism	Emergency cases N (%)	Elective cases N (%)	Total N (%)
1	S. *aureus*	15(13.04)	15(13.04)	30(26.09)
2	CoNS*	1(0.87)	3(2.61)	4(3.48)
3	*Streptococcus* spp.	4(3.48)	4(3.48)	8(6.96)
4	*E. coli*	17(14.78)	16(13.91)	33(28.69)
5	*Klebsiella* spp.	8(6.96)	7(6.09)	15(13.04)
6	*Proteus* spp.	6(5.22)	4(3.48)	10(8.69)
7	*Citrobacter* spp.	2(1.74)	2(1.74)	4(3.48)
8	*Acinetobacter* spp.	2(1. 74)	1(0.87)	3(2.61)
9	*Salmonella* spp.	0(0.00)	1(0.87)	1(0.87)
10	P. *aeruginosa*	4(3.48)	3(2.61)	7(6.09)
	Total	59(51.30)	56(48.69)	115(100)

*Coagulase-negative staphylococci

S. aureus was found in 15 cases each of elective and emergency surgery. The maximum susceptibility (100%) was observed to linezolid and vancomycin. The maximum resistance was observed to ampicillin [22 (73.33%)], followed by trimethoprim-sulfamethoxazole, i.e., co-trimoxazole [20 (66.67%)] (**Table 2**). All 4 coagulase- negative staphylococci (CoNS) isolated were susceptible to linezolid, vancomycin and ceftriaxone. None were however sensitive to ciprofloxacin and chloramphenicol. None of the isolates were vancomycin-intermediate *S. aureus* (VISA). Streptococci were 100% susceptible to linezolid and vancomycin. Overall maximum resistance was observed to chloramphenicol (75%) followed by ciprofloxacin and cotrimoxazole (62.5%).

Table 2: Pattern of antimicrobial susceptibility in *S. aureus* in Emergency and Elective Surgery cases

S. No	Antimicrobial agents	Emergency case n=15			Elective case n=15		
		S	I	R	S	I	R
1	Ampicillin	2	2	11	3	1	11
2	Azithromycin	9	0	6	9	1	5
3	Ceftriaxone	10	1	4	8	0	7
4	Cefazolin	6	1	8	7	0	8
5	Cotrimoxazole	7	0	8	3	0	12
6	Chloramphenicol	10	2	3	10	0	5
7	Ciprofloxacin	7	1	7	4	5	6
8	Linezolid	15	0	0	15	0	0
9	Vancomycin	15	0	0	15	0	0

S = Susceptible; I = Intermediate; R = Resistant

In *E. coli* overall maximum susceptibility was observed to ciprofloxacin [25 (75.76%)], followed by gentamicin (54.5%). Maximum resistance was observed to cotrimoxazole [27 (81.8%)], followed by azithromycin (78.7%) and chloramphenicol (72.7%) (**Table 3**). In *Klebsiella* spp. overall maximum susceptibility (66.6%) was noted with ceftriaxone and gentamicin followed by ciprofloxacin (53.3%). Maximum resistance was seen against cotrimoxazole (73.3%) followed by azithromycin (66.6%) and chloramphenicol (60%) (**Table 3**). In *Proteus* spp. maximum susceptibility (70%) was observed with gentamicin followed by ciprofloxacin (60%). Maximum resistance was seen against azithromycin (80%).

In *Citrobacter spp.* maximum susceptibility was noted with ciprofloxacin (75%) followed by azithromycin, ceftriaxone and cefaclor each showing 50% susceptibility. 100% resistance was noted against co-trimoxazole. In *Acinetobacter spp.* 100% susceptibility was noted with ciprofloxacin and ceftriaxone. In *Salmonella spp.* 100% susceptibility was seen with chloramphenicol, ciprofloxacin, ceftriaxone and gentamicin (**Table 4**).

Table 3: Pattern of antimicrobial susceptibility in *E. coli* and *Klebsiella spp.* in Emergency and Elective Surgery cases

S. N.	Antimicrobial Agents	*E. coli*						*Klebsiella spp.*					
		Emergency cases n=17			Elective cases n=16			Emergency cases n=8			Elective cases n=7		
		S	I	R	S	I	R	S	I	R	S	I	R
1	Azithromycin	4	2	11	0	1	15	3	0	5	2	0	5
2	Chloramphenicol	3	2	12	3	1	12	4	0	4	2	0	5
3	Ciprofloxacin	13	0	4	3	9	4	6	0	2	2	1	4
4	Cotrimoxazole	3	0	14	2	1	13	2	0	6	2	0	5
5	Cefuroxime	6	3	8	5	0	11	4	0	4	4	0	3
6	Ceftriaxone	6	4	7	4	2	10	4	1	3	5	0	2
7	Gentamicin	6	3	8	7	2	7	4	1	3	4	1	2

S = Susceptible; I = Intermediate; R = Resistant

Table 4: Pattern of antimicrobial susceptibility in *Citrobacter spp.*, *Acinetobacter spp.* and *Salmonella spp.* in Emergency and Elective surgery

Microorganism		AZT	CHL	CIP	COT	CEF	CEFT	GE
Citrobacter spp. n=4	S	2	1	3	0	2	2	1
	I	0	0	0	0	0	0	1
	R	2	3	1	4	2	2	2
Acinetobacter spp. N=3	S	1	1	3	1	2	3	1
	I	0	0	0	0	0	0	0
	R	2	2	0	2	1	0	2
Salmonella spp. n=l	S	0	1	1	0	0	1	1
	I	0	0	0	0	0	0	0
	R	1	0	0	1	1	0	0

AZT=Azithromycin; CHL=Chloramphenicol; CIP=Ciprofloxacin; COT=Cotrimoxazole; CEF=Cefaclor; CEFT=Ceftriaxone; GE=Gentamicin

DISCUSSION

The risk of developing surgical-site infection is dependent on a myriad of host (intrinsic) and operative (extrinsic) risk factors[10]. Infection is an unresolved problem while undertaking any surgical operations. Infections occur even though surgeons perform thoroughly clean procedures during surgery and patients are strictly managed before and after surgery. Despite the availability of a large arsenal of antimicrobial agents the ability of bacteria to become resistant to antibacterial agents is amazing. This is more evident in hospital settings where antimicrobial agents are being used profusely. A changing pattern of isolated organisms and their antimicrobial sensitivity; (it varies from hospital to hospital and region to region) is a usual feature. Many a time patients' lives are lost after extensive surgery, owing to microbial infections and improper treatment. Thus, the study is clinically relevant in the present scenario by not only observing the spectrum of microorganisms isolated from surgical patients (nosocomial infections) but also in evaluating their antimicrobial susceptibility pattern.

In this prospective study, samples were collected from 129 patients admitted in the surgery wards. Of 62 cases of emergency surgery 54 (87.09%) were culture positive, and of 67 cases of elective surgery, 54 (80.59%) were culture positive. These observations depicted that a high infection rate (83.72%) was prevailing currently in these setting in this region of the state and that the infection rate was comparable in both the groups, unlike other previous studies where infection in the emergency surgery was significantly higher as compared to elective surgery. This calls for more stringent steps for proper disinfection and sterilization during the elective surgery cases. Moreover, infection leads to protracted hospitalization, patients risk complications associated with additional surgery and antimicrobial treatment as well the possibility of renewed disability.[11]

The predominance of males 71 (65.74%) in culture positive cases is probably due to more exposure to the environment and more chances of accidents while earning livelihood. A higher involvement of males has also been reported by other workers in the field. There was statistically no significant difference in male to female infection rate.

The age distribution of cases ranged between 0-80 years with younger and most productive, 21-40 years age group being maximally involved [48 (37.21%)]. The involvement of younger age group was quite significant. Moreover, the infection rate was also maximal in the age group of 21-40 years and least in 61 to 80 years age group.

An attempt was also made to correlate the presence of fever with culture positivity. Out of 65 febrile cases, positive culture was found in 60 cases (92.30%). Whereas out of 64 afebrile cases, positive culture was found in only 48 cases (75%). This observation was in conformity with general concept that infection is associated with fever. The explanation is infection causes release of pyrogens, which elevate the body temperature.[12]

In regard to patients' factors, several conditions have been recognized to significantly increase the risk of post-operative infection, such as diabetes mellitus, sickle cell anemia, malnutrition, renal failure with haemodialysis, concurrent urinary tract infection (postoperative bladder retention), obesity, smoking, etc.

There was predominance of Gram-negative organisms over Gram- positive organisms. A higher rate of infection due to Gram-negative organisms, [62 (57.41%)] in this study is because the study comprised of nosocomial infections and majority of specimens were collected from abdominal surgery cases. As abdominal surgeries usually involve organ perforation, gut surgeries and handling of gut, that may cause spillage of gut flora which of course is rich in Gram-negative organisms, hence a greater infection rate with Gram-negative organisms. The facultative organisms most commonly isolated from intra-abdominal infection are *Escherichia coli* and *Enterococcus faecalis*.[13,14] Ananthnarayan *et al*[15] reported that enteric Gram-negative bacilli were the most common hospital pathogens. Zaleznik[16] also found that Gram-negative bacteria were the commonest cause of nosocomial infections.

According to Keshari *et al*[17], of 92 isolates, 54 were Gram-negative bacilli and 38 Gram-positive cocci and *Klebsiella spp.* and *E. coli* were more common in a study carried out in septicemic neonates. Khosravi *et al*[18], in a study on post-operative infections of orthopedic bone implants observed *S. aureus* [34 (21.94%)], *Klebsiella ozaenae* [26 (16.77%)] and *P. aeruginosa* [24 (15.50%)] being the most common causative agents in variance to our observations. Further, while investigating the antimicrobial susceptibility of bacterial isolates from tracheal specimens obtained from pediatric patients from Tehran, Jafari *et al*[19] observed *Pseudomonas spp.* to be the most prevalent bacterial isolate (32%) followed by *S. aureus* (27.6%), *Klebsiella spp.* (16%) and *Enterobacter spp.* (9.6%).

In the present study, among the total 115 bacterial isolates there were 101 single isolate culture positive and 7 (6.08%) double isolate culture positive. Khosravi *et al*[18] also reported that among the positive cultures less than 2% were mixed bacterial culture of two organisms.

Regarding the distribution of bacterial isolates in the samples, it was observed that *E. coli* and *S. aureus* were the commonest organisms both in emergency and in elective surgery cases. *Salmonella* (non-typhoidal) was grown in only one case. Mixed infections were noted in 7 (6.48%) cases and most of them were from burn patients. Our findings are in conformity with those of Sanyal *et al*.[20] As most of the samples were from abdominal surgery; hence in these cases more Gram negative bacteria were isolated. Most of the *P. aeruginosa* isolates were from burn patients. Madoff[21] reported that *P. aeruginosa* as one of the commonest bacteria causing infection in burn patients. Ganesamoni *et al*[22] also found *P. aeruginosa* being the commonest (81.1%) and responsible for serious infection in burns.

Our findings in respect to susceptibility pattern of *S. aureus* in emergency and elective surgery cases showed that maximum susceptibility (100%) was noted to linezolid and vancomycin. Our findings are in conformity with those of Shobha *et al*[23] who found all isolates of *S. aureus* were sensitive to vancomycin. Archer and Polk[24] reported that vancomycin is the drug of choice for methicillin resistant *S. aureus* (MRSA). Linezolid is an FDA approved agent for the treatment of infections caused by methicillin susceptible and resistant strains of *S. aureus*.[25] Thus our findings are in agreement with these authors. As far as the susceptibility pattern of CoNS was concerned maximum susceptibility was observed with linezolid and vancomycin in 100% cases.

Since the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptides like vancomycin are frequently being used as agent of choice for the treatment of infection caused by MRSA. Although, the incidence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) has been rising in various parts of the world yet documented reports of VISA/VRSA in India are few. It may be mentioned that in the present study vancomycin-intermediate *S. aureus* (VISA) were not found. Menezes *et al*[26] in their study from a tertiary care hospital of southern India reported that out of a total of 261 Staphylococcal isolates (141 *S. aureus* isolates and 120 coagulase negative *Staphylococci*), there were 6 VISA strains (1 from *S. aureus* isolate and 5 from coagulase-negative *Staphylococci*). Kapil[27] also reported presence of VISA in their study. Moreover, Tiwari *et al*[28] in their study from northern part of India reported the presence of both vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-intermediate (VISA) as well as teicoplanin-intermediate. The authors studied a total of 1681 Staphylococcal isolates consisting of 783 *S. aureus* and 898 coagulase-negative *Staphylococci* (CoNS). Authors reported that out of 783 *S. aureus,* two *S. aureus* strains were found to be vancomycin and teicoplanin resistant and 6 strains of vancomycin-intermediate. One CoNS strain was resistant to vancomycin and teicoplanin and two CoNS strains were intermediate to vancomycin and teicoplanin. Moreover, two VRSA strains were also found to be resistant to several other antimicrobials such as gentamicin, tobramicin, amikacin, norfloxacin, ciprofloxacin, erythromycin, tetracycline, co-trimoxazole, and cefaperazone/sulbactam.

Our findings in respect to susceptibility pattern of *Streptococcus spp*. showed that maximum susceptibility was noticed with Iinezolid and vancomycin in 100% cases. Linezolid is approved by FDA for the treatment of infections caused by *Streptococcus spp*. They are highly susceptible to vancomycin as well.[25]

Regarding the susceptibility pattern of *E. coli* it was observed that maximum susceptibility was noted with ciprofloxacin in 75.76% cases followed by gentamicin 54.54%. Fluoroquinolones are potent bactericidal agents against *E. coli* and ceftriaxone has also been found to be a useful drug for the treatment of infections caused by Enterobacteriaceae.[29] Regarding the susceptibility pattern of *Klebsiella spp*. it was noted that maximum susceptibility was with ceftriaxone in 66.67% cases. In short, antimicrobial susceptibility test revealed a high rate of antimicrobial resistance to the most antibiotics used in this study suggesting a horizontal spreading of resistance among the isolates. It is found that most of the organisms were susceptible to vancomycin, linezolid and ciprofloxacin. Khosravi *et al*[18] reported that the staphylococci which was the major isolate, showed high sensitivity to vancomycin in conformity with our observations. It may be emphasized that the consequences of increased drug resistance are far-reaching.

Bassetti *et al*[30] has observed that accurate information on local epidemiology and antimicrobial resistance pattern of pathogens is essential to select clinically effective antibiotic therapy for infection.

In conclusion, it is observed that both rate of infection and bacterial resistance to commoner antimicrobial agents are fairly high in our study thus necessitating treatment with the second or third generation antimicrobial agents, hence escalating the cost of treatment with remarkable economic impact, and increasing hospital stay. Moreover, increased bacterial resistance is probably due to irrational and inappropriate use of antimicrobial agents, disregard to hospital infection control policies and showing negligible regard to culture susceptibility pattern while administering antimicrobial agents. The study provides important feedback data to choose empirical therapy in cases of surgery based on the knowledge of commonly isolated organisms and their antimicrobial susceptibility pattern.

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