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MICROBIOLOGICAL CHARACTERIZATION AND ANTIBIOTIC SENSITIVITY PATTERN OF THE SURFACE CONTAMINATION IN SURGICAL WOUND AREAS IN A TERTIARY CARE TEACHING HOSPITAL

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ABSTRACT This study included 1000 surgery patients with signs and symptoms indicative of wound infections, who presented over the course of 5 years. Bacterial pathogens were isolated from 712 individuals. A single etiologic agent was identified in 381 patients, multiple agents were found in 331, and no agent was identified in 288. A high preponderance of aerobic bacteria was observed. Among the common pathogens were Staphylococcus aureus (324 patients, 45.51%), Pseudomonas aeruginosa (218 patients, 30.61%), Escherichia coli (54 patients, 10.67%), Staphylococcus epidermidis (32 patients, 4.49%), and Enterococcus faecalis (62 patients, 8.71%). In antibiotic sensitivity pattern it was observed that imipenem and tigecycline is the most sensitive in gram negative bacteria and vancomicyn and ciprofloxacin is the most sensitive to gram positive bacteria.

KEYWORDS : Antibiotics, bacteria, imipenem, surgical wounds and vancomycin

Introduction

A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues. When bacteria breach this barrier, infection can result [1, 7]. The most common underlying event for all wounds is trauma. Trauma may be accidental or intentionally induced. The latter category includes hospital-acquired wounds, which can be grouped according to how they are acquired, such as surgically and by use of intravenous medical devices. Although not intentionally induced, hospital-acquired wounds can be the pressure sores caused by local ischemia, too. They are also referred as decubitus ulcers, and when such wounds become infected, they are often colonized by multiple bacterial species [7]. Most wound infections can be classified into two major categories: skin and soft tissue infections, although they often overlap as a consequence of disease progression [5, 7, 8, 9]. Infections of hospital- acquired wounds are among the leading nosocomial causes of morbidity and increasing medical expense. Routine surveillance for hospital-acquired wound infections is recommended by both the Centers for Disease Control and Prevention [6] and the Surgical Infection Society [2]. The objectives of the present study were to identify the etiologies of surgical wound infections and characterize the antimicrobial susceptibilities of the pathogen isolates.

Materials and Methods

This retrospective study included 1000 patients who underwent surgical treatment during the 5year period from May 2010 to April 2015. All patients presented signs and symptoms indicative of surgical wound infections. A definite case of surgical wound infection was defined as one in which there was any skin eruption or drainage at the surgical site that was positive for bacteria by culture within 60 days of a surgical procedure. On the other hand, a presumptive case was one in which there was any skin eruption or drainage at the surgical site that was either culture negative or unresponsive to appropriate antibiotic therapy for organisms obtained on culture. All Gram-negative bacteria were identified basing on the biochemical results (Table 1, 2), whereas all Gram-negative bacteria were identified basing upon the test, catalase, oxidase, and coagulase results. The results are compared with the colony morphology of the culture result also (Fig 1). In Staphylococcus aureus golden yellow, opaque, circular colonies white butyrous consistency were observed on nutrient agar whereas, yellow colonies were observed on mannitol salt agar, and beta-hemolysis was seen on blood agar. After identification, individual bacterial were tested for antibiotic sensitivity pattern with Kirby-Bauer method. Then, the sensitive or resistances of the used antibiotics were detected by measuring the diameter of inhibitor zone created by the antibiotics (Fig. 2a-d). All the organisms were identified basing on the previous methods [10-17].

Table 1 Morphology and culture characters of clinically isolated bacteria along with MTCC strains.

Bacterium	Agar media	Colony morphology
E. coli	MAC agar	LF, pink, mucoid colonies.
	CLED agar	Yellow and mucoid colonies
Pseudomonas aeruginosa	NA	Large, irregular colonies, with bluish green pigment.
Staphylococcus Sp.	Blood agar	Pale yellow collour, Transparent colony
Enterococcus faecalis	lood agar	White pinpoint colony, transparent with hameolysis

Fig 1a Staphylococcous aureus on nutrient agar

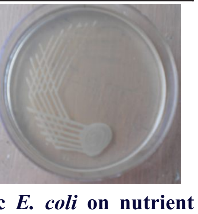

Fig 1c *E. coli* on nutrient agar

Fig 1c *Enterococcous faecalis* on nutrient

Fig 1d *Enterococcous faecalis* on nutrient

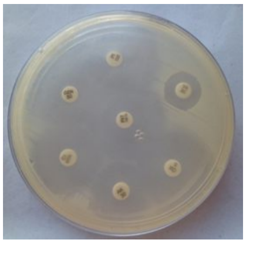

Fig 2aAntibiotic sensitivity pattern of *S. aureus*

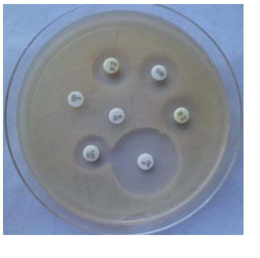

Fig 2b Antibiotic sensitivity pattern of *E. coli*

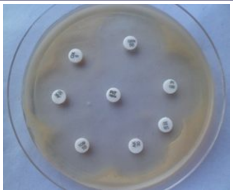

Fig 2c. Antibiotic sensitivity pattern of *E. faecalis*

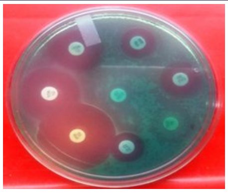

Fig 2d. Antibiotic sensitivity pattern of *P. aeruginosa*

Table 2 Summary of results of biochemical tests of isolated Gram-negative bacteria.

Bacteria	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	M
E. coli	+	ND	+	+	-	-	-	A/AG	+	M
P. aeruginosa	+	+	-	-	-	+	+	ND	+	M

Note: +, positive; -, negative; V, variable; MR, methyl red; VP, Voges-Proskauer; TSI, triple sugar iron; A, acid; K, alkali; G, gas; H2S, H2S production; M, motile; NM, non-motile; ND, not done.

Result

In this study, 1000 surgical wound swabs were cultured and 712 bacteria were isolated. It was observed that, from single clinical sample one type, two types or more than 2 types colonies were observed. Among the common pathogens were Staphylococcus aureus (324 patients, 45.51%), *Pseudomonas aeruginosa* (218 patients, 30.61%), *Escherichia coli* (76 patients, 7.58%), *Staphylo coccus epidermidis* (32 patients, 4.49%), and *Enterococcus faecalis* (62 patients, 8.71%) (Table 3).

Table 3 Total number of bacteria isolated from surgical wound swab.

Sample/ organism	Total	%
S. aurues	324	45.51
P. auregenosa	218	30.61
E. Coli	76	7.58
Staphylococcous epidermis	62	8.71
Enterococcous faecalis	32	4.49

In gram positive bacteria, 16 antibiotics were screened and it was revealed that oxacillin was 87% resistant to *Staphylococcus aureus,* where as it was 58%and 27% resistant to *E. faecalis* and *S. epiderdimi* respectively. Vancomycin was resistant to *S. aureus, E. faecalis, S. epiderdimis* at the rate 27%, 91% and 18% respectively. On the other hand Tigecycline and Colistin was more sensitive to *S. aureus, E. faecalis, S. epiderdimis* 3-9%. The details antibiotic resistance pattern of these 3 gram positive bacteria were noted in table 4, 5.

Table 4 Percentage of antibiotic resistance to *S. aureus.*

Bacteria	Susceptibility to prescribed antibiotics															
	Ak	Ge	Ac	Am	Ox	P	Ctr	Cf	Of	Tei	Va	E	Az	Cd	TGC	COL
S. aureus	75	46	43	38	87	59	85	68	53	48	27	43	62	39	05	09
E. faecalis	69	48	81	25	58	91	34	64	52	16	91	92	58	69	03	05
S. epidermidis	32	12	23	23	27	14	37	42	10	12	18	23	27	24	05	09

Note: Antibiotics (µg/disc): Ak, amikacin 30; Ac, amoxyclav 30; Am, ampicillin 10; Cf, cefpodoxime 10; Ctr, ceftriaxone 30; Ge, gentamicin 30; Of, ofloxacin 5; Ox, oxacillin 1; P, penicillin 10, Az, azithromycin 15; Cd, clindamycin 2; Ch, chloramphenicol 30; Cot, co-trimoxazole 25; E, erythromycin 15; Tei, teicoplanin 30; Va, vancomycin 30.

Table 5. Percentage of antibiotic resistance to Gram negative bacteria

Bacteria	Susceptibility to prescribed antibiotics															
	AK	GEN	NET	TOB	AT	PI	PIT	CPM	CPZ	CFS	CAZ	CTR	IPM	GAT	TGC	COL
E. coli	78	56	51	32	60	78	34	61	56	24	74	68	42	16	15	22
P. aeruginosa	68	59	28	17	51	67	23	83	73	31	73	74	39	31	11	17

Note: Antibiotics (µg/disc): AK, amikacin 30; GEN, gentamicin 30; NET, netillin 30; TOB: tobramycin 10; AT: aztreonam 30; PI: piperacillin 100; PIT: piperacillin/tazobactam 100/10; CPM: cefepime 30; CPZ: cefoperazone 75; CFS: cefoperazone/sulbactam 75/30; CAZ: ceftazidime 10; CTR: ceftriaxone 10; IPM: imipenem 10 (Carbap., carbapenem); GAT: gatifloxacin 30; TGC: tigecycline 55; COL, colistin 10.

During the study period 2 other gram negative bacteria were also found. They were *E. coli* and *P. aeruginosa*. In antibiotic sensitivity pattern, it was revealed that Tigecycline and Colistin is more sensitive i.e. 11-20% respectively where as Cefepime wasa resistant 61-83% to *E. coli and P. aeruginosa* respectively. The aminoglycoside group of antibiotics AK, GEN, NET, TUB were resistant up to 28-78%.

Discussion

Results from cultures and susceptibility tests performed before antibiotic therapy are summarized in Table 1. Independently of culture results, antibiotic treatment was started for all patients. During treatment, 681 T1 control specimens were obtained from 582 (95.0%) of the abovementioned 712 culture-positive (C1) individuals, while 71 specimens were obtained from 55 (88.7%) of the 288 culturenegative (C2) patients. Overall, bacterial pathogens were isolated from 131 (21.3%) C1 patients, while the C2 patients remained culture negative, with the exception of two patients positive for the presence of *P. aeruginosa and Stenotrophomonas maltophilia.* Finally, successive control specimens were obtained at the end of antibiotic treatment from all the 131 patients with T1 control specimens positive for bacterial pathogens. Nineteen individuals out of these 131 patients had persistently positive culture results in spite of specific antibiotic treatment. Overall, on the basis of clinical and microbiological data, 595 (96.9%) out of 712 C1 individuals were classified as having definite cases of surgical wound infection, while the abovementioned 288 C2 patients and 19 (3.1%) out of 712 C1 patients were classified as having presumptive cases of surgical wound infection. The susceptibility patterns of the 1,060 bacterial strains, divided into three 2-year periods, to several antimicrobial agents are summarized in Table 2. Some consequential observations arose from the data in Table 2. More than 50% of the *Enterobacteriaceae* tested were resistant to ampicillin, while only a few (,20%) were resistant to the combination of amoxicillin and clavulanate. This finding suggests that the resistance observed was due mainly to the production of b-lactamase by the organisms. In addition, most isolates were susceptible to ceftriaxone but more than 50% were resistant to cefazolin. Most *P. aeruginosa* isolates were susceptible to piperacillin, ceftazidime, and imipenem, although a gradual emergence of resistance to these b-lactams has been observed. In addition, only a few isolates were resistant to netilmicin, while a severe decrease in ciprofloxacin activity has been noted in the last few years.

In this study *S. aureus* was the most common cause of surgical wound infections. Methicillin resistance was documented in 104 (54.4%) of 191 *S. aureus* isolates. Although amoxicillinclavulanate, cefazolin, and imipenem were shown to be active in vitro against more than 60% of the isolates, according to National Committee for Clinical Laboratory Standards recommendations, the methicillin-resistant staphylococci were considered resistant to all b-lactams, including penicillins, cephalosporins, b-lactam–b-lactamase inhibitor combin ations, and carbapenems, since these agents may be clinically ineffective against such organisms. *Enterococci,* a frequent cause of infection in surgical wounds, were isolated from 48 patients. Nearly all of the 38 *Enterococcus faecalis* isolates were susceptible in vitro to glycopeptides and gentamicin (data not shown). In contrast, most of the strains were resistant to cefazolin. Finally, good in vitro activities were shown by amoxicillin-clavulanate andimipenem.

Anaerobic species (36 strains) were isolated from 21 distinct patients. Overall, the anaerobic gram-positive cocci (27 isolates) were susceptible to all the drugs tested, while the gramnegative isolates (nine *Bacteroides* spp. strains) were shown to be resistant to ampicillin and cefazolin.

Epidemiological data about the emergence of antibiotic resistance were drawn by dividing the susceptibility patterns of the the isolates on the basis of the microbiological results obtained during three 2-year periods (Table 2). The susceptibility data collected in this study suggest that some antibiotics would have very limited usefulness for the prophylaxis or the empirical treatment of wound infections. For instance, most of the gram-negative isolates were found to be resistant to ampicillin and cefazolin while the majority of staphylococcal strains were resistant to methicillin. These are remarkable data, since virtually all the patients received first- or second-generation cephalosporins as antibiotic prophylaxis. Overall, a progressive variation in causative pathogens and resistance patterns has been observed throughout the study. In fact, the susceptibility to antibiotics constantly decreased while multiresistant *Pseudomonas* and staphylococcal strains were isolated with increasing frequency. According to literature data, perioperative prophylaxis can decrease the incidence of wound infection (2, 3, 6, 7, 10–12, 14, 16). Cefazolin is the most used agent for surgical prophylaxis in our hospitals but can be ineffective against the increasingly common wound pathogens methicillin- resistant *S. aureus,* methicillin-resistant coagulase-negative staphylococci, *P. aeruginosa*, and other species of gramnegative rods. The inappropriate usage of antimicrobials in surgical perioperative prophylaxis is still a problem, and a close collaboration between surgeons and microbiologists is needed (4, 15).

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