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Bacteriological Study of Wound Infection and Antibiotic Susceptibility Pattern of the Isolates

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Abstract
Wound infections result in sepsis, limb loss, long hospital stays, higher costs, and are responsible for significant human mortality and morbidity worldwide. The present study was conducted to isolate and identify the causative organisms of wound infection, to determine the antibiotic susceptibility pattern of the isolates and to study the risk factors for wound infection. The study was conducted for six months in which pus specimens collected from 244 patients were processed to investigate etiological agents using standard technique. Disc susceptibility of bacterial agents were then determined. A total of 244 pus samples were collected and 147 (60.20%) samples showed growth. A total of 150 bacterial isolates were isolated; of which 118 (78.67%) were Gram positive and 32 (21.33%) were Gram negative bacteria. *Staphylococcus aureus* (72.00%) was most common followed by *Escherichia coli* (6.67%), *Citrobacter freundii* (5.34%), *Staphylococcus epidermidis* (4.00%), *Pseudomonas aeruginosa* (3.33%), *Proteus mirabilis* (2.00%), *Enterococcus faecalis* (1.33%), *Acinetobacter* spp. (1.33%), *Klebsiella oxytoca* (1.33%), *Klebsiella pneumoniae* (1.33%), *Streptococcus pneumoniae* (0.67%) and *Streptococcus pyogenes* (0.67%). The most effective antibiotic for Gram positive isolates was Gentamicin (77.97%). For Gram negative isolates (except *Ps. aeruginosa*), Amikacin (74.07%), for *Ps. aeruginosa*, Amikacin, Ciprofloxacin, Gentamicin and Ofloxacin each with the susceptibility of 80.00% and for *Staph. aureus*, Cloxacillin (79.63%) were the most effective antibiotics. Hence, the most common isolate in wound infection was *Staph. aureus* followed by *E. coli*. Therefore, routine microbiological analysis of the wound specimens and their antibiotic susceptibility testing is recommended that will guide clinician for treatment of wound infection.

Key words: antibiotics, bacterial isolates, risk factors

Introduction
Skin, the largest organ in the human body, plays a crucial role in the sustenance of life through regulation of water and electrolyte balance, thermoregulation, and by acting as a barrier to external noxious agents including microorganisms, however, when the epithelial integrity of skin is disrupted, a wound results (Zafar *et al*. 2008). This may be characterized by the classic signs of redness, pain, swelling, raised temperature and fever (Sanjay *et al*. 2010).

The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors, including the type, site, size, and depth of the wound, the extent contamination, the level of blood perfusion to the wound, the general health and immune status of the host, the microbial load and the virulence expressed by the types of microorganisms involved (Bowler *et al*. 2001).

Wound can be infected by a variety of microorganisms. The common gram-positive organisms are *Staphylococcus aureus* and *Streptococcus pyogenes.* The gram-negative organisms are *Escherichia coli*, *Klebsiella* species, *Pseudomonas aeruginosa*, *Enterobacter* species, and *Proteus* species. The fungal organisms are *Candida* species and moulds such as *Aspergillus* species (Mordi & Momoh 2009).

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to a greater incidence of infections caused by methicillin-resistant *Staph. aureus* (MRSA) and polymicrobic flora (Akinjogunla *et al*. 2009). Although MRSA accounts for some wound infections, the majority are caused by methicillin-susceptible strains (Kernodle *et al*. 1998).

Methods

A cross-sectional descriptive analytical analysis was carried out in the Laboratory of Microbiology, Alka Hospital in order to isolate the causative organisms of wound infection along with their antibiotic susceptibility pattern, from June 1, 2011 to November 30, 2011. A total of 244 samples from patients suspected of wound infection were collected and were processed according to the standard laboratory methods (Procedures/Guidelines for the Microbiology Laboratory 2010). For laboratory investigation, two pus swabs were collected; one for the direct smear stains microscopy and the other for culture (Collee *et al*. 1999). The pus specimens were cultured onto the MacConkey agar and Blood agar plates and incubated at 37ºC for 24 to 48 hours. After overnight incubation, the culture plates were examined for bacterial growth and identified using standard microbiological techniques such as colony characteristics, staining reactions and biochemical properties. The antibiotic susceptibility test of all isolates was then performed by Kirby Bauer's disc diffusion method on Mueller Hinton agar. The antibiotics used were Gentamicin (10mcg/disc), Ofloxacin (5mcg/disc), Ciprofloxacin (30mcg/disc), Cefixime (5mcg/disc), Amoxycillin (30mcg/disc), Cotrimoxazole (25mcg/disc), Cephalexin (30mcg/disc), Erythromycin (15mcg/disc), Cloxacillin (10mcg/disc), Amikacin (30mcg/disc), Cefotaxime (30mcg/disc), Ceftriaxone (30mcg/disc), Ceftazidime (30mcg/disc), Piperacillin (100mcg/disc), Carbenicillin (100mcg/disc) and Piperacillin/ Tazobactam (100/ 10mcg/disc). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included as control strains.

Results and Discussion

Out of 244 samples collected, 155 (63.52%) were aspirated pus and 89 (36.48%) were pus swab. 147 (60.25%) samples showed growth while 97 (39.75%) samples showed no growth. Out of 155 aspirated pus, 94 (60.65%) samples were growth positive and 61 (39.35%) samples were growth negative. On the other hand, in case of pus swab, out of 89 samples, 53 (59.55%) samples were growth positive while 36 (40.45%) were growth negative (P=0.867). Out of 147 growth samples; 144 (97.96%) samples showed single isolate and 3 (2.04%) showed multiple isolates. The single isolates were found in 92 (97.87%) aspirated pus and 52 (98.11%) pus swabs while multiple isolates were found in 2 (2.13%) aspirated pus and 1 (1.89%) pus swab (P=0.92). A total of 150 bacterial isolates were obtained of which 118 (78.67%) were Gram positive and 32 (21.33%) were Gram negative bacteria (P=0.528) (Table 1).

Table 1. Number and percentage of Gram positive and Gram negative bacteria

S.N	Sample Type	Gram positive bacteria		Gram negative bacteria		Total	P- value
		No.	%	No.	%	No.	
1	Aspirated pus	74	77.08	22	22.92	**96**	P>0.05
2	Pus swab	44	81.48	10	18.52	**54**	
Total		**118**	**78.67**	**32**	**21.33**	**150**	

Table 2 illustrates the age and sex distribution of patents with wound infections. Out of 244 cases, the patients of age group 30-40 and 70-80 had higher growth of organism, each with 69.23% (P=0.608) and out of 133 (54.50%) male patients and 111 (45.50%) female patients, the growth was found to be higher in male patients (63.16%) than in female patients (56.76%) (P=0.309).

Out of total 244 samples, 183 (75.00%) were from Outpatients, while 61 (25.00%) were from Inpatients. Among outpatient samples, 112 (61.20%) and among inpatient samples, 35 (57.38%) were growth positive (P=0.597). Among the pattern of microbial growth in pus samples collected from different wards, the highest growth rate were from Cabin (83.33%) (P=0.262) and infection due to multiple organisms was only found in Cabin (40.00%) (P=0.028). *Staph. aureus* was the predominant isolates in the orthopedic ward (52.94%), general ward (57.13%), cabin (57.13%) and gynaecology ward (100.00%). In post-operative ward (POW), *E. coli* (50.00%) was the most common isolates.

Table 2. Age and sex distribution of patents with wound infections

Variables		No. (%) of culture positive	P-value
Age groups (years)	≤10(19)	10 (52.63)	
	10-20(29)	16(55.17)	
	20-30(74)	48(64.86)	
	30-40(39)	**27(69.23)**	
	40-50(27)	13(48.15)	P>0.05
	50-60(21)	11(52.38)	
	60-70(19)	12(63.16)	
	70-80(13)	**9(69.23)**	
	≥80(3)	1(33.33)	
Gender	Male (133)	84 (63.16)	P>0.05
	Female(111)	63(56.76)	
	Total	**147**	

Out of total 244 samples, 116 patients were found to be involved in smoking. Out of 116 patients who are involved in smoking, 76 (65.52%) showed growth. On the other hand, out of 128 patients who aren't involved in smoking, 71 (55.47%) showed growth (P=0.109).

Among Gram positive bacteria, *Staph. aureus* (108) and among Gram negative bacteria, *E. coli* (10) were the predominant isolate in both the samples (Table 3). *Staph. aureus* (2) and *Staph. epidermidis* (2) were present as a mixed growth and *E. coli* (1) and *Pr. mirabilis* (1) were present as a mixed growth.

Table 3. Frequency of Gram positive and Gram negative isolates

S.N.	Organism isolated	Aspirated pus		Pus swab		Total
		Count	%	Count	%	No.
1	**Gram positive**					
	Staph. aureus	68	62.96	40	37.04	**108**
	Staph. epidermidis	4	66.67	2	33.33	**6**
	Ent. faecalis	2	100.00	-	-	**2**
	Strep. pneumoniae	-	-	1	100.00	**1**
	Strep. pyogenes	-	-	1	100.00	**1**
S.N.	**Organism isolated**	**Aspirated pus**		**Pus swab**		**Total**
		Count	**%**	**count**	**%**	**No.**
2	**Gram negative**					
	E. coli	8	80.00	2	20.00	**10**
	Kl. pneumoniae	2	100.00	-	-	**2**
	Kl. oxytoca	1	50.00	1	50.00	**2**
	Cit. freundii	4	50.00	4	50.00	**8**
	Ps. aeruginosa	3	60.00	2	40.00	**5**
	Pr. mirabilis	2	66.67	1	33.33	**3**
	Acinetobacter spp.	2	100.00	-	-	**2**
	Total	**96**	**64.00**	**54**	**36.00**	**150**

Among 118 Gram positive isolates, Gentamicin (77.97%) was drug of choice followed by Cloxacillin (74.58%). Co-trimoxazole (38.13%) was found to be the least effective antibiotic (Table 4).

Table 4. Antibiotic susceptibility pattern of Gram positive isolates

	Sensitive		Intermediate		Resistant		Total
Antibiotic used	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**
Amoxycillin	66	55.93	3	2.54	49	41.53	118
Cotrimoxazole	45	38.13	5	4.24	68	57.63	118
Ciprofloxacin	84	71.19	11	9.32	23	19.49	118
Cefixime	74	62.71	9	7.63	35	29.66	118
Cloxacillin	88	74.58	1	0.85	29	24.57	118
Cephalexin	68	57.63	3	2.54	47	39.83	118
Erythromycin	86	72.88	5	4.24	27	22.88	118
Gentamicin	92	77.97	3	2.54	23	19.49	118
Ofloxacin	84	71.19	6	5.08	28	23.73	118

Among 27 Gram negative isolates (Except *Ps. aeruginosa*), Amikacin (74.07%) was drug of choice and Amoxycillin (29.63%) was the least effective antibiotic (Table 5).

Table 5. Antibiotic susceptibility pattern of Gram negative isolates (Except *Ps. aeruginosa*)

	Sensitive		Intermediate		Resistant		Total
Antibiotic used	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**
Amoxycillin	8	29.63	-	-	19	70.37	27
Amikacin	20	74.07	2	7.41	5	18.52	27
Cotrimoxazole	15	55.56	-	-	12	44.44	27
Ciprofloxacin	19	70.37	1	3.70	7	25.93	27
Cefixime	14	51.85	2	7.41	11	40.74	27
Cefotaxime	19	70.37	-	-	8	29.63	27
Ceftriaxone	19	70.37	-	-	8	29.63	27
Gentamicin	19	70.37	2	7.41	6	22.22	27
Ofloxacin	18	66.67	-	-	9	33.33	27

Amikacin, Ciprofloxacin, Gentamicin and Ofloxacin were the drug of choice for *Ps. aeruginosa* each with 80.00%, followed by Piperacillin, Carbenicillin and Piperacillin/Tazobactam each with susceptibility of 60.00%. Cefixime was found to be the least effective antibiotic with resistance of 100.00% followed by Ceftazidime with susceptibility of 40.00%. For *Staph. aureus*, Cloxacillin (79.63%) was the most effective antibiotic followed by Gentamicin (78.70%). Co-trimoxazole (36.11%) was found to be the least effective antibiotic.

In our present study, 60.20% of pus samples showed bacterial growth. This isolation rate of pathogen is relatively lower (70.50%) than that previously observed in Ethiopia (Azene & Beyene 2011) but higher (55.50%) than that previously observed in Skopje (Kaftandzieva *et al*. 2012).

According to our findings, no significant difference was found in the wound infections among gender. However, the predominance of males in culture positive cases is probably due to more exposure to the environment and more chances of accidents while earning livelihood (Verma *et al*. 2012) and due to our social behavior where males are given superiority to the female and if get diseased are brought immediately to hospitals (Zafar *et al*. 2008).

The highest percentage of positive culture was found in the age group 30-40 and 70-80, each with 69.23%, followed by the age group 20-30 (64.86%). This may be due to the weakening of the immune response as the age of the patient increases.

In our study, no significant difference was found in the positive culture among inpatients and outpatients. The mixed infection (2.04%) observed in this study was lower than 18.5% (Azene & Beyene 2011) and 6.48% (Verma *et al*. 2012) reported in previous studies.

In this study, a total of 150 bacterial species were isolated and no significant difference was found in infection rate among Gram positive and Gram negative isolates. Altogether, 12 different bacterial species were isolated with *Staph. aureus* (72.00%) being the most prominent one. *Staph. aureus* is the most important pathogen for wound infection and its nasal carriage is the main risk factor for the infection since carriers are two to nine times more likely to acquire *Staph. aureus* wound infection than noncarriers (Barbos *et al*. 2010). The high incidence of Gram-negative organisms confirms the observation that most wound infections arising from abdominal procedures are presently acquired from the patient's own faecal flora (Isibor *et al.* 2008).

From POW, *E. coli* (50.00%) and from orthopedic ward, *Staph. aureus* (52.94%) were the most common in our study. This agrees with another study where *E. coli* (31.63%) and *Staph. aureus* (32.25%) were predominant in POW and Orthopedic ward respectively (Kumari 2008). From gynaecology ward, the only organism isolated was *Staph. aureus* (100%). In contrast, *Cit. freundii* (22%) was the predominant isolate in another study (Jayavanth *et al*. 2011). From cabin and general ward *Staph. aureus* (57.13%) was common in each ward. However, the difference in the single isolate/ multiple isolate with different ward was statistically significant.

In our study, no significant difference was found in the positive growth and smoking. However, in another study, the wound infection rate in smokers was 12% compared with 2% in never-smokers (Sorensen *et al*. 2003). Smoking, increase the risk of wound infection presumably through reduction of the oxidative killing mechanism of neutrophils (Sorensen *et al*. 2005).

Among Gram positive isolates, the most effective antibiotic was Gentamicin and the least effective antibiotic was Co-trimoxazole. Similar result was observed in a study of Nigeria, where Gentamicin was the most effective antibiotic (Adegoke *et al*. 2010). However, Shrestha and Basnet (2009) reported Cloxacillin being the most effective antibiotic and Amoxycillin being the most resistant antibiotic. Among Gram negative isolates (except *Ps. aeruginosa*), the most effective antibiotic was Amikacin and the least effective antibiotic was Amoxycillin. Our result agrees with the study carried in Kathmandu (Shrestha & Basnet 2009). However, in a study carried out in Gujarat, the gram negative isolates were resistant to cefuroxime (Goswami *et al.* 2011). For *Ps. aeruginosa*, the drug of choice was Amikacin, Ciprofloxacin, Gentamicin and Ofloxacin. The least effective antibiotic was Cefixime with 100.00% resistance. In a study carried out in Dhaka, *Ps. aeruginosa* was more resistant to Azithromycin (100%) and the only drug found least resistant was Imipenem (Rashid *et al*. 2007). Also, *Ps. aeruginosa* showed the highest resistance to Erythromycin in a study carried out in North East Ethiopia (Azene and Beyene 2011) and to Ceftriazone in a study carried out in Kathmandu (Shrestha and Basnet 2009). For *Staph. aureus*, the most effective antibiotic was Cloxacillin and the least effective antibiotic was Co-trimoxazole followed by Amoxycillin. In another study, Cloxacillin was the most effective antibiotic against *Staph. aureus* and the least effective antibiotic was Amoxycillin (Shrestha and Basnet 2009). However, *Staph. aureus* was shown to have high level of resistance to tetracycline in a study carried out in North West Ethiopia (Fantahun *et al*. 2009) and to Oxacillin in a study carried out in Gujarat (Goswami *et al.* 2011). The 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 (Verma *et al.* 2012).

Combination of trimethoprim (TMP) and sulfonamides i.e. Co-trimoxazole is thought to have a synergistic effect . A single amino acid substitution in the *dhfr* (dihydrofolate reductase) gene and altered chromosomally encoded DHFR has been considered responsible for resistance to TMP among *Staph. aureus* (Huovinen 2001) and *Strep. pneumoniae* (Pikis *et al*. 1998). Similarly, mutations in the chromosomal *dhps* (dihydropteroate synthetase) gene are prevalent in nature. In isolates of sulfonamide-resistant *Strep. pneumoniae,* resistance is based on 2 amino acid duplications in the *fol*P gene (a *dhps* gene) that alter the tertiary structure of the enzyme (Huovinen 2001) while in *Staph. aureus* as many as 14 mutations are thought to be involved in conferring resistance to sulfonamides (Padayachee and Klugman 1999). Strains of sulfonamide-resistant *Strep. pyogenes* emerged rapidly after the introduction of sulfonamides; it is more likely that the changes in the *dhps* gene were introduced by means of transformational recombinations than by a series of mutations (Swedberg *et al*. 1998).

Resistance to penicillins and cephalosporins (Levy and Marshall 2004) by gram-negative bacteria is most commonly due to the production of â-lactamase, either chromosomally encoded or, more often, plasmid mediated. Other important mechanisms of resistance include alterations in penicillin-binding proteins (PBPs), decreased permeation of the antibiotic into the bacterial cell, or combinations of these resistance strategies (Deloney & Schiller 2000). Active efflux pumps in gram negative bacteria which excrete drugs, including multidrug efflux pumps, can also confer resistance to â-lactams (Nikaido 1996). Some *E. coli* strains develop resistance to third-generation cephalosporins and monobactams (i.e., aztreonam) through the acquisition of ESBLs, commonly arising through mutation of TEM-, SHV-, or CTX-M–type enzymes. The ESBLs are not active against cephamycins, such as cefoxitin and cefotetan; however, resistance to cephamycins and other â-lactams may arise as a result of changes in the porins in the outer membrane (Tenover 2006).

Resistance to aminoglycosides such as gentamicin, amikacin is widespread, with more than 50 aminoglycoside-modifying enzymes. Most of these genes are associated with gram-negative bacteria. Depending on their type of modification, these enzymes are classified as aminoglycoside acetyltransferases (AAC), aminoglycoside adenyltransferases (also named aminoglycoside nucleotidyltransferases [ANT]), and aminoglycoside phosphotransferases (APH). Aminoglycosides modified at amino groups by AAC enzymes or at hydroxyl groups by ANT or APH enzymes lose their ribosome-binding ability and thus no longer inhibit protein synthesis. Besides aminoglycoside-modifying enzymes, efflux systems and rRNA mutations have also been involved in resistance (Byarugaba 2009).

Fluoroquinolone (ciprofloxacin, ofloxacin) resistance can be increased by mutations in the genes encoding the targets gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*), by increased levels of the multidrug efflux pump AcrAB and by the presence of plasmid-borne mechanisms QnrA, QnrB, QnrS, and Aac(6Œ§)-Ib-cr (Morgan-Linnell *et al*. 2009).

Also, bacteria resist macrolide (erythromycin) antibiotics in 3 ways; through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, through efflux of the antibiotic, and by drug inactivation. The erythromycin ribosome methylase *erm*(A) and *erm*(C) determinants are predominant in staphylococci. The spread of *erm* genes belonging to the *erm*(B) class and, rarely, to the *erm*(TR) subset of the *erm*(A) class accounts for the vast majority of resistance caused by ribosomal methylation in streptococci and enterococci (Leclercq 2002).

Staph. aureus and E. coli remain the most frequently isolated etiological agents from pus samples. The results obtained from this study will help policy makers in evaluating the infection control measure in hospitals and to prevent the increment of drug resistance microorganisms in the health institution as well as in the community. The result has proven itself to be of advantage and helpful and the need for further research required. A research on cleaning and handling of the sterile equipments also would be another angle to research on wound infections as it is impossible to discover exactly where infections arise. We did not investigate the impact of hygiene in the development of wound infection but we suggest that education of patients on personal hygiene will be helpful in enhancing wound healing and management of patients. Although complete eradication of wound infections is not possible however by taking the preventive measures and adopting prompt clean surgical procedures and proper care of wounds, the incidence of wound infection may be limited to minimum.

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