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Full length research paper

Aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern: a hospital based cross-sectional study

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Post-operative wound infections as well as emergence and spread of drug resistant strains have been found to pose a major problem in the field of surgery. This study investigated common aerobic bacterial isolates and their antimicrobial susceptibility patterns in patients with clinical diagnosis of post-surgical wound infections. Microbial analysis was carried on pus samples obtained from 194 patients with clinical diagnosis of post-surgical wound infections at Hawassa Teaching and Referral Hospital, from November 2010 to March 2011. The isolation rate of aerobic bacteria was 138 (71.1%). *S. aureus* was the most frequent isolates (37.3%); followed by *E. coli* (25.4%), *Klebsiella species* (13.6%), *Proteus* (10.2%), *P. aeruginosa* (10.2%) and *coagulase* negative *Staphylococci* (3.4%). Single and multiple antimicrobial resistances were observed in 6.8% and 93.2 % of the isolates, respectively. No bacterial isolates was found to be sensitive to all antibiotics tested. The high isolation rate of aerobic bacteria and their increased resistance to the commonly used antibiotics warrants the need to practise aseptic procedures and rational use of antimicrobial agents leading to minimize infection rate and emergence of drug resistance.

Keywords: Aerobic bacteria; post-surgical wound; antimicrobial; susceptibility

Introduction

Post-operative wound infections have been found to pose a major problem in the field of surgery. It may occur as a primary wound infections following surgical operation from sources in the ward or as a secondary wound infection due to some other complications (Masaadeh and Jaran, 2009). In fact, most post-operative wound infections are hospital acquired (nosocomial infection) and varies from one hospital to the other (Isibor et al., 2008).

The global epidemiology of surgical site infection (SSI) has not been well described due to lack of standardized criteria for diagnosis. The global prevalence of nosocomial infections was estimated at 3-21% of which, wound infections account for 5-34% (Samuel, 2010; Mangram *et al.,* 1999). Patients with SSIs had a significantly extended hospital stay, incurred higher costs and increased risk of mortality compared to those without these infections (Suchitra and Lakshmidevi 2009). Bacterial factors such as inoculum size, virulence, and invasive capability as well as immunological and physiological state of the host influence occurrence of surgical wound infection (Masaadeh and Jaran, 2009).

Advances in control of infections have not eliminated the risk of post-operative wound infections due to emergence and spread of resistant microbes. The condition is serious particularly in developing countries where irrational prescription of antimicrobial agents is common. Measures including new antimicrobial production, better infection control program and rational use of existing antimicrobial agents have been suggested

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to reduce the problem (Gottrup, 2000;Anguzu and Olila, 2007; Andhoga *et al*., 2002; Hart and Kariuki, 1998).

In Ethiopia, studies showed prevalence of post-surgical wound bacterial infections varying from 14.8% to 60%. *Staphylococcus aureus*, *Kelbsiella species*, *Escherichia coli*, *Proteus* species, *Streptococcus* species, *Enterobacter species*, *Pseudomonas species* and *coagulase* negative *Staphylococci* (CoNS) were among the most commonly isolated species (Taye, 2005;Endalafer *et al*.,2011;Biadglegne *et al*.,2009; Mulu *et al*.,2006; Tesfahunegn *et al*.,2009). However, limited information about the epidemiology of bacterial pathogens causing post-surgical wound infections and their drug susceptibility profile in southern part of the country make rational use of drugs impracticable. This study, therefore, described the distribution of common aerobic bacteria and their antimicrobial susceptibility patterns in patients with clinical diagnosis of post-surgical wound infections. Thus, it helps clinicians in selection of appropriate treatment regimen against bacteria causing wound infections.

Materials and methods

A cross-sectional study was conducted in patients with clinical diagnosis of post-surgical wound infections at Hawassa Teaching and Referral Hospital, from November, 2010 to March, 2011. Hawassa is located 270 km south of Addis Ababa and the capital city of the Southern Nations, Nationalities and Peoples' Region (SNNPR) (HCA, 2006). The hospital is the largest in the regional state with bed capacity of 300 and provides better health care for large number of population. Patients seeking medical care are served in different outpatient and inpatient units (Surgery, Gynaecology and Obstetric, Internal Medicine, Paediatric, Ophthalmology). The study population consisted of patients of all age groups who undergone surgery and developed SSI as diagnosed clinically by physicians within 30 days of having post surgical procedure. The sample size was estimated considering 14.8 % prevalence (10), 5% precision, and 95% confidence level; thus, a total of 194 patients were included.

Socio-demographic data was collected from patients at surgical, pediatrics and gynaecology/obstetrics wards using structured and predesigned questionnaire. Experienced laboratory personnel collected two post-operative wound swabs aseptically from surgical sites ahead of cleansing the wound with antiseptic solutions and delivered to Microbiology Laboratory within five minutes of collection. Gram stain preparations were made from one swab and culture are processed from another swab. In brief, would swab was inoculated on blood agar (BA), Mannitol salt agar (MSA) and MacConkey agar (all Oxoid, Ltd., England). The plates were incubated in an aerobic atmosphere at 37℃ for 18–24 hours in an incubator. The plates were read the following day but extended to 48 hours if there was no bacterial growth within 24 hours. Colonies were counted using colony counter, and counts were categorized and interpreted as follows: counts <5 were considered as contamination; 5 -15 were colonization; 16-30 were critical colonization; and >30 were infection. Isolated colonies were subjected to Gram staining and biochemical tests for identification. Identification was carried out according to the standard biochemical tests (Cheesbrough, 2006).

Antimicrobial susceptibility test was performed on Müller-Hinton agar medium (Oxoid Basingstoke, UK) using disk-diffusion method according to the direction of the Clinical and Laboratory Standards Institute (Barry and Thornberry, 1991). Briefly, 1-3 similar colonies of the isolates were inoculated on nutrient broth to prepare inoculums; broths were incubated at 37℃ for 4hrs. Turbidity of broths was standardized at 0.5 McFarland using sterile phosphate buffered saline (pH, 7.2). Bacterial suspensions were inoculated on Mueller–Hinton agar plate (Oxoid, UK) and antibiotic disks (Oxoid, UK), representing antibiotics commonly prescribed in the study area, were placed on the media. After 18-24 hours of incubation, the diameter of each inhibition zone was measured using caliper and interpreted as per the standard. All isolated gram- positive and gram-negative aerobic bacteria were tested against ampicillin (10μg), chloramphenicol (30μg), ciprofloxacin (5μg), gentamicin (10μg), amoxacillin-clavunilic acid (30μg), trimethoprim-sulphamethoxazole (25μg), and ceftriaxone (30μg). In addition, gram-positive bacteria were tested against penicillin G (10 IU), erythromycin (15μg), and vancomycin (30μg). Reference strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC-27853) and *S. aureus* (ATCC 25923) were tested as controls.

The study was approved by the Research and Ethical Review Committee of the Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University and the College of Medicine and Health Sciences, Hawassa University. Participation was fully voluntary and study subjects or their parents gave informed oral consent. Physicians manage those patients with infection. Data were analyzed using SPSS Version-16 software and results were summarized using means and percentages. Pearson Chi-square test was used to evaluate differences in proportions and p-value <0.05 was considered to be significant

Results

A total of 194 patients' pus specimens were analyzed in the study. Majority of the study subjects were rural residents (75.3%) and males (59.8%). The mean age of

Table 1: Socio-demographic characteristics of post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Ethiopia (November 2010-March 2011).

Characteristics	Number (%) of tested	Number (%) of culture positive	P-value
Sex			
Male	116 (59.8)	93(80.2)	0.001
Female	78(40.2)	45(57.7)	
Total	194(100.0)	138(71.1)	
Age (in years)			
≤10	33(17.0)	21(63.6)	0.095
11-20	41(21.1)	30(73.2)	
21-30	60(30.9)	39(65.0)	
31-40	18(9.3)	15(83.3)	
41-50	21(10.8)	15(71.4)	
51-60	15(7.7)	15(100)	
≥60	6(3.1)	3(50.0)	
Ward			
Surgery	87(44.8)	69(79.3)	0.073
Paediatrics	50(25.8)	33(66.0)	
Gynaecology	57(29.4)	36(63.2)	
Residence			
Urban	48(24.7)	33(68.8)	0.674
Rural	146(75.3)	105(71.9)	
Location of wound			
Back	6(3.1)	6(100)	0.006
Thorax	6(3.1)	3(50)	
Abdomen	107(55.2)	72(67.3)	
Head and neck	9(4.6)	3(33.3)	
Leg	66(34.0)	54(81.8)	

the study participants was 28 years (range, 6/12 to 100 years) and the modal age group was 21-30 years (30.9%). Eighty seven patients (44.8%) were from surgical ward and 55.2% had wound on the abdomen (Table 1).Of the total analysed specimens, 138 (71.1%) were found culture positive. And isolation rate was higher in males (80.2 %) compared to females (57.7%) (P = 0.001) (Table 1). In total, 177 aerobic bacteria were isolated; of which, 105 (59.3 %) were gram-negative and 72 (40.7%) were gram-positive organisms. *S. aureus* was the most frequent isolates (37.3%); followed by *E. coli* (25.4%), *Klebsiella species* (13.6%), *Proteus* (10.2%), *P. aeruginosa* (10.2%) and CoNS (3.4%) (Table 2). Patients with single and dual infections accounted for 99(51%) and 39 (20.1%), respectively. *S. aureus* most frequently occurred in combination with *E. coli* or *Klebsiella* species, (23.1%) (Table 3).

As presented in table 4, *S. aureus* isolates showed the highest resistance to penicillin (100%), ampicillin (95.5%) and ceftriaxone (81.8%) while their resistance rate to amoxacillin-clavunilic acid was 30.3%. Isolates of CoNS showed 100% resistance to vancomycin, ceftriaxone, ampicillin and penicillin; but sensitive to chloramphenicol. All isolates of *E. coli*, *Proteus* and *P. aeruginosa* were resistant to ampicillin. Also, no isolates of *Klebsiella* and *P. aeruginosa* were sensitive to gentamicin and

Table 2: Characterization of organisms isolated from post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Ethiopia (November 2010- March 2011).

Gram-positive bacteria		Gram-negative bacteria			
S. aureus	**CoNS**	***E .coli***	***Klebsiella* species**	***Proteus* species**	***P. aerugenosa***
66(37.3%)	6(3.4%)	45 (25.4%)	24(13.6%)	18(10.2%)	18(10.2%)

CoNS = Coagulase negative *Staphylococci*

Table 3: Multiple infections among post surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010- March 2011).

Pattern of multiple infection	Frequency	Percent (%)
S. aureus and E. coli	9	23.1
S. aureus and Klebsiella species	9	23.1
S. aureus and Proteus species	6	15..3
S. aureus and P. aeruginosa	3	7.7
E. coli and Proteus species	3	7.7
E. coli and P. aeruginosa	3	7.7
Klebsiella species and CoNS	3	7.7
Proteus species and CoNS	3	7.7
Total	39	100

CoNS= coagulase negative *Staphylococci*

Table 4: Antimicrobial susceptibility pattern of aerobic bacterial isolates from post-surgical wound infected patients at Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010 - March 2011).

	Bacterial isolates resistant to each drug tested (%)									
Organisms	**VAN**	**SXT**	**CN**	**C**	**AMP**	**AMC**	**CRO**	**P**	**E**	**CIP**
S.aureus	43(65.2)	37(56.1)	26(39.4)	43(65.2)	63(95.5)	20(30.3)	54(81.8)	66(100)	29(43.9)	26(39.4)
E. coli	-	27(60.0)	21(46.7)	18(40.0)	45(100)	21(46.7)	18(40.0)	-	-	18(40.0)
Klebsiella Species.	-	21(87.5)	24(100)	21(87.5)	21(87.5)	12(50.0)	21(87.5)	-	-	15(62.5)
Proteus Species	-	3(16.7)	6(33.3)	12(66.7)	18(100)	9(50.0)	6(33.3)	-	-	0
P. aeruginosa	-	12(66.7)	9(50.0)	18(100)	18(100)	18(100)	18(100)	-	-	0
CoNS	6(100)	3(50.0)	3(50.0)	0	6(100)	3(50.0)	6(100)	6(100)	3(50.0)	3(50.0)
Total	49(68.1)	103(58.2)	86(48.6)	112(63.3)	171(96.6)	83(46.9)	123(69.5)	72(100)	32(44.4)	62(35)

CoNS= Coagulase negative *Staphylococci*; VAN= Vancomycin; SXT= Trimethoprim-sulphamethoxazole; CN= Gentamicin; C= Chloramphenicol; AMP= Ampicillin; AMC= Amoxacillin-clavunilic acid; CRO= Ceftriaxone; P= Penicillin; E= Erythromycin; CIP= Ciprofloxacin

chloramphenicol, respectively. While, *Proteus* and *P. aeruginosa* isolates were susceptible to ciprofloxacin. Single and multiple antimicrobial resistances were observed in 6.8% and 93.2 % of the isolates, respectively. No bacterial isolates was found to be sensitive to all antibiotics tested, and three isolates of S. aureus (4.5%) were shown to be resistant to all antibiotics tested (Table 5)

Discussion

We described aerobic bacterial isolates in patients with clinical diagnosis of post-surgical wound infections and characterized the antimicrobial susceptibilities of these isolates. The rate of bacterial isolation in this study was 71.1%, which is similar to a previously reported result from Ethiopia (70.5%) (Mulugeta and Bayeh,2001). However, the lower rates of isolation (14.8%- 53%) reported from different localities in Ethiopia (Taye, 200510, Biadglegne *et al*.,2009; Tesfahunegn *et al.,*2009) may be due to varying distribution of bacterial aetiology with geography, time and infection prevention practice in diverse settings. Difference in identification methods are also known to influence the relative prevalence of bacteria which makes comparison of results difficult. The rate of isolation was higher among males compared to females, but it was not found to be

Table 5: Multiple drug resistance patterns of Gram-positive and Gram-negative aerobic bacteria in post-surgical wound infected patients attending Hawassa Teaching and Referral Hospital, Hawassa, Ethiopia (November 2010-March 2011).

	Antibiogram pattern No (%)										
Organisms	**R_0**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**
S. aureus **(n=66)**	-	3(4.5)	-	9(13.3)	6(9.1)	12(18.2)	3(4.5)	9(13.3)	12(18.2)	9(13.3)	3(4.5)
CoNS **(n=6)**	-	-	-	-	3(50)	-	-	-	-	3(50)	-
E. coli **(n=45)**	-	9(20)	9(20)	-	6(13.3)	6(13.3)	12(26.7)	3(6.7)	-	-	-
Klebsiella **spp.(n=24)**	-	-	-	-	3(12.5)	-	6(25)	15(62.5)	-	-	-
Proteus **spp. (n=18)**	-	-	6(33.3)	6(33.3)	-	6(33.3)	-	-	-	-	-
P. aeruginosa **(n=18)**	-	-	-	-	-	15(83.3)	3(16.7)	-	-	-	-
Total	-	12(6.8)	15(8.5)	15(8.5)	18(10.2)	39(22)	24(13.6)	27(15.3)	12(6.8)	12(6.8)	3(1.7)

CoNS - coagulase negative *staphylococci*; R_0 - sensitive to all antibiotics tested; R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10} - resistant to one, two, three, four, five, six, seven, eight, nine, ten antibiotics, respectively.

influenced by age group. Among the isolated aerobic bacterial pathogens were *S. aureus, E. coli, Klebsiella spp., Proteus spp., P. aeruginosa* and CoNS. The preponderance of *S. aureus* was in agreement with other reports from Ethiopia and other countries (Anguzu and Olila, 2007; Biadglegne *et al*.,2009; Mulu *et al.,*2006; Nwachukwu *et al*, 2009), which may be due to its presence as common flora on the skin as well as in the hospital environment, facilitating its way into surgical sites (Giacometti et al.,2000). The occurrence of gram negative bacteria, especially *enterobacteria* may also be due to the contamination of wound by enteric organisms during surgical procedure and/or faecal contamination thereafter as majority of the operations was undertaken on abdomen. These groups of organisms are also becoming endemic in hospital environment as they easily transfer from object to object resisting common antiseptics (Odedina *et al*, 2007; Amrita *et al*., 2010). The rate of poly-microbial pathogens in the current study (20.1%) was higher compared to a previous finding from Ethiopia (18.4%) (Mulugeta and Bayeh,2001).

The in vitro antimicrobial sensitivity studies showed that isolates react differently to various antibiotics. Similar to previous reports documented in Ethiopia (Biadglegne *et al.,*2009; Mulu *et al*.,2006; Gebre-Selassie, 2007), most isolates showed higher rate of resistance to antibiotics such as ampicillin and penicillin. The higher susceptibility of about 90% of gram negative isolates to ciprofloxacin in Gondar (Mulu *et al*., 2006) has now declined to 68.6% of the isolates in present study, which may indicate a dropping efficacy of the drug with time. The high resistance rate reported from different localities in Ethiopia may be because of the extensive use of antimicrobials without getting evidence about bacterial etiologies and appropriate treatment. Most of the isolates in this study, as elsewhere (Mulugeta and Bayeh,2001), were also multiple drug resistant. However, this study did not attempt to isolate etiologies of wound infections other than aerobic bacteria; thus, failure to detect other pathogens did not necessarily indicate the unequivocal absence of the infection. Moreover, we tested limited number of antimicrobials in some isolates. The high isolation rate of aerobic bacteria and increased drug resistance to the commonly used antibiotics warrants the need for immediate measures ensuring effective infection prevention and rational use of antimicrobial agents leading to minimize infection rate and emergence of drug resistance. Ciprofloxacin is relatively effective for most isolates and becomes the best choice when empiric treatment of wound infection is unavoidable. Periodic monitoring of etiology and antimicrobial susceptibility both in the community and hospital settings is recommended.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LD was the principal investigator for the study; LD, TS,ET and SG contributed to the design of the study; LD and ET carried out the laboratory work; ET and TS supervised data collection; LD and TS performed the statistical analyses; LD, SG and TS interpreted the result; all authors contributed to the write up and approved the final manuscript.

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