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Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia

MULUGETA K. AZENE[1*] and BAYEH A. BEYENE[2]
[1]*Department of Biology, Science College, Bahir Dar University, P.O. Box 79, Bahir Dar, Ethiopia*
[2]*Department of Microbiology, Parasitology and Immunology, College of Medicine and Health Sciences, Bahir Dar University, P.O. Box 79, Bahir Dar, Ethiopia*

Abstract: Wound infections result in sepsis, limb loss, long hospital stays, higher costs, and are responsible for significant human mortality and morbidity worldwide. The aim of this study was to investigate the profile of pathogens cultured from wound infection and determine the antimicrobial susceptibility patterns. A retrospective analysis of bacterial pathogens and their antimicrobial susceptibility was done on wound swab samples that have been cultured at Dessie Regional Laboratory from 2003 to 2010. Antimicrobial susceptibility tests were done using disc diffusion technique as per the standard of Kirby-Bauer method. The mean age of male and female patients was 31.2 and 29.8 years, respectively with male to female ratio of 1:1.6. Out of 599 wound swab samples analyzed, 422 (70.5%) were culture positive. A total of 500 bacteria from 422 positive cases were identified. Seventy eight (18.5%) of the culture had double infections. *Staphylococcus aureus* was the most frequently isolated pathogen which accounted for 208 (41.6%) of isolates followed by *Pseudomonas* spp. 92 (18.4%), *E. coli* 82 (16.4%), *Proteus* spp. 55 (11.0%), *Enterobacter* spp. 21 (4.2%), and *Citrobacter* spp. 21 (4.2%), *Klebsiella* spp. 12 (2.4%) and Coagulate negative staphylococcus 9 (1.8%). Amoxicillin had the highest resistance rate 78.9%, followed by tetracycline 76.1% and erythromycin (63.9%). The sensitivity rates of norfloxacin, ciprofloxacin and gentamicin were 95.1%, 91.8% and 85%, respectively. The overall multiple antimicrobial resistances rate was 65.2% and only 13% of the isolates were sensitive to all antimicrobial agents tested. The most frequently isolated bacteria were sensitive to ciprofloxacin, gentamicin, cloxacillin and norfloxacin. These antimicrobials are considered as appropriate antimicrobials for empirical treatment of wound infections. Periodic surveillance of aetiology and drug susceptibility both in the community and hospital settings is recommended.

Key words: wound infection, antimicrobial resistance, Ethiopia

Introduction

Wound infections are responsible for significant human mortality and morbidity worldwide (Green & Wenzel, 1977). Wound infection results in sepsis, limb loss, long hospital stays and higher costs (Bowler *et al*., 2001; Branom, 2002; Cutting & White, 2004). Wound infections are caused by both gram-positive and gram-negative bacteria, especially by *Staphylococcus aureus*,

* Correspondence: Mulugeta Kibret; E-mail: mulugetanig@gmail.com

Escherichia coli, and *Pseudomonas aeruginosa* (Bowler & Davies, 1999; Larson, 2002). Studies conducted in Ethiopia have shown that wound infections are caused by *Staphylococcus aureus, E. coli, Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp. and Coagulase negative staphylococci (Biadeglegne *et al*., 2009; Tesfahunegn *et al*., 2009; Gedebou *et al*., 1987). Similar results have been obtained in Ghana (Newman *et al*., 2006), Nigeria (Okesola & Oni, 2000; Shittu *et al*., 2002) and Uganda (Anguzu & Olila, 2007).

The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. Antimicrobial resistance among pathogens of wound infections is on the increase (Taiwo *et al*., 2002; Anguzu & Olila, 2007; Okesola & Kehinde, 2008). 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 *S. aureus* (MRSA) and polymicrobic flora (Akinjogunla *et al*., 2009).

The prevalence of different bacteria in infected wounds varies and the knowledge of prevalence in an institution cannot be extrapolated to others. Apart from inter-institutional variation, trans-institutional variation also exists (Sule *et al*., 2002). In Ethiopia few studies have been done on the prevalence and antimicrobial resistance patterns of wound infections (Gedebou *et al*., 1987; Mulu *et al*., 2006; Fantahun *et al*., 2009; Tesfahunegn *et al*., 2009; Yismaw *et al*., 2010). These studies indicated that many of the bacterial isolates showed high levels of resistance to amoxicillin, tetracycline, chloramphenicol, erythromycin while low levels of resistance to gentamicin, cloxacillin, norfloxacine and ciprofloxacin were documented (Taiwo *et al*., 2002; Yismaw *et al*., 2010). Studies conducted so far focused on inpatients however data on antimicrobial susceptibility from outpatients is scarce. Knowledge of the causative agents of wound infection in a specific geographic region will therefore be useful in the selection of antimicrobials for empiric therapy. The objective of the present study was to identify the etiologies of infections over the course of eight years and determine the antimicrobial susceptibilities of the pathogens.

Material and methods

Study design

A retrospective review of culture results of wound swabs performed from 2003 to 2010 at Dessie Regional Health Research Laboratory was done. Demographic variables, the organism isolated and the antimicrobial resistance patterns were collected from the registration records using a standard data collection form.

Culture and identification

The laboratory standard operation procedure showed that wound swabs were collected using sterile cotton swabs. Wound specimens were inoculated on blood agar (for gram-positive bacteria), mannitol salt agar (selective media for *S. aureus*) and MacConkey agar (for gram-

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negative bacteria) (Oxoid, England). The plates were incubated in atmosphere at 37°C for 24 - 48 h. All positive cultures were identified by their characteristic appearance on their respective media, gram staining and confirmed by the pattern of biochemical reactions using the standard method (Cheesbrough, 2004). Members of the family enterobacteriaceae and other gram-negative rods were identified by indole production, H_2S production, citrate utilization, motility test, urease test, carbohydrate utilization tests and other tests. For gram-positive bacteria coagulase, DNase, catalase, bacitracin and other tests were used (Cheesbrough, 2004).

Antimicrobial susceptibility tests

According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, Hampshire, England) using Kirby Bauer disk diffusion method (Bauer *et al*., 1966). Morphologically identical 4-6 bacterial colonies from overnight culture were suspended in 5ml nutrient broth and incubated for 4 hours at 37°C. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. The surface of Mueller Hinton agar plate was evenly inoculated with the culture using a sterile cotton swab. The antibiotic discs were applied the surface of the inoculated agar. After 18-24 hours of incubation, the diameter of growth inhibition around the discs were measured and interpreted as sensitive, intermediate or resistant according to Clinical and Laboratory Standards Institute (CLSI, 2006) (formerly known as National Committee for Clinical Laboratory Standards / NCCLS). The antimicrobial agents tested were: tetracycline (30 µg), nitrofurantoin (300 µg), erythromycin (15 µg), chloramphenicol (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), cephalotin (30 µg), cotrimoxazole (25 µg), ceftriaxone (30µg), norflaxocin (10 µg), cloxacillin (5 µg) and amoxicillin (10µg) (Oxoid, England). Resistance data were interpreted according to (CLSI, 2006). Reference strains of *E. coli* ATCC 25922 and S. *aureus* ATCC 25923 were used for quality control for antimicrobial susceptibility tests.

Statistical analysis

Chi-square test was employed to compare the proportion of bacterial isolates with patient sex and age; and comparison of antimicrobial resistances. P-value of < 0.05 was considered to indicate statistical significant difference.

Ethical consideration

Ethical approval was secured from Research Ethics Committee of Bahir Dar University. Permission from Dessie Regional Health Research Laboratory was also obtained.

Results

Between 2003 and 2010, a total of 599 wound swab samples analyzed. Four hundred twenty two (70.5%) samples were culture positive while 177 (29.5%) of wound swab cultures showed no growth (Table 1). A total of 500 bacteria were isolated from 422 cases (Table 2). Three hundred forty four (81.5%) sample had single pathogens while 78 (18.5%) had double infections. The

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number of isolates was higher than the number of cases because two pathogens were isolated from 78 (18.5%) cases (Table 3).

Table 1: Age and sex distribution of patents with wound infections at Dessie regional laboratory, Ethiopia, from 2003 to 2010.

Demographic characteristics		No. (%) of culture positive	P-value
Age groups (years)			
	< 4 (n=16)	11 (68.8)	0.01
	5-14 (n=71)	49 (69.0)	
	15-25 (n=205)	151 (73.7)	
	26-44 (n=176)	109 (61.9)	
	> 44 (n=131)	102 (77.9)	
Gender			
	Male (n=368)	271 (64.2)	0.09
	Female (n=231)	151 (35.8)	
	Total	422 (100)	

The age of the patients ranged from 1 year to 90 years, with mean age of 30.7 (SD=17.5) years. The mean age of male and female patients was [(31.2 (SD=18.1) and 29.8 (SD=16.3)] years, respectively. In this study males 368(61.4%) constituted and females 231 (38.6%) with male to female ratio of 1:1.6. Majority of the pathogens were isolated from males with isolation rate of (64.2%) (CI=0.45) and 35.8% (CI=0.36), respectively (Table 1).

Table 2: Type and frequency of pathogens isolated from wound infections at Dessie regional laboratory, Ethiopia, from 2003 to 2010.

Bacteria Isolated	Number (n=500)	Percent
S. aureus	208	41.6
Pseudomonas spp.	92	18.4
E. coli	82	16.4
Proteus spp.	55	11.0
Enterobacter spp.	21	4.2
Citrobacter spp.	21	4.2
Klebsiella spp.	12	2.4
CNS	9	1.8
Total	500	100.0

Key: CNS = Coagulase negative staphylococci

Table 2 illustrates the frequency of the pathogens isolated. Gram negative and gram positive bacteria constitute 56.6% and 43.4% of the bacterial isolates. *S. aureus* was the most frequently isolated pathogen which accounts for (41.6%), followed by *Pseudomonas* spp., (18.4%), *E. coli* (16.4%), *Proteus* spp. (11.0%), *Enterobacter* spp. and *Citrobacter* spp. (4.2%), *Klebsiella* spp. 2.4% and CNS (1.8%).

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S. aureus and *Pseudomonas spp.* were the most frequently isolated mixed infection (38.5%). Infections with *S. aureus* and *Proteus* spp., *S. aureus* and *E. coli*, *S. aureus* and *Citrobacter* spp. with rates of 20.5%, 17.9% 9% and 6.4%, respectively were among the double infections recorded in this study (Table 3).

Table 3: Type and number of pathogens in mixed wound infection at Dessie regional laboratory, Ethiopia, from 2003 to 2010

Pathogens	Frequency	Percentage
S. aureus and *Pseudomonas* spp.	30	38.5
S. aureus and *Proteus* spp.	16	20.5
S. aureus and *E. coli*	14	17.9
S. aureus and *Enterobacter* spp.	7	9.0
S. aureus and *Citrobacter* spp.	5	6.4
Enterobacter spp. and *Pseudomonas* spp.	3	3.8
S. aureus and *Klebsiella* spp.	1	1.3
Klebsiella spp. and CNS	1	1.3
E.coli and *Citrobacter* spp.	1	1.3
Total	78	100

Key: CNS = Coagulase negative staphylococci

Analysis of species specific resistance rates indicated that most of *S. aureus* was mostly resistant to amoxicillin (79.1%) and tetracycline (71.0%). On the other hand, *S. aureus* was susceptible to norflaxocin, ciprofloxacin, cloxacillin and gentamicin with resistance of only 8.0%, 8.8%, 10.3% and 12.4%, respectively. *Pseudomonas* spp. showed the highest resistance to erythromycin (90.5%), tetracycline (90.3.0%) and amoxicillin (82.4%) while the resistance rates to ciprofloxacin and gentamicin were 4.6% and 7.8%. *E. coli* isolates had resistant rates of 85.0% to amoxicillin and 71.4 % to tetracycline whereas their resistance rates to ciprofloxacin and gentamicin were 7.7% and 14.4%, respectively. *Proteus* spp. had resistance rate of 92.9% to erythromycin, 87.5 % to tetracycline and 86.4 % to amoxicillin. However, *Proteus* isolates were sensitive to gentamicin with resistance rate of only 9.6% and 16.7 % resistance to ciprofloxacin (Table 4).

In this study, the overall multiple antimicrobial resistances rate was 65.2% and only 13% of the isolates were sensitive to eleven antimicrobial agents tested. The resistances against two or more antimicrobial agents were 47.6%, 82.6%, 75.6%, and 87.3% for *S. aureus*, *Pseudomonas* spp., *E. coli* and *Proteus* spp., respectively (Table 5).

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Table 4: Susceptibility patterns of bacterial isolates from wound infections at Dessie regional laboratory, Ethiopia, 2003-2010

Antimicrobial agents	*S. aureus* (n=208)		*Pseudomonas* spp. (n=92)		*E.coli* (n=82)		*Proteus* spp. (n=53)		*Citrobacter* spp. (n=21)		*Enterobacter* spp. (n=21)		*Klebsiella* spp. (n=12)		CNS (n=9	
	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]	#T[a]	% R[b]
Tetracycline	138	71	72	90.3	70	71.4	48	87.5	18	66.7	17	52.9	9	66.3	4	25.0
Gentamicin	194	12.4	90	7.8	78	14.4	52	9.6	18	16.7	21	4.8	11	-	7	14.3
Chloramphenicol	141	39.0	77	75.3	72	36.1	42	71.4	17	47.1	19	47.4	6	66.7	3	0.00
Cotrimoxazole	134	67.1	71	77.5	70	67.1	42	81.0	18	50.0	19	47.4	8	62.5	7	42.9
Cephalotine	73	20.6	34	64.7	34	64.7	17	64.7	6	33.3	10	80.0	-	-	-	0.00
Ciprofloxacin	68	8.8	65	4.6	26	7.7	24	16.7	15	0.00	16	6.3	-	-	2	50.0
Amoxicillin	67	79.1	17	82.4	20	85.0	22	86.4	6	66.7	4	100	7	100	4	0.00
Norfloxacin	25	8.0	-	-	-	-	-		-	-	-	-	5	100	3	0.00
Erythromycin	139	34.5	63	90.5	54	51.9	28	92.9	16	93.8	17	94.1	-	-	5	80.0
Ceftriaxone	73	17.8	33	51.5	12	66.7	25	64.0	12	41.7	4	25	-	-	2	50.0
Doxycycline	39	10.3	11	54.5	6	33.3	5	20	-	-	-	-	-	-	-	-
Cloxacillin	39	10.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: CNS = Coagulase negative staphylococcus; a = number of isolates tested against each antimicrobial agent, b = Percent of isolates resistant to the antimicrobial agent

Table 5: Multiple antimicrobial resistance patterns of bacterial isolates wound infections at Dessie regional laboratory, 2003-2010

Bacteria	Antibiogram R0	R1	R2	R3	R4	R5	R6	R7
S. aureus (n=208)	40 (19.2)	69 (33.2)	40 (19.2)	33 (15.59)	18 (8.7)	4 (1.9)	4 (1.9)	
Pseudomonas spp. (n=92)	7 (7.6)	9 (9.8)	10 (10.9)	18 (19.6)	27 (29.3)	14 (15.2)	6 (6.5)	1 (1.1)
E. coli (n=82)	9 (11.0)	11 (13.4)	17 (20.7)	23 (28.0)	14 (17.1)	6 (7.3)	1 (1.2)	1 (1.2)
Proteus spp. (n=55)	-	7 (12.7)	11 (20.0)	9 (16.4)	11 (20.0)	14 (25.5)	3 (5.5)	-
Citrobacter spp. (n=21)	3 (14.3)	2 (9.5)	4 (19.0)	5 (23.8)	4 (19.0)	-	3 (14.3)	-
Enterobacter sp. (n=21)	2 (9.5)	7 (33.3)	1 (4.8)	5 (23.8)	4 (19.0)	1 (4.8)	1 (4.8)	-
Klebsiella spp. (n=12)	-	1 (8.3)	4 (33.3)	4 (33.3)	3 (25.0)	-	-	-
CNS (n=9)	4 (44.4)	1 (11.1)	1 (11.1)	2 (22.2)	1 (11.1)	-	-	-
Total Isolates = 500	65 (13)	107 (21.4)	88 (17.6)	99 (19.8)	80 (16.0)	39 (7.8)	18 (3.6)	2 (0.4)

CNS = Coagulase negative staphylococci; R0= Sensitive to all antimicrobials tested; R1, R2, R3, R4, R5, R6, R7 -Resistant to one, two, three, four, five, six, seven antimicrobials, respectively

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Discussion

Wound infections are responsible for significant mortality and morbidity, and incur substantial costs in hospitals. Antimicrobial resistance in wound pathogens is on the rise which can increase complications and costs associated with procedures and treatment (Bowler *et al*., 2001; Mulu *et al*., 2006; Wariso & Nwachukwu, 2003). Identification of pathogens and determination of antibiograms from clinical specimens is beneficial to the patient and assists in selection of chemotherapy.

The isolation rate of pathogens in this study is higher compared to previous studied (Anguzu & Olila, 2007; Mulu *et al*., 2006) however relatively lower than that reported from Ethiopia (Gizachew *et al*., 2010) and Nigeria (Wariso & Nwachukwu, 2003). Statistically significant association was found between age of the patients and isolation rate of bacteria (p=0.01). Wariso & Nwachukwu (2003) reported a similar result. In this study, as in several previous reports (Mulu *et al*., 2006; Gizachew *et al*., 2010; Wariso & Nwachukwu, 2003; Surucuoglu *et al*., 2005) the most frequently isolated bacterium was *S. aureus*. It has been noted that *S.aureus* is the single causative bacterium in 25-655 of cutaneous abscess (Mahdi *et al*., 2000). *Pseudomonas* spp. was the second most common organism isolated followed by *E. coli*, *Proteus* spp., *Enterobacter* spp. and *Citrobacter* spp., *Klebsiella* spp. and CNS. The proportion of bacterial isolates was in agreement with several previous studies (Taiwo *et al*., 2002; Giacometti *et al*., 2000). Bacterial etiology can show geographic variations and may even vary over time within a population (Leegaard *et al*., 2000; Grude *et al*., 2001). Difference in identification methods are also known to influence the relative prevalence of bacteria which makes comparison of results difficult (Barret *et al*., 1999). `

High resistance rates were recorded to amoxicillin, tetracycline and erythromycin. Significantly high resistance rate was demonstrated for amoxicillin, erythromycin and erythromycin (p<0.001). High level of resistance has been reported to tetracycline and erythromycin from studies conducted in Ethiopia and elsewhere (Mulu *et al*., 2006; Biadgelegne *et al*., 2009, Petkovsˇek *et al*., 2009). The sensitivity rates of norflaxocin, ciprofloxacin, cloxacillin and gentamicin were high. Gentamicin, ciprofloxacin, cloxacillin and norfloxacine were the most effective antimicrobials against most isolates. This finding is in line with the results documented from previous studies for ciprofloxacin (Anguzu & Olila, 2007; Wariso & Nwachukwu, 2003; Misra & Yogesh, 2000), cloxacillin and gentamicin (Endalafer *et al*., 2011; Taiwo *et al*., 2002) and norfloxacin (Petkovsˇek *et al*., 2009). The relatively low level of resistance to these drugs can be because these drugs had been in the market for a relatively short period of time as compared to drugs such as tetracycline, amoxicillin and erythromycin (Neweman *et al*., 2006). Majority of the isolates (65.2%) showed resistance to two or more antimicrobials. Previous studies in Ethiopia have demonstrated comparable results (Mulu *et al*., 2006; Yismaw *et al*., 2010).

This retrospective study is based on the results microbiological tests and susceptibility testing carried out between 2003 and 2010. Due to the nature of the retrospective analysis we couldn't trace patient's history. This study did not consider etiology of wound infections other

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than bacteria and anaerobic bacteria due to lack of facility. The number of antimicrobials were tested were also limited in some isolates.

In conclusion, the results of this study showed the prevalence and antimicrobial susceptibility patterns of wound infection over a period of eight years. The most frequently isolated bacteria were sensitive to ciprofloxacin, gentamicin, cloxacillin and norfloxacin. These antimicrobials are considered appropriate for empirical treatment of wound infections in the study area. Periodic monitoring of etiology and antimicrobial susceptibility both in the community and hospital settings is recommended.

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