

ORIGINAL ARTICLE**BACTERIAL ISOLATES FROM WOUND INFECTION AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN FELEGE HIWOT REFERRAL HOSPITAL, NORTH WEST ETHIOPIA**

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ABSTRACT

BACKGROUND: *Wound infection is a major health problem particularly in developing countries like Ethiopia. It's most frequent causes are Staphylococci, Streptococcus species and Entrobacterriaceae groups. Information on the relative frequency of the isolation and susceptibility patterns of these pathogens is critically important. The objective of the study was to identify the specific causal agents of wound infections and their sensitivity to commonly prescribed antibiotics.*

METHODS: *This laboratory based retrospective study of wound swabs was conducted in the microbiology department at Bahir Dar Regional Health Research Laboratory from September 2003 to June 2008. Wound swabs were collected using sterile cotton swabs from in and out patient departments of Felege Hiwot Hospital and processed following the standard bacteriological methods. Antimicrobial susceptibility test for isolated organisms was done as per the standard disk diffusion method. The data were entered in to a computer and analyzed using Microsoft Excel.*

RESULTS: *From the total of 379 wound swabs, bacterial isolates were found on 201 patients with an isolation rate of 53.0%. Staphylococcus aureus was the predominant isolate 140 (69.7%) followed by proteus species 19 (9.5%) and kelebsiella species 10 (5.0%). The overall multiple drug resistance patterns in ten antibiotics was 97.5%.*

CONCLUSION: *Single and multiple drug resistance to the commonly used antibiotics were very high among bacterial isolates from wound. Therefore, further studies to explore the causes for increased drug resistance in the area need to be carried out.*

KEY WORDS: Bacterial pathogens, Drug resistance, wound infection, Bahir Dar, Ethiopia

INTRODUCTION

In developing countries large number of people dies daily of preventable and curable diseases such as wound infections. Wound infection is one of the health problems that are caused and aggravated by the invasion of pathogenic organisms in different parts of the body. Previous studies from different parts of the country showed that *S. aureus*, *Kelebsiella* species, *E. coli*, *proteus* species, *streptococcus* species,

entrobacter species, *pseudomonas* species and coagulase negative staphylococci were the most common pathogens isolated from wound (1). The wound sometimes gets infection by either single or multiple organisms. Wound infections are mostly due to nosocomial pathogens that differ from country to country and from hospital to another with in the same region (1), which remains the major source of post operative morbidity (2).

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On the other hand, wound infection by resistant bacteria worsens the condition (3). Rapid spread of resistant microbes affected the effectiveness of antimicrobials creating worldwide problem (1). The condition is serious in developing countries owing to irrational prescription of antimicrobial agents (4). The battle between bacteria and their susceptibility to drugs is yet problematic among public, researchers, clinicians and drug companies looking for effective drugs. Measures to control problem include development of new antimicrobial, better infection control program and more appropriate use of existing antimicrobial agents (5, 6, 7). Many researchers made different recommendations on the susceptibility of micro-organisms to drugs (8).

Data on anti microbial susceptibility pattern among bacterial isolates from wound infections is limited in Ethiopia in general and in the study area in particular. The presence of drug resistant bacteria in the natural environment is a great threat for clinicians. Timely information is crucial for clinicians on local pathogens and their drug susceptibility patterns to decide on appropriate treatment regimen. Therefore, this study was aimed to identify the specific causal agents of wound infection and their sensitivity to commonly prescribed antibiotics.

SUBJECTS AND METHODS

This cross-sectional study was conducted based on retrospective review of records of 379 patients for whom wound swabs were processed in the microbiology department of Bahir Dar Regional Health Research Laboratory at Felege Hiwot Hospital, Bahir Dar, from September 2003 to June 2008.

Standard operation procedures were checked for laboratory techniques and data were collected from registration book using a format. Data on socio-demographic variables such as age, sex, patient settings and microbiological data were abstracted from patients' records from July 1-30, 2008. The laboratory standard operation procedure showed that wound swabs were collected using sterile cotton swabs (9). Culture and susceptibility test was processed by microbiologist and/or senior technologist of the department. Specimens were inoculated onto 5% Sheep's blood, chocolate agar, mannitol salt agar and MacConkey agar plates (Oxoid Ltd, Basing stoke Hampshire, UK) for aerobic and facultatively anaerobic organisms. The plates were incubated at 37 °C aerobically (MacConkey, mannitol salt agar) and under 5% carbon dioxide (5% sheep's blood and chocolate) and examined at after 24 and 48 hours. Records showed that antimicrobial susceptibility testing was done on Mueller-Hinton agar (Oxoid, Basing stoke Hampshire, UK) as per the standard disc-diffusion method recommended by the National

Committee for Clinical Laboratory Standards (NCCLS) (10) against Ampicillin (10µg), chloramphenicol (30µg), gentamicin(10µg), penicillin G (10IU), tetracycline (30µg), erythromycin (30µg), co-trimoxazole (25µg), streptomycin (10 µg), carbenicillin (100 µg) and cephalotin (30 µg). Morphologically identical 4-6 bacterial colonies from overnight culture were suspended in 5ml Muller Hinton broth (Oxoid, Basingstoke, and Hampshire, UK) and incubated for 3 to 5 hours at 37°C. Turbidity of the broth culture was equilibrated to match that of 0.5 McFarland standards. Then, a loop full of this bacterial suspension was placed at the center of the Muller Hinton agar medium and evenly spread using dry cotton swabs. After 18-24 hours of incubation, diameters of growth inhibition around the discs were measured and interpreted as per the standards. Reference strains *E. Coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as controls (11). The data was entered in to a computer and analyzed using Microsoft Excel. Ethical clearance was obtained from the ethical committee of College of Medicine and Health Sciences, Bahir Dar University.

RESULTS

Wound swabs from 379 patients were analyzed in the study. Male patients constituted 222 (58.6%) and their age ranged from 3 months to 90 years (Table 1). Bacterial isolates were found on 201 (53.0%) patients. The isolation rate was significantly higher in males (63.2%) compared to females (36.8%). The predominant isolates were gram positive bacteria 149 (74.1%). The most frequently isolated organisms were *S. aureus* 140 (69.7%) followed by proteus species 19(9.5%), kelesiella species 10 (5%), pseudomonas species 9(4.5%) and Streptococcus species 8 (4.0%) (Table 2).

Among gram positive organisms *S. aureus* showed high level of drug resistance for tetracycline 94 (71.4%), streptomycin 91(65.0%), chloramphenicol 86 (61.4%), penicillin G 79 (65.4%), ampicillin 71 (50.7%), carbencillin 48 (34.3%), erythromycin 42 (30%), co-trimoxazole 37 (26.4%), cephalotin 35 (25.0%) and gentamycin 34 (24.3%) (Table3). Of the gram negative bacteria *E. coli*, proteus species, pseudomonas species showed high level of drug resistance. All *E. coli* isolates were found to be resistant to ampicillin, tetracycline, chloramphenicol. Isolate specific drug resistant rates of each bacterial species to each drug tested were well summarized in (Table 3). Multiple drug resistance was found in 97.5% of the isolates, while 0.9% of the bacteria were resistant to one antibiotic and only 1.5% was found to be sensitive to all antibiotics tested (Table 4).

Table 1. Age and sex distribution of patients investigated for wound infection in Felege Hiwot Referral Hospital, North West Ethiopia, 2008.

Variables	Number(n=379)	Percent
Age		
< 5 Years	25	6.6
5-15	48	12.7
16-49	275	72.5
>49	31	8.2
Sex		
Male	222	58.6
Female	157	41.4
Sources:		
OPD	165	43.5
Wards	214	56.5

OPD – out patient department

DISCUSSION

The data presented in this study could provide information to clinicians on the selection of antimicrobial agents in treating patients suffering from wound infections. In the present study the isolation rate of bacteria from wound swabs was 53.0%. This result is in accordance with other previous studies (1, 2, 12). In this study, the most commonly isolated organisms were *S. aureus*, followed by proteus species and klebsiella species which are also in agreement with similar studies reported in Gondar, Sidamo and Jimma Hospitals in Ethiopia (1, 12, 13). The frequency of

isolation of *E. coli*, klebsiella species and coagulase negative Staphylococci was decreased in this study while that of proteus species was increased as compared to previous study (1). The results of our study showed that the predominant agents in wound swabs belong to gram positive cocci mainly of *S. aureus* which is in line with previous studies conducted in Ethiopia (1, 12) and Pakistan (14). This may be due to contamination of wounds by the normal flora of bacteria on the skin. The proportion of gram negative bacteria isolate was also high which could be explained by the chronic nature of most infected wounds (1).

Table 2. The etiology and frequency of wound pathogens isolated at Bahir Dar Regional Laboratory of Felege Hiwot referral Hospital, North West Ethiopia, 2008

Organisms isolated	Number(n=201)	Percent
<i>S. aureus</i>	140	69.7
Proteus spp.	19	9.5
Kelebsilla spp.	10	5.0
Pseudomonas spp.	9	4.5
Streptococcus spp.	8	4.0
Enterobacter spp.	7	3.5
<i>E. coli</i>	4	2.0
Citrobacter spp.	2	0.9
Serratia spp.	1	0.6
CN Staphylococci	1	0.5

CN- Coagulase negative

The present study showed a high rate of resistance to antimicrobial agents, which is consistent with reports in different studies (2, 15, 16). Remarkably high frequency of resistance to tetracycline, chloramphenicol, ampicillin, penicillin, streptomycin,

carbenicillin, cephalotin and erythromycin has been observed in our study. This may be due to the easily availability and indiscriminate use of the drugs with out prescription (17).

Table 3. Susceptibility pattern of isolated wound pathogens, Felege Hiwot Referral Hospital, North west Ethiopia, 2008.

Organisms	Resistance percentage rate of each drug tested No (%)									
	Amp	CN	P	TE	C	E	SXT	S	CB	KF
<i>S. aureus</i>	71(50.7)	34(24.3)	79(56.4)	94(71.4)	86(61.4)	42 (30)	37(26.4)	91(65.0)	48(34.3)	35(25.0)
<i>Proteus spp.</i>	16(84.2)	6 (31.6)	8 (42.1)	13(68.4)	12(63.2)	10(52.6)	5 (26.3)	12(63.2)	7 (36.8)	9 (47.4)
<i>Kelebsilla spp.</i>	7 (70.0)	-	-	10(10.0)	5 (50.0)	1 (10.0)	1 (10.0)	7 (70.0)	5 (50.0)	7 (70.0)
<i>Pseudomonas spp.</i>	6 (66.7)	3 (33.3)	7 (77.8)	7 (77.8)	8 (88.9)	3 (33.3)	3 (33.3)	6 (66.7)	4 (44.4)	5 (55.6)
<i>Streptococcus spp.</i>	5 (62.5)	2 (25.0)	3 (37.5)	2 (25.0)	3 (37.5)	2 (25.0)	2 (25.0)	4 (50.0)	5 (62.5)	4(50.0)
<i>Enterobacter spp.</i>	6 (85.7)	3 (42.9)	1 (14.2)	6 (85.7)	6 (85.7)	1 (14.2)	2 (28.5)	6 (85.7)	3 (42.9)	5 (71.4)
<i>E. coli</i>	4(100.0)	1 (25.0)	-	4 100.0)	4 100.0)	-	1 (25.0)	2 (50.0)	2 (50.0)	2 (50.0)
<i>Citrobacter spp.</i>	1 (50.0)	-	1 (50.0)	-	2(100.0)	-	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
CNStaphylococci	1(100.0)	-	1(100.0)	1(100.0)	1(100.0)	-	1(100.0)	-	-	-
<i>Serratia spp.</i>	-	-	-	1(100.0)	1(100.0)	1(100.0)	1(100.0)	-	-	-
Total	117(59.2)	49(24.4)	138(49.8)	140(69.7)	128(63.7)	60(31.8)	54(26.9)	75(37.3)	75(37.3)	68(33.8)

Amp- Ampicillin, CN- Gentamycin, P- penicillin G, TE- Tetracycline, C- Chloramphenicol, E- Erythromycin, SXT- co-trimoxazole, S- Streptomycine, CB- Carbenicillin, Kf- Cephalotin,

Studying Bahir Dar area, people have easy access to most of the antimicrobial agents with out any prescription. Most people in Bahir Dar also visit only private diagnostic laboratories and provided with drugs with out being seen by clinicians. Most of the isolates in Bahir Dar, as else where, were multiple drug resistant indicating that plasmids could have played an

important role for the spread of drug resistant organisms (18), that will be associated with the increased chance of cross infection among in patients, are known to account for circulating resistant bacterial strains (19). There fore, the observed high level of drug resistance is probably due to the above mentioned factors.

Table 4. Multiple drug resistance patterns in bacterial pathogens from wound, Felege Hiwot referral Hospital, 2008

Organisms	Antibiogram pattern (%)						
	R0	R1	R2	R3	R4	R5	
<i>S. aureus</i>	2 (1.4)	2 (1.4)	6 (4.3)	16 (11.4)	17 (12.1)	97 (69.4)	
<i>Proteus spp.</i>	-	-	1 (5.3)	1 (5.3)	3 (15.8)	14 (73.6)	
<i>Kelebsilla spp.</i>	-	-	-	1 (10)	3 (30)	6 (60)	
<i>Pseudomonas spp.</i>	-	-	-	2 (22.2)	1 (11.1)	6 (66.7)	
<i>Streptococcus spp.</i>	1 (12.5)	-	2 (25.0)	2 (25.0)	-	3 (37.5)	
<i>Enterobacter spp.</i>	-	-	-	-	1 (14.3)	6 (85.7)	
<i>E. coli</i>	-	-	-	-	-	4 (100.0)	
<i>Citrobacter spp.</i>	-	-	-	-	-	2 (100.0)	
CN Staphylococci	-	-	-	-	-	1 (100.0)	
<i>Serratia spp.</i>	-	-	-	-	-	1 (100.0)	
Total	3 (1.5)	2 (0.9)	9 (4.5)	22 (10.9)	25 (12.4)	140 (69.7)	

R₀- sensitive to all antibiotics, R₁ – resistant to 1 antibiotic, R₂- resistant to 2 antibiotics, R₃ – resistant to 3 antibiotics

R₄- resistant to 4 antibiotics, R₅ – resistant to 5 or more than 5 antibiotics, CN – Coagulase Negative

Multiple drug resistance to the commonly used antibiotics was 95.5% in this study which is very high compared to previous findings (1, 2, 15, 16, 20, 21). The change in the pattern of bacterial resistance has importance both clinical setting and epidemiological purpose. To help physician to choose effective antibiotics and detect local epidemics of resistant bacteria, surveillance at local level is important (1). As the study was a retrospective analysis we could not access patients' detail clinical information rather the study was limited to characterize type of wounds per bacterial species.

In conclusion, single and multiple drug resistance to the commonly used antibiotics were very high among bacterial isolates from wound. There fore, we recommended further studies to identify the causes for high level of drug resistance in the area.

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