

Spectrum and Drug Susceptibility Profile of Bacteria Recovered from Patients with Wound Infection Referred to Arsho Advanced Medical Laboratory

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Abstract: Wound infection still remains a significant cause of morbidity and mortality. Hence, studying the spectrum of bacterial etiological agents and their drug susceptibility profile is critical. A prospective study was conducted at Arsho Advanced Medical Laboratory from June 2016 to July 2017. Wound specimens were collected from 366 patients following standard procedures. Specimens were plated and incubated at 37°C for 48 hours. Identification and drug susceptibility testing of cultures were carried out by using the VITEK 2 compact system. Among 366 wound samples cultured, bacteria grew in 271(74%) samples. The highest (81.9%) wound infections were documented among patients with an age group of 15-64 years. Two hundred twenty one bacterial isolates were recovered of which 43.2% were Gram-negative while, 56.8% were Gram-positive. *Staphylococcus aureus* and Coagulase-Negative Staphylococci were major Gram-positive bacteria while *Escherichia coli* and *Pseudomonas spp.* were the commonest Gram-negative bacteria. Gram-negative bacteria had the highest overall drug resistance rate against ampicillin. Tobramycin and piperacillin/tazobactam combination were effective antimicrobial agents against Gram-negative bacteria. The highest overall resistance rate to Gram-positive bacteria was observed against erythromycin. Vancomycin and linezolid were the most active antimicrobial agents against Gram-positive bacteria. High culture positivity rate of wound infections reported in the present study initiates many similar studies to be conducted on wound in the country. High level of drug resistance to the commonly prescribed drugs dictates a search for better choices.

Keywords: Wound Infections, Drug Susceptibility Pattern, Etiological Agents, Ethiopia

1. Introduction

The human skin is one of our first line innate immunities that prevents infections of internal tissues by micro-organisms physically. Sweat and sebaceous secretions produced by the skin also deny microbial infections of internal tissues by a virtue of their acidic pH (3-5). Moreover, fatty acids that have antifungal properties and lysozyme that dissolves bacterial cell wall also play a major role in the protection of internal tissues by microbial pathogens. Wound is, therefore, a break in the skin that exposes internal tissues to pathogens. It provides moist, warm, and conducive situation that is favorable for bacterial colonization and

propagation [1]. Accidental (e.g., thermal wound infection) or intentionally (surgical or use of intravenous medical devices) induced trauma is an indispensable incident for all wound colonization. Wounds develop into an infected state when the balance between microorganism and the host shifts in favour of the micro-organism [2].

Wound infections can be classified into skin infection and soft tissue infection. Wound infections can also be classified into community acquired and hospital acquired infection [3] where the latter is one of the prominent nosocomial causes of morbidity. Hospital- acquired wound infections result in

repeated hospitalization, lengthy hospital stay, increased demand of wound care, and treatment cost. It is also a major cause of anxiety in health workers causing wound management practices much more challenging [4, 5].

Bacterial etiologies of wound infections have been excellently reviewed by Howell-Jone et al [6]. Although bacterial etiologies of wound infections vary within countries and hospitals in the same country [7], *S. aureus* and Coagulase-Negative Staphylococci have been the major bacteria isolated irrespective of the type of study [6].

Wound infection still remains a substantial cause of morbidity and mortality particularly in developing countries, although major achievements in its control and management have been achieved [8]. This is because, wound infections are one of the major sources of post-operative disorder, that cause mortality among burn patients [9], and accounts for roughly one-fourth of all hospital acquired infections [10]. To this effect, identification and determining drug susceptibility pattern of bacteria associated with wound infections for efficient management of patients with the problem is still an active field of research. Although numerous researches have been conducted on wound infections in Ethiopia, a change in etiologic agents and poor laboratory set up coupled with the development of drug resistance warranted additional investigation.

In Ethiopia, like other developing countries, diagnostic microbiology laboratories are poorly organized. Diagnostic laboratories that isolate and characterize bacteria by using even few routine biochemical tests are rare. Furthermore, drug susceptibility testing of bacterial isolates has also been determined by using agar diffusion technique with all its limitations. Consequently, treatment of bacterial wound infection in Ethiopia has remained empirical. In addition, agreement with respect to the distribution of bacterial species associated with wound infections and their drug susceptibility pattern among different local studies is lacking. In view of this, application of fully automated systems for bacterial characterization and for the assessment of their antimicrobial susceptibility profile has become important. The VITEK 2 compact (bioMérieux, France) is a machine capable of running bacterial identification and drug susceptibility testing at the same time. Reduced turnaround times, better specimen management, enhanced quality control, reproducibility, precision, and the ability to track results are other benefits of the VITEK 2 compact system over conventional methods. With regards to Identification, the machine characterizes a total of 135 Gram-negative fermenting and non-fermenting bacilli and 115 Gram-positive cocci and non-spore-forming bacilli to the species level by using 64 biochemical tests and substrates. Identification of bacterial isolates to species level provides indispensable information on its pathogenic potential and is of greatest importance for the correct explanation of antibiotic susceptibility testing. Against this background, the objective of this study was to characterize and evaluate drug susceptibility profile of bacteria associated with wound infections from patients referred to Arsho Advanced Medical

Laboratory by employing the fully automated VITEK 2 compact system.

2. Materials and Methods

This study was carried out at Arsho Advanced Medical laboratory, Addis Ababa, Ethiopia from June 2016 to July 2017. Arsho is the oldest Medical Laboratory where patients are referred to culture and drug sensitivity testing. On the average about 50 patients per day are referred to Arsho for culture and drug sensitivity testing. It is also the only diagnostic laboratory in the country where automated machines such as the VITEK 2 compact system is employed for routine diagnostic and/or research activity. The requisition form filled out by physicians was used as standard proforma to document socio-demographic characteristics, history of antibiotic treatment and other information about study subjects. Patients to be included in the study, they must be clinically diagnosed for wound infection, consent to participate in the study, and no anti-bacterial therapy is administered within two weeks prior to their attendance.

Wound specimens were collected aseptically from study participants following standard procedures. Clinical samples such as biopsy and tissue materials collected and referred from respective health institutions were also used in this study. All wound samples were then streaked onto primary isolation culture media (Blood Agar base (Oxoid, Basingstoke, Hampshire, UK) to which 10% sheep blood is incorporated, Mannitol salt agar (Oxoid, Basingstoke, Hampshire, UK), MacConkey agar (Oxoid, Basingstoke, Hampshire, UK), and Chocolate agar), incubated at 37°C for 18-24 hours aerobically. Pure isolates of significant bacterial pathogen per sample were preliminarily characterized by colony morphology, Gram-stain, lactose fermentation, and catalase test before inoculating them into AST-GN72 and AST-GP71 cards.

Identification and drug sensitivity testing of pure cultures were carried by the VITEK 2 compact system following the procedures of the manufacture (bioMérieux, France). AST-GN72 cards (kits used for the identification and susceptibility testing Gram-negative bacteria) were used for the identification and susceptibility testing of fermenting and non-fermenting Gram-negative bacilli, while the AST-GP71 cards (kits used for the identification and susceptibility testing of Gram-positive bacteria) were used for the identification and susceptibility testing of non-spore-forming Gram-positive bacteria. Detailed description of inoculum size determination, bacterial identification and drug sensitivity testing by the machine, and the antimicrobial agents used with their concentration can be obtained from Bitew et al [11].

3. Ethics and Consent to Participate

All ethical considerations and obligations were duly addressed. The study was carried out after the approval of research and ethical committee of Arsho Advanced Medical

Laboratory private limited company (AAMLRERC). Data collection was started after obtaining written informed consent from study subjects and assent form was completed and signed. All the information obtained from the study subjects were coded to maintain confidentiality.

4. Results

Out of 366 wound samples studied, 153 (41.8%) were collected from female and 213 (58.2%) from male study subjects. Among wound samples studied, 271 (74%) showed

bacterial growth where 109 (40.2%) were collected from female and 162 (59.8%) from male study subjects. Two hundred twenty two (81.9%; 222/271) wound infections were documented from young and middle age patients with an age group of 15-64 years. Of a total of 271 individuals with wound infections, pediatric study subjects (0-14 years) accounted for 3.7% while elderly study subjects (≥ 65 years) accounted for 14.4% (Table 1). The highest wound infection (39.5%) was recorded in patients of age group 45-64 followed by age group of 25-44.

Table 1. Frequency of wound infection in relation to gender and age (n=366).

Variables	category	Sample size	Culture positive samples, n (%)	Culture negative samples, n (%)
Gender	Overall	366	271 (74)	95 (26)
	Female	153 (41.8)	109 (40.2)	44 (46.3)
	Male	213 (58.2)	162 (59.8)	51 (53.7)
	Total	366 (100)	271 (100)	95 (100)
Age in years	<1	4 (1.1)	3 (1.1)	1 (1.0)
	1-14	14 (3.8)	7 (2.6)	7 (7.4)
	15-24	68 (18.6)	51 (18.8)	17 (17.9)
	25-44	86 (23.5)	64 (23.6)	22 (23.2)
	45-64	145 (39.6)	107 (39.5)	38 (40)
	65+	49 (13.4)	39 (14.4)	10 (10.5)
	Total	366 (100)	271 (100)	95 (100)

Out of 271 bacterial isolates recovered, 117(43.2%; 117/271) were Gram-negative while 154 (56.8%; 154/271) were Gram-positive bacteria. *S. aureus* and Coagulase-Negative Staphylococci were the major Gram-positive bacteria, comprising of (40.6%; 110/271) and 12.9%

(35/271) of the total isolates, respectively. The four major genera of Gram-negative bacteria isolated include *Escherichia* 49 (18.1%), *Pseudomonas* 15 (5.5%), *Klebsiella* 14 (5.2), and *Proteus* 12 (4.4%) (Tables 2 and 3).

Table 2. Distribution and percentage frequency of Gram- negative bacterial species (n=117).

Genus	Species	n (%) of the total isolates
<i>Acenitobacter</i>	<i>A. baumannii</i>	4 (1.5)
	<i>A. calcoocticus</i>	2 (0.7)
<i>Burkholderai</i>	<i>B. cepacia</i>	1 (0.4)
	<i>C. diversus</i>	3 (1.1)
<i>Citrobacter</i>	<i>C. freundii</i>	1 (0.4)
<i>Escherichia</i>	<i>E. coli</i>	49 (18.1)
<i>Enterobacter</i>	<i>E. cloacae complex</i>	6 (2.2)
	<i>K. pneumonia</i>	12 (4.4)
<i>Klebsiella</i>	<i>K. oxytoca</i>	2 (0.7)
<i>Morganella</i>	<i>M. morganii</i>	3 (1.1)
	<i>P. mirabilis</i>	7 (2.6)
<i>Proteus</i>	<i>P. vulgaris</i>	5 (1.8)
	<i>Providentia retgerii</i>	1 (0.4)
<i>Providentia</i>	<i>P. aeruginosa</i>	14 (5.2)
	<i>P. luteola</i>	1 (0.4)
<i>Pseudomonas</i>	<i>R. planticola</i>	2 (0.74)
	<i>R. ornithinolytica</i>	2 (0.7)
<i>Raoultella</i>	<i>S. enterica</i>	1 (0.4)
<i>Salmonella</i>	<i>S. marcescens</i>	1 (0.4)
<i>Serratia</i>		
Total (13)	21	117 (43.2)

The overall drug susceptibility profile of Gram-positive bacteria against the sixteen antimicrobial agents evaluated is presented in Table 4. The highest overall resistance rate to Gram-positive bacteria was recorded against erythromycin (60.4%). The resistance rates to tetracycline (51.3%) and clindamycin (52.2%) were also high. Vancomycin with a

sensitivity rate of 99.1% and linezolid with a sensitivity rate of 98.7% were the most active drugs against Gram-positive bacteria. The sensitivity rates of *S. aureus* strains and Coagulase-Negative Staphylococci were 100% to vancomycin, linezolid, and daptomycin.

Table 3. Distribution and percentage frequency of Gram- positive bacterial species (n= 154).

Genus	Species	n (%) of the total isolates
<i>Enterococcus</i>	<i>E. faecalis</i>	1 (0.4)
	<i>E. avium</i>	1 (0.4)
<i>Kocuria</i>	<i>K. intermides</i>	1 (0.4)
	<i>K. varians</i>	1 (0.4)
<i>Staphylococcus</i>	<i>S. aureus</i>	110 (40.6)
	<i>S. epidermidis</i>	15 (5.5)
	<i>S. haemolyticus</i>	9 (3.3)
<i>Coagulation negative staphylococci</i>	<i>S. hominis</i>	5 (1.8)
	<i>S. lentus</i>	3(1.1)
	<i>S. intermidus</i>	2 (0.7)
	<i>S. lugdunensis</i>	1 (0.4)
<i>Streptococcus</i>	<i>S. pyogens</i>	5 (1.8)
Total (4)	13	154 (56.8)

Table 4. Percentage in vitro antibacterial susceptibility pattern of all Gram-positive bacteria isolates (n =154).

Species	Antibiotics																
	P	CIP	CM	E	GM	LEV	LIN	MNO	MFx	FT	QDA	RA	TE	TRM	VA	TGC	DAP
<i>S. aureus</i> (110)	S	91.0	33.6	22.7	85.5	80.1	100	77.2	83.6	79.1	91.8	68.2	17.3	66.4	99.1	96.4	98.2
	I	2.7	9.1	10.9	12.7	14.5	0	6.4	6.4	9.1	0.9	9.1	27.3	10.9	0.9	2.7	1.8
	R	6.3	57.3	63.4	1.8	4.5	0	16.4	10.0	11.8	6.3	31.8	55.5	27.7	0	0.9	0
<i>S. epidermidis</i> (15)	S	86.7	20.0	33.3	93.3	86.7	100	80	86.7	73.3	80	60	46.7	53.3	100	86.7	100
	I	6.7	26.7	13.3	0	6.7	0	6.7	6.7	20.0	6.7	26.7	6.7	20.0	0	6.7	0
	R	6.7	53.3	53.3	6.7	6.7	0	13.3	6.7	6.7	13.3	13.3	46.7	26.7	0	6.7	0
<i>S. haemolyticus</i> (9)	S	77.8	33.3	55.6	88.9	66.7	100	77.8	77.7	77.8	78.8	44.4	33.3	44.4	100	88.9	100
	I	11.1	11.1	11.1	0	22.2	0	0	0	11.1	11.1	33.3	11.1	22.2	0	11.1	0
	R	11.1	55.6	33.3	11.1	11.1	0	22.2	22.2	11.1	11.1	22.2	55.6	33.3	0	0	0
<i>S. hominis</i> (5)	S	40.0	40.0	40.0	100	60	100	80	80	80	80	60	40	60	100	80	100
	I	0	0	0	0	20	0	20	0	0	0	0	20	20	0	0	0
	R	60.0	60.0	60.0	0	20	0	0	20	20	20	40	40	20	0	20	0
<i>S. lentus</i> (3)	S	66.7	33.3	33.3	66.7	66.7	100	67.3	100	100	33.3	33.3	33.3	66.7	100	66.7	100
	I	33.3	0	33.3	33.3	33.3	0	33.3	0	0	33.3	33.3	33.3	0	0	33.3	0
	R	0	66.7	33.3	0	0	0	0	0	0	33.3	33.3	33.3	33.3	0	0	0
<i>S. intermidus</i> (2)	S	50	50.0	50.0	100	50	100	50	100	100	50	0	100	50	100	100	100
	I	0	50.0	0	0	50	0	50	0	0	0	50	0	0	0	0	0
	R	50	0	50.0	0	0	0	0	0	0	50	50	0	50	0	0	0
<i>S. lugdunensis</i> (1)	S	0	0	0	100	100	100	100	0	0	100	100	100	0	100	100	100
	I	0	0	0	0	0	0	0	100	100	0	0	0	100	0	0	0
	R	100	100	100	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. pyogens</i> (5)	S	80	60.0	40	60	80	100	80	40	40	80	40	20	20	100	100	100
	I	0	0	20	20	0	0	0	60	20	0	20	40	60	0	0	0
	R	20	40.0	40	20	20	0	20	0	20	20	40	40	20	0	0	0

Table 4. Cont'D.

Species	Antibiotics																
	P	CIP	CM	E	GM	LEV	LIN	MNO	MFX	FT	QDA	RA	TE	TRM	VA	TGC	DAP
<i>E. faecalis</i> (1)	S	100	0	0	0	100	100	0	0	100	100	0	100	0	100	0	100
	I	0	100	100	100	0	0	0	100	0	0	100	0	100	0	100	0
	R	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
<i>E. avium</i> (1)	S	100	0	100	0	100	100	0	0	100	0	100	100	0	100	0	100
	I	0	100	0	100	0	0	0	100	0	0	0	0	100	0	100	0
	R	0	0	0	0	0	0	100	0	0	100	0	0	0	0	0	0
<i>Kocuria intermides</i> (1)	S	100	0	0	100	100	0	0	0	100	0	0	0	100	0	0	100
	I	0	100	100	0	0	100	100	100	0	100	100	0	0	100	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	0
<i>K. varians</i> (1)	S	100	0	0	100	100	0	0	0	100	0	0	0	100	100	100	0
	I	0	100	100	0	0	100	100	100	0	100	100	100	0	0	0	100
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total 154	S	86.4	31.2	27.3	85.1	77.9	98.7	75.3	80.5	79.2	85.7	61.7	24.7	61.0	99.1	92.2	98.7
	I	4.5	13.6	13.3	11.7	14.3	1.3	8.4	10.0	10.4	4.5	24.0	24.0	15.6	0.9	5.2	1.3
	R	9.1	52.2	60.4	3.2	8.5	0	16.2	8.4	10.4	9.7	14.0	51.3	22.4	0	2.6	0

CIP= ciprofloxacin, CM= clindamycin, E= erythromycin, GM= gentamicin, LEV= Levofloxacin, LIN=Linezolid, MNO= minocycline, MFx= moxifloxacin, FT= nitrofurantoin, QDA= quinupristin/dalfopristin, RA=rifampicin, TE= tetracycline, TRM= trimethoprim/sulfamethoxazole, VA= vancomycin, TGC= Tigecycline, DAP= daptomycin, S= Sensitive, I=Intermediate, R=Resistance, P=Pattern.

Table 5. Percentage in vitro antibacterial susceptibility pattern of all Gram-negative bacteria isolates (n =117).

Species		Antibiotics																		
		P	AM	AMC	CIP	CZ	CXM	CXMA	FOX	CF	CPD	CAZ	CRO	FEP	GM	LEV	FT	TZP	TM	TE
<i>E. Coli</i> (49)	S	16.3	18.4	77.6	40.8	34.7	38.8	30.6	16.3	28.6	71.4	65.3	67.3	73.5	61.2	91.8	93.9	91.8	28.5	34.7
	I	12.2	10.2	12.2	36.7	26.5	40.8	12.2	40.8	8.2	12.2	14.3	4.1	16.3	20.4	2.0	2.0	4.1	24.5	20.4
	R	71.4	71.4	10.2	22.4	38.8	20.4	64.3	42.9	4.1	16.3	20.4	28.6	10.2	38.8	6.1	4.1	4.1	46.9	44.9
<i>K. pneumonia</i> (12)	S	0	0	75	16.7	25	33.3	41.7	25	16.7	66.7	41.7	33.3	66.7	75	58.3	91.7	83.3	16.7	33.3
	I	0	0	8.3	16.7	16.7	8.3	8.3	25	8.3	8.3	0.0	0	16.7	16.7	16.7	0	8.3	16.7	25
	R	100	100	16.7	66.7	58.3	58.3	50.0	50	75.0	25.0	58.3	66.7	16.7	8.3	25.0	8.3	8.3	67.7	41.7
<i>P. aeruginosa</i> (14)	S	0	0	85.7	21.4	14.3	7.1	7.1	14.3	7.1	64.3	21.4	21.4	85.7	64.3	57.1	85.7	85.7	0	21.4
	I	0	0	7.1	28.6	14.3	14.3	7.1	42.9	7.1	7.1	0	7.1	7.1	14.3	7.1	7.1	7.1	7.1	14.3
	R	100	100	7.1	50	71.4	78.6	85.7	42.9	85.7	28.6	78.6	71.4	7.1	21.4	35.7	7.1	7.1	92.9	64.3
<i>E.cloacae</i> complex (6)	S	0	0	66.7	16.7	33.3	33.3	16.7	33.3	33.3	66.7	50	33.3	66.7	66.7	66.7	66.7	66.7	16.7	33.3
	I	33.3	50	0	50	33.3	33.3	16.7	16.7	16.7	33.3	33.3	33.3	0	16.7	0	16.7	16.7	16.7	33.3
	R	66.7	50	33.3	33.3	33.3	33.3	66.7	50	50.0	0	16.7	33.3	33.3	16.7	33.3	16.7	16.7	66.7	33.3
<i>P. mirabilis</i> (7)	S	14.3	14.3	71.4	57.1	57.1	57.1	28.6	42.9	28.6	100	28.6	42.9	71.4	71.4	85.7	87.5	100	14.3	28.6
	I	14.3	28.6	0	28.6	14.3	42.9	14.3	14.3	14.3	0	0	0	0	14.3	14.3	0	0	0	14.3
	R	71.4	57.1	28.6	14.3	28.6	0	57.1	42.9	57.1	0	71.4	57.1	28.6	14.3	0	14.3	0	85.7	67.0
<i>P. vulgaris</i> (5)	S	0	0	60	0	0	0	60	20	20.0	80	40.0	40	100	80	20	80	80	0	40.0
	I	40	60	20	40	40	40	20	40	20	0	0	20	0	20	40	0	20	40	0
	R	60	40	20	60	60	60	20	20	60	20	60.0	40	0	0	40	20	0	60	60.0
<i>A. baumannii</i> (4)	S	0	0	25	0	0	0	0	0	0	25	25	25	25	50	0	25	50	0	0
	I	0	0	25	0	0	0	0	0	0	0	25	25	0	25	25	50	50	0	0
	R	100	100	50	100	100	100	100	100	100	75	50	50	75	25	75	25	0	100	100
<i>M. morganii</i> (3)	S	0	0	66.7	0	0	0	66.7	33.3	100	100	0	100	66.7	100	66.7	66.7	100	0	33.3
	I	0	33.3	33.3	0	0	0	33.3	33.3	0	0	100	0	0	0	0	33.3	0	0	66.7
	R	100	66.7	0	100	100	100	0	33.3	0	0	0	0	33.3	0	33.3	0	0	100	0

Table 5. Cont'D.

Species	Antibiotics																			
	P	AM	AMC	CIP	CZ	CXM	CXMA	FOX	CF	CPD	CAZ	CRO	FEP	GM	LEV	FT	TZP	TM	TE	SXT
<i>K. oxytoca</i> (2)	S	0	0	100	0	0	0	2	0	100	100	50	100	100	50	0	100	100	0	0
	I	0	50	0	0	0	0	0	0	0	0	50	0	0	50	50	0	0	0	0
	R	100	50	0	100	100	100	0	100	0	0	0	0	0	0	50	0	0	100	100
<i>A. calcooeticus</i> (2)	S	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	50	100	0	50
	I	0	0	50	0	0	0	50	0	50	50	0	50	50	100	0	50	0	0	50
	R	100	100	50	100	100	100	50	100	50	50	100	50	0	0	100	1	0	100	0
<i>S. marcescens</i> (1)	S	0	0	100	0	0	0	0	0	0	100	100	100	100	100	0	0	100	0	100
	I	0	0	0	0	0	0	100	0	100	0	0	0	0	0	0	100	0	0	0
	R	100	100	0	100	100	100	0	100	0	0	0	0	0	0	100	0	0	100	0
<i>R. ornithinolytica</i> (2)	S	0	0	50	0	0	0	0	0	50	50	50	50	50	50	0	50	50	0	50
	I	0	0	0	0	0	0	50	0	0	0	0	0	0	0	100	50	0	0	50
	R	100	100	50	100	100	100	50	100	50	50	50	50	50	50	0	0	50	100	0
<i>R. planticola</i> (2)	S	0	0	100	0	0	0	50	0	100	100	50	100	100	100	50	100	100	0	0
	I	0		0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	50
	R	100	100	0	100	100	100	50	100	0	0	50	0	0	0	0	0	0	100	50
<i>B. cepacia</i> (1)	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
<i>C. diversus</i> (3)	S	0	0	100	0	0	0	0	0	100	100	100	0	100	100	0	0	0	0	0
	I	0	0	0	0	0	0	100	100	0	0	0	100	0	0	100	100	100	0	100
	R	100	100	0	100	100	100	0	0	0	0	0	0	0	0	0	0	0	100	0
<i>C. freundii</i> (1)	S	0	0	100	0	0	0	0	0	100	100	0	100	100	100	100	0	100	0	100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0
	R	100	100	0	100	100	100	100	100	0	0	100	0	0	0	0	0	0	100	0

Table 5. Cont'D.

Species	Antibiotics																			
	P	AM	AMC	CIP	CZ	CXM	CXMA	FOX	CF	CPD	CAZ	CRO	FEP	GM	LEV	FT	TZP	TM	TE	SXT
<i>Providentia retgerii</i> (1)	S	0	0	0	0	0	0	100	0	100	100	0	0	0	100	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100	100	100	0	100
	R	100	100	100	100	100	100	0	100	0	0	100	0	100	0	0	0	0	100	0
<i>P. luteola</i> (1)	S	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	100	0	0	0
	I	0	0	100	0	0	100	0	0	100	100	100	100	100	100	100	0	100	0	100
	R	100	100	0	100	100	0	0	100	0	0	0	0	0	0	0	0	0	100	0
<i>S. enterica</i> (1)	S	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
	I	0	0	100	100	0	0	100	0	100	100	100	0	100	100	100	100	100		100
	R	100	100	0	0	100	100	0	100	0	0	0	0	0	0	0	0	0	100	0
Total (117)	S	7.7	8.5	63.2	16.2	24.8	26.5	25.6	17.1	26.5	69.2	41.0	56.4	72.6	77.8	64.1	79.5	83.8	15.4	29.9
	I	9.4	11.1	9.4	20.5	19.7	26.5	13.7	31.6	9.4	8.5	14.5	9.4	10.3	11.1	13.7	13.7	10.3	15.4	23.1
	R	82.9	80.3	27.3	63.2	55.6	47.0	60.7	51.3	64.1	22.2	44.4	34.2	17.1	11.1	22.2	6.8	5.9	69.2	47.0

AM= ampicillin, AMC= amoxicillin/clavulanic Acid, CIP= ciprofloxacin, CZ= ceftazidime, CXM= cefuroxime, CXMA= cefuroxime axetil, FOX= cefoxitin, CF= cefalotin, CPD= cefpodoxime, CAZ= ceftazidime, CRO= ceftriaxone, FEP= cefepime, GM= gentamicin, LEV= levofloxacin, FT= nitrofurantoin, TZP= piperacillin/tazobactam, TM= tobramycin, TE= tetracycline, SXT=trimethoprim/sulfamethoxazole, S= Sensitive, I=Intermediate, R=Resistance, P=Pattern

The overall antimicrobial sensitivity pattern of Gram-negative bacteria against the nineteen agents evaluated is illustrated in Table 5. The antimicrobial resistant rates of Gram-negative bacteria in their descending order were 82.9% to ampicillin, 80.3% to amoxicillin, and (69.2%) to tetracycline. Tobramycin and piperacillin/tazobactam with the overall resistance rates of 4.3% and 6.8%, respectively were better active against Gram-negative bacteria. *E. coli* had equal resistance rates of 71.4% to both ampicillin and amoxicillin while the resistance rate of the bacterium to tetracycline was 46.9%. Piperacillin/tazobactam, nitrofurantoin, and tobramycin were better active against *E. coli*. *P. aeruginosa* exhibited resistance rates of 100% to ampicillin and amoxicillin/clavulanic acid combination. The least resistance rates of the bacterium (7.1%) was observed against Piperacillin/tazobactam tobramycin, gentamycin, and ciprofloxacin. *K. pneumonia* showed a resistance rate of 100% for ampicillin and amoxicillin/clavulanic acid combination. The drug susceptibility rates of *Acinetobacter baumannii* were $\leq 50\%$ and the bacterium was 100% resistant to ten drugs out of the nineteen drugs tested.

5. Discussion

Out of 366 wound specimens collected from study subjects, bacterial colonies were observed in 271 giving a culture positivity rate of 74%. The culture positivity rate of wound infections in the present study was relatively higher than the culture positivity rates reported in similar studies [12-15]. However, it was lower than the culture positivity rate (87.3%) reported by Mohammedaman et al [16]. Studies conducted in Ethiopia reported culture positivity rates of wound infections in the range of 52% to 87.3%. Differences in the nature and site of wound infections may explain disparities in the culture positivity rates of wound infections in the present and earlier studies.

The spectrum and the relative frequencies of bacteria implicated in causing wound infections vary greatly among studies. In this study, *S. aureus* and *E. coli* were the major bacteria associated with wound infection. *S. aureus* and *E.*

coli as main isolates have been reported by Mulu et al [12], Mohammedaman et al [16], and Mulugeta et al [13]. Oladeinde et al [15] and Giacometti et al [17], however, reported *S. aureus* and *P. aeruginosa* as the commonest bacterial isolates. *E. coli* as a third major isolate following *S. aureus* and *P. aeruginosa* has been documented by Oladeinde et al [15] and Giacometti et al (17). In the present study, Coagulase-Negative Staphylococci were the third most frequent isolated organisms following *S. aureus* and *E. coli*. Our finding was consistent with the finding of Howell-Jones et al [6]. Cross contamination of wound from nasal colonization by *S. aureus* could be one possible explanation for high isolation rate of *S. aureus*. In the present study Coagulase Negative Staphylococci were the third dominant isolates accounting for 12.9% of the total isolates. This is predictable because Coagulase Negative Staphylococci are the dominant bacteria in our skin. A breach of skin, the physical barrier, may facilitate colonization of the human skin with Coagulase Negative Staphylococci.

Although the isolation of major predictable Gram-negative and Gram-positive bacterial pathogens from wound infections in the present study was consistent with most similar studies conducted locally [12, 13, 16], there were some striking differences. Isolation of 34 bacterial species belonging to 17 genera in the present study was higher than the previous studies. Furthermore, their studies apparently could not isolate bacteria such as *P. aeruginosa*, *P. luteola*, *A. baumannii*, *A. calcoaceticus*, *Burkholderia cepacia*, *Raoultella planticola*, *R. ornithinolytica*, *Kocuria intermides* and *K. varians* that made 10.2% of bacterial isolates in our study. The isolation of more bacterial species in the present study could be explained with caution by the fact that, bacteria that were not commonly isolated from wound infections may replace the ones that have been commonly isolated from wound infections under selective pressure of drugs that might have emanated from the current empirical based treatment of wound infection in Ethiopia. Isolation of *P. aeruginosa*, *P. luteola*, *A. baumannii*, *A. calcoaceticus*, *B. cepacia*, *R. planticola*, *R. ornithinolytica*, *K. intermides* and *K. varians* that were 100% resistant for six to nineteen drugs

may support our suggestion. Secondly, in recent years, non-fermenting Gram negative bacilli, in particular *P. aeruginosa* and *A. baumannii*, have been associated with opportunistic nosocomial infections, wound being one of the main sites of infection [18]. Application of automated method of identification (the VITEK 2 compact system) in this study could be another explanation for an increase in the diversity of bacterial isolates as identification of these bacteria by routine biochemical methods is unsatisfactory.

Identification of bacterial pathogens down to a species level is important because (a) different species have different antibiotic susceptibilities (b) serious bacterial infections caused by predictable “pathogens” have decreased in recent years in proportion to those caused by opportunistic bacteria that once were considered to be of low virulence (i.e. the incidence of opportunist infections is increasing) and (c) such infections cannot be traced epidemiologically or documented without identification of bacteria to a species level. In line with this, identification of all bacterial isolates to the species level including staphylococci that were grouped as Coagulase Negative staphylococci was another striking difference in the present study and earlier local [12, 13, 16] and international studies [6, 14, 15].

In this study, wound infection rate was more in male patients than female patients. Furthermore, the present study revealed that the proportion of wound infections was the highest in age groups of 45-64 years. Underlying diseases such as diabetes mellitus, obesity, and a decrease in immune system at advanced age could be possible explanation for the situation.

The overall drug resistance rates to Gram-negative bacterial isolates ranged from 4.3% for tobramycin to 82.9% for ampicillin. The resistance rates to amoxicillin and tetracycline were also high. The highest overall resistance rate to Gram-positive bacteria was observed against erythromycin (60.4%), followed by tetracycline (51.3%), and clindamycin (52.2%). This may demonstrate that old generation antimicrobial agents (ampicillin, amoxicillin, tetracycline, erythromycin, clindamycin etc.) as a single agent for empirical treatment of wound infections would not cover the majority of wounds infected by Gram-negative and gram positive bacteria in the study area. High level of drug resistance to the old generation antimicrobial agents in the present study was compatible with the results of similar studies conducted locally [13] and internationally [19, 20]. Availability of these anti-microbial agents without prescription and inappropriate dosing schedules may explain the isolation of high level of drug resistance against these drugs.

A notable observation was that the majority of Gram-negative bacterial isolates were more sensitive towards tobramycin and piperacillin/tazobactam combinations. Both antimicrobial agents were the most effective agents against *E. coli*, the most frequently isolated Gram-negative bacterium. Our finding was similar to the result described by Manikandan and Amsath [21] and Lu et al [22]. Bours et al [23] reported that 93% *E. coli* were susceptible to

nitrofurantion. Our result was in line with their finding as 91% *E. coli* were susceptible to this antimicrobial agent. Of the nine cephalosporins tested, except the extended β -lactam cephalosporins, the resistance rates of *E. coli* to the first and second generation of cephalosporins was very high. Our result was comparable to the reports of Manikandan and Amsath [21] and Lu et al [22].

K. pneumoniae, the third most commonly isolated Gram-negative bacterium was sensitive to tobramycin, Piperacillin/tazobactam combination, and fluoroquinolones (ciprofloxacin levofloxacin) as seen in other studies [22, 24]. However, eight drugs tested against the isolate failed to achieve a sensitivity rate above 35%. The two extended β -lactam cephalosporins were relatively better active against the bacterium and our result was comparable to that of Lu et al [22], Mojtahedzadeh [24], and Juyal et al [25]. A higher sensitivity rates of the bacterium against the two extended β -lactam cephalosporins (cefepime and ceftazidime) than ours was reported by Manikandan and Amsath [21]. On the other hand Mojtahedzadeh et al [24] revealed that greater than 90% of the isolates were resistant to ciprofloxacin, ceftazidime, cefepime and ceftriaxone.

Other enterobacteria isolated in the present study such as *P. mirabilis*, *P. vulgaris*, *M. morganii*, *S. marcescens*, and *Citrobacter* species were also susceptible to tobramycin and piperacillin/tazobactam combinations. However, their sensitivity towards the old generation antimicrobial agents was high as seen in another local study [13]. *E. cloacae* complex on the other hand was relatively resistant to most antimicrobial agents tested against Gram-negative bacteria particularly of the first and second generation cephalosporins. Our result in this regard was comparable to Lu et al [22].

The sensitivity rates of thirteen drugs tested against *P. aeruginosa*, the second most common isolate were below 25%. However, the sensitivity rates of the isolate against ciprofloxacin, gentamycin, tobramycin, and Piperacillin/tazobactam were above 80%. Our result was comparable to the results of earlier studies [15, 20, 21]. Among cephalosporins tested, the two extended β -lactams (cefepime and ceftazidime) were better active against the pathogen as seen in a study conducted by Lu et al [22] but contrary to the findings of Mojtahedzadeh et al [24]. Mojtahedzadeh et al [24] revealed that *P. aeruginosa* was 100% resistant to cefepime, ceftazidime, ceftriaxone and ciprofloxacin. A non-fermenting Gram negative bacillus, *B. cepacia* was 100% resistant to all antimicrobial agents tested.

Interestingly, none of the tested drugs achieved sensitivity rates above 50% for *A. baumannii* and *A. calcoaceticus*. Similar result was obtained in a study conducted by Lu et al [22], Sivaraman et al [26], Mostof et al [27], and Benachinmardi et al [28]. An inherent resistance resulting from the bacterial cell structure, together with a gradual acquisition of genetic determinants of resistance over time have been incriminated as cause of drug resistance development in the bacterium [18].

Changes in the susceptibility of Gram-positive cocci in hospital and community settings have been reported

worldwide [29]. Similar to many earlier studies [4, 30, 31], the level of drug resistance of Gram-positive cocci to erythromycin (60.4%), clindamycin (52.2%), and tetracycline (51.3%), the most commonly prescribed drugs in Ethiopia was very high. However, the overall drug sensitivity rates of Gram-positive bacterial isolates towards many antimicrobial categories such as lipopeptides (daptomycin), glycopeptides (vancomycin) oxazolidinones (linezolid), glycylicyclines (tigecycline), fluoroquinolones (ciprofloxacin, levofloxacin & moxifloxacin), aminoglycosides (gentamycin) tetracycline (minocycline) and streptogramins (quinupristin-dalfopristin) were very high. The susceptibility rate of Gram positive bacteria extends from 75.3% for minocycline to 99.1% for vancomycin. Except one isolate of *S. aureus* (intermediate), all isolates of *S. aureus* and all isolates of Coagulase-Negative staphylococci were 100% susceptible to vancomycin. Our finding was in line with the findings of earlier studies [16, 32]. Tiemersma et al [32] analyzed 50,759 *S. aureus* isolates collected from 1999-2002 in 495 hospitals in 26 countries in Europe for their drug susceptibility profile and their result revealed that none of the isolates was resistant to vancomycin. However, vancomycin resistant coagulase-positive and Coagulase-Negative Staphylococci have been isolated in many countries. Studies conducted in Nigeria by Moses et al [33], in Bangladesh by Hasan et al [34], and in Ethiopia by Ten et al [35] reported 5.3%, 4.2% and 14% vancomycin resistant *S. aureus*, respectively. Similar studies conducted in Ethiopia by Ten et al [35] and Amare et al [36] documented a 13.4% and 4.5% prevalence rate of vancomycin resistant Coagulase -Negative Staphylococci. Tigecycline and linezolid as the most active antimicrobial agents against Gram-positive pathogens including enterococci, streptococci and staphylococci has been reported [37]. The relatively low level of resistance to these drugs may be, these drugs had been in the market for a relatively short period of time as compared to drugs such as tetracycline, amoxicillin and erythromycin [38]. In conclusion high culture positivity rate of wound infection was depicted. The resistance rates of bacterial isolates to the commonly prescribed drugs were very high.

6. Conclusions

The culture positivity rate recorded in the present study was compatible higher than similar studies conducted locally. Bacteria implicated in causing wound infection reported in this study were so diverse compared to previous local and international studies. High culture positive rate of wound infection and identification of bacteria that were not reported in similar studies warrants a continuous epidemiological survey of wound infection in health institutions across the country. The antimicrobial susceptibility pattern of bacteria against the commonly prescribed drugs such as erythromycin, tetracycline, ampicillin and amoxicillin demonstrated that these drugs would not cover the majority of bacterial wound infections as a single agent for empirical treatment. As the result, other alternatives should be considered.

Strength and limitations of the study

Isolation and characterization of diverse bacterial species some of which have never been reported in previous studies, Identification of all bacteria associated with wound infection including Coagulase Negative staphylococcus to the species level, and testing the drug susceptibility of bacterial isolates against a large number of antimicrobial agents by automated methods were the strengths of this study over similar earlier studies. However, the study was not without limitations. Lack of information whether wound infections were hospital acquired or community-acquired and lack of information about the site of infection were major limitations. In addition to these, mechanisms of drug resistant development in our isolates were not studied as a result of problems associated with facilities. Instead, we have maintained all isolates in culture for further studies.

Competing of Interests

The work does not have no financial and/or non-financial competing interest. The authors declare that there is no conflict of interests regarding the publication of this paper. The study was approved by the Internal Review Board (IRB) of Arsho Advanced Medical Laboratory private limited company.

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