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Identification of Diverse Multiple Drugs Resistant Bacterial Pathogens using Microscan Panel

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Abstract

Bacterial isolates from clinical sources have increased resistance to antimicrobial agents available and routinely used in developing countries like Ethiopia. One of the control measures of antimicrobial resistance is to know the susceptibility of pathogenic bacteria from clinical specimens and treat patients accordingly. Therefore the objective of the present study is to isolate bacterial pathogens from different clinical specimens and determine their antimicrobial susceptibility patterns. Clinical samples (urine, blood, pus and discharges from different body sites were cultured and isolation of bacterial pathogens were done following standard bacteriological methods using media recommended by Cheesbrough. Identification of bacterial pathogens and antimicrobial susceptibility tests were done using Micro Scan Identification Panel method. The Panels were read by Micro Scan Auto Scan 4 reader after incubating for 18 to 24 hours at 35^{o}c aerobically. The retrospective data of microbiological culture and antimicrobial susceptibility test results were analysed**.** A total of 995 clinical specimens were cultured at Bethzatha Bacteriology Lab., Bethzatha Hospital, from May 2021 to February 2022. The most frequent specimens were, urine 87 (32%), blood 77(28%), pus and discharges from different body sites 65(24%). Out of these, 273(27%) yielded different bacterial pathogens. The most dominant gram negative bacterial isolates from urine samples 43/87(49%), 6/87(7%), 7/87(8%) were *E. coli, Acinetobacter and Klebsiella spp,* respectively in that order. *Klebsiella pneumoniae* 12/77 (16%) were most frequently isolated from blood culture followed by diverse coagulase negative *Staphylococci* (CoNS) 26/77 (33.7%) and *S. aureus* 15/77(19.5%). The most frequent 20/65(31%) isolates from pus and discharges were *S. aureus* followed by 12/65(18.5%) CoNS. In the present study, most bacterial isolates from different clinical samples were multiply resistant to ampicillin, amoxicillin, trimethoprim sulphametoaxzole, levofloxacillin and ampicillin-sulbactam. On the other hand the most frequent Gram negative bacteria, *E.coli* and *Klebsiella pneumonia* were most susceptible to amikacin and ertapenem, and the gram positive isolates, *S. aureus* were most susceptible to levofloxacillin and gentamicin; whereas coagulase negative staphylococci (CoNS) were most susceptible to gentamicin, tecioplanin, rifampicin, vancomycin, daptomycin. Therefore clinicians should be guided by antimicrobial susceptibility test. In the absence of antimicrobial susceptibility test we suggest that the above mentioned drugs to be most appropriate for empirical treatment in the study hospitals and health settings in Addis Ababa. Furthermore critical measures need to be taken to curb the increasing spread of AMR bacteria.

Keywords: KASA; Quality Assessment; Pharmaceutical Quality; Knowledge-Aided Assessment; OPQ

Abbreviations: AMC: Amoxicillin; Azk: Azithromycin; CIP: Ciprofloxacin; Gm: Gentamicin; IP: Imipenem; LE: Levvofloxacin; TEC: Tetracycline; ToR: Tobramycin; TSM: Trimethoprim sulfamethoxazole; CFT: Cefotaxime; Am: Ampicillin; Dap: Daptomycin; CD: Clindamycin; ER: Erythromycin; OC: Oxacillin; TP: Tecioplanin; RF: Refampin; FM: Fosfomycin; VAN: Vancomycin; LZL: Linezolid; SYA: Synercid; P: Penicillin.

Introduction

Antimicrobial resistant bacteria are global health problems causing 700,000 deaths annually and it has been predicted that if appropriate control and prevention measures are not taken antimicrobial resistance (AMR) would become one of the main reasons of death among hospitalized and non-hospitalized patients in developing and developed countries [1]. Proper antibiotic usage and administration are essential for treatment of bacterial infections. Thus inappropriate prescription and misuse of antibiotics could contribute to emergence of AMR pathogenic bacteria, restriction of therapeutic options, increase of hospitalization time and high treatment costs and finally a greater death rate [2].

Reports on AMR related deaths from Sub-Saharan Africa were very high in 2019 [2]. Detection of resistance and monitoring of its spread requires appropriate laboratory based surveillance. Thus to maintain the useful life of antimicrobial agents in African countries there is needs to improve access to diagnostic laboratories and improved surveillance of the emergence of resistance [2,3].

In Ethiopia, although limited, there have been studies on bacterial isolates and antimicrobial susceptibility for decades from different parts of the country [4-8]. The data on antimicrobial resistance of bacterial pathogens against commonly used drugs of humans and animals shows high resistance of bacterial pathogens [7,8]. Among the frequently isolated bacterial pathogens from clinical samples are Enterobacteriaceae such as *E.coli, Klebsiella spp, Acinetobacter*, *Pseudomonas spp.* and *Proteus spp* [9-13]. Some of these pathogenic bacteria have been found to produce extended spectrum Beta lactamase causing paediatric septicaemia, urinary tract infection and surgical site infections. Most of these bacterial pathogens are multidrug resistant and it is important to perform antimicrobial susceptibility tests to control the spread of AMR [14].

Clinical bacteriology laboratories need to be equipped to diagnose bacterial infection and perform antibiotic susceptibility testing. Correct identification of bacteria in clinical samples is a cornerstone for proper management of bacterial infections because both empirical and direct antibiotic treatments depend on identification of organisms and antimicrobial susceptibility testing [15]. Current "Golden standard" identification methods in high–resource settings, such as matrix–assisted laser desorption/ionization time –of high flight spectrometry and molecular techniques are not adapted to diagnostic laboratories in law resource settings (LRS). Most Laboratories in LRS still rely on "conventional" phenotypic identification techniques in which isolates are inoculated on different culture media containing different carbohydrates and enzyme substrates, and interpretation of test results is carried out using dichotomous decision trees [16,17]. Commercialized panels consisting of phenotypic Dried overnight MicroScan ID panels by Bekman Coulter (Brea, CA , USA) currently chosen because they have a long shelf life and can be read without automated or with semi-automated instruments. Previous studies Ombelet S, et al. [18] indicate that the accuracy of the Microscan identification panels was excellent for gram negative species and good for gram positive species. Improvements in stability, robustness, and ease of use have been identified to assure adaptation to LRS constrains.

Ethiopia has realised the problem of antimicrobial resistance and committed to join global partners in the detection and prevention of AMR. In a region where AMR data is under-represented and often lacking, the country has made some progress in the establishment of its National Antimicrobial Resistance Surveillance System to properly understand and address the prevailing problem in the country [19]. Nevertheless, due to different limiting factors it has not attained the desired goal in this area so far. Identification of bacterial pathogens and their susceptibility to Antimicrobial drugs helps physicians and policy makers to find solutions for resistance problems in their countries. Lack of general AMR surveillance programs in developing and several developed countries will lead to inappropriate use among patients and health care staff. Therefore, investigating AMR patterns are very critical and important, mainly in developing countries such as Ethiopia. On the other hand, it is necessary to analyse the patterns of antibiotic resistance for Gram positive bacteria (GPB) and Gram Negative Bacteria (GNB) to help both clinicians and policy makers in implementing empirical therapy. Therefore, it is necessary to emphasise on the importance of performing periodic culture and antimicrobial susceptibility tests and continuously monitor the spread of antimicrobial resistance (AMR). Therefore the objective of present study is to isolate bacterial pathogens from different clinical specimens and determine their antimicrobial susceptibility.

Materials and Methods

Specimen and Bacteriological Culture

Different clinical specimens (urine, blood culture, pus and discharges from different body sites) sent to Bethzatha Laboratory from different wards of Bethzatha Hospital and other Health Institutions in Addis Ababa for culture and antimicrobial susceptibility tests. These were cultured for isolation and identification of bacterial pathogens and testing antimicrobial susceptibility. The cultures were done on conventional culture media such as MacConkey, Blood agar, Nutrient Agar, Mannitol salt agar, Chocolate agar and Salmonella-Shigella agars depending on the types of the specimen by Cheesbrough [16].

Bacterial Identification and Antimicrobial Susceptibility Test

Bacterial identification and antimicrobial susceptibility were done using Microscan panel identification methods (Beckman Coulter, Brea, CA, USA). Identification of gram negative organisms is done by inoculating dried overnight Negative COMBO and for Gram Positive organism's dried overnight positive COMBO panels. Microscan dried overnight COMBO Panel is a Panel containing both dried biochemical reagents and antimicrobials. In Microscan COMBO Panels, susceptibility up to 28 different antimicrobials was tested by the minimum inhibitory concentration methods, with break point referenced to CLSI (Clinical and Laboratory Standard Institute) guide line. According to MicroScan Panel identification methods, 3-4 pure bacterial colonies were picked from 18-24 hours' aerobic culture by means of a wand designed for holding bacterial material from primary isolation media mentioned above and inoculated into 30 ml of Prompt inoculation water (Beckman Coulter, Brea, CA, USA). Then the bacterial suspension was transferred into Seed Tray Inoculator D sets (Beckman Coulter, Brea, CA, USA). The COMBO panel wells' are inoculated from bacterial suspension in the Seed Tray using a device known as Microscan Renok (Beckman Coulter, Brea, CA, USA) which delivers 115 µL of broth suspension to each well. According to manufacturer's instruction (Beckman Coulter, Brea, CA, USA) three drops of mineral oil was added to the wells containing glucose, urea, lysine, H_2S, arginine, ornithine, for gram negative COMBO panels; and for gram positive COMBO Panels only arginine and urea containing wells were overlaid with the mineral oil. Some reagents recommended by the manufacturer were added to the panels after incubating for 18 to 24 hours at 35°c aerobically.

The panels were read by MicroScan AutoScan 4 automated reader (Beckman Coulter, Brea, CA, USA). The Microscan automated reader gives the identification for each bacterial biotype with probability scores. Results with high probability scores (>85%) were considered reliable while results with probability scores (<85%) "unconfirmed". If the biochemical profile did not much any identification in Program's software database, the result generated was "very rare bio type". Compared to the manual biochemical identification conventionally used in traditional microbiology laboratory of low-resource settings, diverse bacterial biotypes were generated by the automated system.

Results

A total of 995 clinical specimens were received for culture and antimicrobial susceptibility tests by Bethzatha Laboratory from May 2021 to February 2022. The most frequent specimens were, urine 87 (32%), blood culture 77(28%), pus and discharges from different body sites 65(23%), and body fluid including cerebrospinal fluid 45(16%). Out of the total 995 clinical specimens, 273(27%) yielded different bacterial pathogens. The most frequent gram negative bacterial isolates from urine samples were *E. coli 42/87(48.3%); Klebsiella spp [K. Pneumonia(4) K.oxytoca (1)and K.ozaenae(1)] 6/87 (7%), Acinetobacter spp. [Acinetobacter baumanii (4) and A. lwofii (3] 7/87(8%)* and other diverse gram negative bacteria 14(16 %). Bacterial isolates and antimicrobial susceptibility pattern of the uropathogens is depicted on Table 1. *E. coli* isolates from urine were multiply resistant to levofloxacillin, ampicillin, trimethoprim-sulphamethoxazole, and ampicillin-sublactam, 26(61 %%), 22(51%), 21(49%), 12(28%) respectively in that order (Table 1). Most *E. coli* 35(85%), 29(67%), 26(60%) and 25(58%) were susceptible to amikacin, meropenem, gentamicin, imipenem and tetracycline, respectively. Three out of four *Klebsiella Pneumonia* isolated from urine samples were resistant to ampicillin. On the other hand all the *K. pneumoniae* isolated from urine were susceptible to tetracycline and piperacillin-tazobactam. *K. ozaenae* isolate was multiply resistant to most antimicrobial drugs including meropenem. Similarly K. oxytoca isolate was multiply resistant to amoxacillin, azetronam, ampicillin and ampicillin-sublactam. Three out of four *Acinetobacter baumannii* isolated from urine were resistant to gentamicin, tobramicine and trimethoprim-sulfamethoxazole. Similarly diverse coagulase negative staphylococci strains from urine samples and their antimicrobial susceptibility patterns are given (Tables 2-5).

ANTI MICROBIAL AGENT	E .coli			Klebsiella oxytoca		Klebsiella pneumonia		Klebsiella ozaenae		Enterobacter cloacae			Acinetobacter baumanii ,N=4	
	N=43			N=1		N=4		N=1		N=5				
	S	I	R	S	R	S	R	S	R	S	I	R	S	R
AK	35	1	1	-		-	-100	1		4(80) -20	1	- -50	2 -50	2
AMC	18(42)	1 -2.3	1 -2.3	-	1 -100	-		1(100)		-	- -100	5	-	-
AZK		-			1(100)			-100	1	-20	1	-	-	-
CIP	4(9.3)	-	1 -2.3	1(100)				-100	1	2(40) -20	1	-50	2 -50	2
GM	26(60)	1 -2.3	8 -19	1(100)		-		1(100)		2(40) -20	1	1(20)	1(25)	3(75)
IP	25(58)	-	4 -9.3	1(100)		-		1(100)		4(80)	-	1(20) -50	2 -25	1
LE	6(14)	2 -4.6	26 -61	1(100)		-				4(80)		-50	2 -50	2
MRP	29(67)	-	-			3(75)	1(25)	-100	1	3(60)			2(50)	2(50)
TEC	25(58)		1(2.)	1(100)		4(100)		-100	1	4(80)			-	-
TOR	18(42)	2 -4.6	11 -26	1(100)		3(75)		1(100)		2(40) -20	1	1(20) -25	1 -75	3
TSM	8(19)	-	21 -49		1(100)	3 (75)		1(100)		1(20)		2(40)	- -75	3
COL	19(44.2)	-	4 -9.3		1(100)	3(75)		1(100)						
A/S	9(20)	10 (23(	12 -28		1(100)	3(75)	1(25)	1(100) -100	1	1(20)		3(60) -50	2 -50	2
CFX	11(26)	-	6 -14			3(75)		1(100)		1(20)		2(40)		
AM	-	-	22 -51		1(100)		3(75)	-100	1			4(80)		
PIP				1(100)		4 -100		1(100)		4(80)		1(2)		

Table 1: Antimicrobial Susceptibility Patterns of Bacterial Isolates from Urine Samples.

Table 2 shows most frequent bacterial isolates from pus and discharges. From among the gram negative bacteria, *E.coli* 9(14%), *Klebsiella pneumoniae* 8(12%), *Acinetobacter baumannii* 7(11%) and from the gram positives cocci, *S. aureus* 20 (31%) and diverse coagulase negative staphylococci biotypes (shown on Table 4 and 5). *E. coli*

from Pus and discharges were also multiply resistant. Out of nine *E. ccoli* isolated from pus and discharges 5/9(56%) were resistant to both ampicillin and trimethoprim-sulfamethoaxzole, and 4/9(44%) to ampicillin-sublactam, gentamicin and levefloxacillin, but 9/9 (100%) of the *E.coli* isolates were susceptible to both amikacin and tetracycline. Most 5/8 (63%) *Klebsiella pneumoniae* isolated from pus were resistant to ampicillin. Simillarly 6/8 (75%) of *Klebsiella pn*eumoniae isolates were resistant to both ertapenem and gentamicin. On the other hand *K. oxytoca* isolate was resistant to all drugs except amikacin, colistin, ertapenem and meropenem whereas *K. ascorbita* was only susceptible to colistin, amikacin and piperacillin-tazobactam and resistant to all other antimicrobial drugs tested (Table 2). *S. aureus* from pus and discharge were 14/20(70%) 13/20(65%), 11(55%), 10/20(50%) were resistant to oxacillin, imipenem, tetracycline and amoxicillin respectively in that order. *S. aureus* isolated from pus and discharges were most susceptible to linezolid, synercid, fosfomycin and refampin (Table 2).

Pathogenic bacterial isolates from pus and discharges											
Antimicrobial Agents	**S. aureus**		**E. coli**		**Acinetobacter baumannii**	**Klebsiella pneumoniae**		**K.oxytoca**		**K.ascorbata**	
	N=20		**N=9**		**N=7**	**N=8**		**N=1**		**N=1**	
	S	R	S	R	S	S	R	S	R	S	R
AK			9(100)		5(71)	5(63)	2(25)	1(100)	-	1(100)	
AMC	10(50)	10(50)	6(67)	2(22)	-	3(38)	4(50)		1(100)		1
AZK	8(40)	5(25)							1(100)		1(100)
CIP	9(45)	2(10)	3(33)	3(33)	3(43)	3(38)	4(50)	-	1		1(100)
EP			7(78)	2(22)	-	2(25)	5(63)	1(100)			
GM	12(60)	8(40)	6(67)	4(44)	3(43)	2(25)	5(63)	-	1(100)		1(100)
IP	7(35)	13(65)	7(78)	2(22)	-	5(63)	2(25)				
LE	12(60)	8(40)	4(45)	4(44)	4(57)	4(50)	3(38)	1(100)			1(100)
MRP	2(10)	18()	8(89)	1(11)	6(86)	4(50)	3(38)		1(100)		1(100)
PIP			6(67)	3(33)		4(50)	3(38)	1(100)		1(100)	
TC	9(45)	11(55)	9(100)	-	2(29)	6(75)	1(13)	-	1(100)		1(100)
TOR	10(50	10(50)	5(56)	3(33)	3(43)	3(38)	4(50)	-	1(100)		1(100)
TSM	9(45)	2(10)	3(33)	5(56)	-	2(25)	5(63)	-	1(100)		1(100)
COL			3(33)	3(33)	1(14)	6(75)	1(13)	1(100)		1(100)	
A/S			3(33)	4(44)	1(14)	1(13)	6(75)		1(100)		1(100)
CFT									1(100)		1(100)
AM			4(44)	5(56)		-	7(88)		1(100)		1(100)
CZ			3(33)	1(11)		1(13)	3(38)		1(100)		
DAP	9(45)										
CD	7(35)	1(5)									
ER	8(40)	2(10)									
OC	6(30)	14(70)									
TP	12(60)	8(40)									
RF	17(85)	3(15)									
FM	18(90)	2(10)									
VAN	12(60)	2(10)									
LZL	19(95)	1(5)									
SYA	18(90)	2(10)									

Table 2: Antimicrobial Susceptibility Patterns of Bacterial Isolates from Pus and Discharges.

Most frequent isolates from blood culture and their susceptibility patterns to antimicrobials are depicted onTable3. Twenty six (76.5%) different coagulase negative *Staphylococci biotypes* (Table 4) followed by *S. aureus* 15/34 (44%) were isolated from blood culture. *Klebsiella Pneumoni*ae was most frequent 12/34 (35%) from among gram negative bacteria followed by *E. coli*, *Acinetobacter baumannii*, *Yersinia enterocolitica* each 2/34 (5.9%) and *Serratia marcescens* 1/34(2.9%) (Table3). Multiple resistances to antimicrobials tested were observed in *Klebsiella pneumonia* isolated from blood culture. *Klebsiella pneumonia* were resistant 10/12(83%), 8/12(67%), 7/12(58%), to ampicillin, trimethoprim-sulfamethoaxzole and ampicillin-sublactam respectively in that order. Most 10/12(83%) *K. pneumonia* was susceptible to colistin. All *E. coli* isolated from blood culture were resistant to ampicillin and trimethoprim sulfamethoxazole. Half (50%) of *Acinetobacter baumannii* from blood culture were multiply resistant to most antimicrobials tested, but 2/2(100%) were susceptible to ciprofloxacillin, gentamicin, trimethoprim-sulphamethoxazole and ampicillin-sublactam (Table 3).

Antimicrobial Agents	S. aureus		E. coli		Acinetobacter baumannii	Klebsiella pneumoniae		Yersinia pseudo tuberculosis		Serratia marcescens	
	N=15(25%)		N=2(3%)		N=2(3%)	N=12(20%)		N=2(3%)		N=1(2%)	
	S	R	S	R	S	S	R	S	R	S	R
AK	7(47)		2		1(50%)	9(75)	3	2(100)		1(100)	
AMC	5(33)	10(50)	1(50%)	1 (50%)	-	4(33)	8(67)	2(100)			
AZK	8(53)	5(25)				6(50)	3(25)	2(100)		1(100)	
CIP	3(20)	2(10)	1(50%)	1(50%)	2(100)	8(67)	2(17)	2(100)		1(100)	
CTX					1(50%)			2(100)		1(100)	
CFN								1(50)	1(50)	1(100)	
EP			2(100)			9(75)	3(25)	2(100)		1(100)	
GM	8 (53)		2(100)		2(100)	5(41)	7(58)	2(100)		1(100)	
IP	4(27)	2(10)	2(100)		1(50%)	9(75)	3(25)	2(100)		1(100)	
LE	10(67)	1(5)	1(50%)	1(50%)	2(100)	9(75)	3(25)	2(100)		1(100)	
MRP	2(13)		2(100)			8(67)	4(33)	2(100)		1(100)	
PIP			1(50%)	1(50%)		9(75)	3(25)	2(100)		1(100)	
TC	9(60)		1(50%)	1(50%)		8(67)	4(33)	2(100)		1(100)	
TOR		1(5)	1(50%)	1(50%)	1(50%)	6(50)	5(41)	2(100)		1(100)	
TSM	9(45)	2(10)	-	2(100)	2(100)	4(33)	8(67)	2(100)		1(100)	
COL			1(50%)	1(50%)		10(83)	2(17)		2 -100	1(100)	
NFT								2(100)		1(100)	
A/S			1(50%)	1(50%)	2(100)	5(41)	7(58)	2(100)		1(100)	
CFT	3(20)		1(50%)			3(25)	3(25)	2(100)		1(100)	
CXM			1(50%)			3(25)	5(41)	2(100)		1(100)	
AM		5(25)	-	2(100)		2(17)	10(83)	2(100)		1(100)	
CZ			1(50%)	1(50)					1(50)		
DAP	6(40)		--								
CD	5(33)	1(5)	-								
ER	6(40)	2(10)	-								

OC	4(27)	7(35)	-								
TP	12(80)		-								
RF	9(60)	3(15)	-								
FM	5(33)		-		1(50%)						
VAN	12(80)	2(10)	-								
LZL	5(33)		-								
SYA	9(60)	1(5)	-								
P		5(25)	-								

Table 3: Antimicrobial Susceptibility Patterns of Pathogenic Bacteria from Blood Culture.

Isolates	Specims			
	Urine	Blood	Pus	Total
S. auricularis	5(38.4)	4(30.7)	4(30.7)	13(100)
S. intermedius	1(33.3)	1(33.3)	1(33.3)	3(100)
S. xylosus	-	1(50)	1(50)	2(100)
S.hycus	-	1(50)	1(50)	2(100)
S. haemolyticus	2(50)	1(25)	1(25)	4(100)
S.cohnni	1(33.3)	1(33.3)	1(33.3)	3(100)
S .epidermidis	4(40)	3(30)	3(30)	10(100)
S.canis	1(100)	-	-	1(100)
S.scium	4(100)	-	-	4(100)
total	18(42.9)	12(28.6)	12(28.6)	42(100)

Table 4: Biotypes of Coagulase Negative Staphylococci (Cons) Isolated from Different Clinical Samples.

Among the Most frequent coagulase negative staphylococci, *S. epidermis* isolates were multiply resistant to amoxicillin, ampicillin, oxacillin, penicillin and imipenem. Similarly, *S. scuri* was multiply resistant to the same antibiotic drugs tested. On the other hand half of *S. auricularis* isolates were resistant to ampicillin and fosfomycin but susceptible to most other antimicrobials tested (Table 5).

Anti microbial Agents	S. auricularis		S intermedius		S. xylosus		S. hyicus		S. haemolyticus		S. cohnii		S. Epidermidis	
	N=13		N=3		N=2		N=2		N=4		N=3		N=10	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
AMC	4(30.7)	9(69)	-	-	0	2 -100			1(25)	3(75)	1 -33	2 -67	3 -30	7 -70
AZK	3(23)	10(76.9)	2(67)	1(33)	0	2 -100	2(100)	0	2(50)	2(50)	1 -33	2 -67	3 -30	7 -70
CIP	9(69)	4(30.7)	2(67)	1(33)	2(100)	0	2(100)	0	1(25)	3(75)	1(33)	2(67)	6 -60	4 -40
GM	13(100)	0	2(67)	1(33)	2(100)	0			3(75)	1(25)	1 -33	2(67)	8 -80	2 -20

IP	4(30.70)	9(69)	-		0	2 -100			2(50)	2(50)	1 -33	2(67)	3 -30	7 -70
LE	9(69)	4(30.7)	2(67)	1(33)	2(100)	0	2(100)	0	2(50)	2(50)	1 -33	2(67)	7 -70	3 -30
TC	8(61.5)	5(38)					2(100)	0	1(25)	2(50)	1 -33	2(67)	2 -20	8 -80
TSM	13(100)	-					2(100)	0	1(25)	3(75)	2 -67		4 -40	6 -60
AM	2(15.4)	11(84.6)	-	-	0	2 -100	-	-	1(25)	2(50)			3 -30	7 -70
DAP	12(92)	1(7.7)	1	2(67)	1(50)	1(50)					1 -33	2(67)	6 -60	4 -40
CD	8(61.5)	5(38)	2(67)	1(33)					2(50)	1(25)	1 -33	2(67)	4 -40	6 -60
ER	8(61.5)	5(38)	2(67)	1(3)	0	2 -100			2(50)	2(50)	1 -33	2(67)	4 -40	6(60)
OC	7(53.8)	6(46.2)	-				2(100)	0			1 -33	2(67)	3 -30	7 -70
TP	13(00)	-	3(100)	0			2(100)	0	1(25)	3(75)	1 -33	2(67)	5 -50	5 -50
RF	12(92)	1(7.7)	3(100)	0			2(100)	0	2(50)	2(50)	1 -33	2(67)	7 -70	3 -30
FM	8(61.5)	5(38)	3(100)	0	2(100)	0	2(100)	0	3(75)	1(25)	1 -33	22(67)	5 -50	-50
VAN	13(100)	-	1(33)	2			1(50)	1(50)	2(50)	2	1 -33	2(67)	6	4 -40
LZL	11(84.6)	2(15.4)	3(100)	0			2(100)	0	2(50)	2(50)			5 -50	5 -50
SYA	7(53.8)	6(46.2)	2(67)	1(33)			2(100)	0	2(50)	2	1 -33	2(67)	5 -50	5 -50
P	3(23)	10(76.9)	-	-	1(50)	1(50)					1 -33	2(67)	5 -50	5 -50

Table 5: Antimicrobial Susceptibility Patterns of Cons Staphylococci from Different Clinical Samples.

Table 6 below shows cumulative antibiogram susceptibility of bacterial isolates from all clinical samples. In the present study *E. coli* isolates were most 51/54 (94%), 39/54 (72%), 38/54 (70%) susceptible to amikacin, ertapenem, meropenem and gentamycin respectively. *Klebsiella* isolates were most susceptible to amikacin 24(86%) and ertapenem 17(60%). On the other hand *Acinetobacter species* 17(60%) and 15(54%) were susceptible to amikacin and ciprofloxacin respectively. From the gram positive isolates *S. aureus* 27(47%) and 26(46%) were susceptible to levofloxacillin and gentamicin respectively. Similarly Coagulase negative staphylococci (other staphylococci) were most susceptible to vancomycin, daptomycin and gentamycin 47(64%), 45(62%) 44(60%) respectively (Table 6).

Antimicrobial agents= 20	E. coli	Klebsiella spp.	Acinetobacter spp.	S. aureus	Other staphylococci spp.
Total	54(100)	28(100)	28(100)	39(100)	89(100)
AK	51(94)	24(86)	17 (60)	9(23)	2(2.2)
EP	39(72)	17(60)	-	-	-
MRP	3 9(72)	12 (43)	13 (46)	-	-
GM	38(70)	13(46)	13 (46)	26 (67)	44(49.4)
AMC	31(57)	12 (43)	1	14 (36)	14(16)
TOB	30 (56)	14 (50)	13 (46)	1	-
CFX	24(44)	6 (21)	-	-	-
AZK	19 (35)	3 (11)	-	14 (36)	8(9)
A/S	17(31)	7 (21.8)	9 (32)	2	-
TSM	17 (31)	11 (39.4)	8 (29)	16 (41)	38(43)
LE	14(26)	16 (57)	12 (43)	27 (69)	35(39)
CFT	12 (22)	4 (14)	5(17)	10 (26)	15(17)
AM	12 (22)	3 (11)	-	1(3)	5(6)
CTX	11 (20)	6 (21)	3 (11)	-	-
CIP	9 (17)	8 (29)	15 (54)	14 (36)	32(36)
CD				12 (31)	21(24)
OC				15(38)	15(17)
VAN				16 (41)	47(53)
DO				9 (23)	3(3.4)
DAP				12 (31)	45(51)

Table 6: Cumulative Antibiogram of susceptible bacterial isolates from all types clinical specimens.

Discussion

In the present study E. coli is a dominant isolate from urine samples. A previous study from Addis Ababa [8] focused on isolation and antimicrobial resistance patterns of uropathogens reported that *E . coli* was the leading gram negative bacteria in urinary tract infections. It was also reported by many workers from Ethiopia and elsewhere Tesfa T, et al. [20-24] that E. coli was frequently isolated from UTI infections. However the rate of isolation of *E. coli* 42/87(48%) from urine samples in the present study is greater than 9/38 (24%) reported Elale AK, et al. [21], 25 % reported by Tesfa T, et al. [20], 42% isolation rate reported by Abayineh Girima and Aemiro A, et al. [22] and 47.*35*% reported by Yitayeh L, et al. [23] from Gamby Teaching General Hospital, *Bahir Dar,* Northwest Ethiopia. Although their sample size was larger than the sample size in the present study, Kibret M, et al. [14] found that E. coli (63.6%) was dominant isolate followed by *Klesiella spp* (8.5%) from urinary tract infections. Although the rate of isolation of *E.coli* in the present study differed from previous studies [20-23] the dominance of *E. coli* as a pathogen in urinary tract infections fits to the previous findings. The variation in the rate of isolation may be due to the sample size, isolation and identification methods etc. The frequency of *Klebsiellla spp.* from urine samples in the present study is also comparable to other reports from elsewhere in Ethiopia [9,10]. In the previous studies, isolation of S*. aureus* from urine samples was reported as most frequent from among the gram positive cocci. In contrary to these reports coagulase negative staphylococci (CoNS) were isolated most frequently18 (21%) in the present study. This may be attributed to method in the present study (MicroScan AutoScan Panel) which identifies to the level of biotype that is not very common in conventional laboratories.

On the other hand, in the present study S. aureus was most frequent followed by E. coli from pus and discharge samples. The present finding is comparable to the finding by Seni, et al. [3] from surgical patients in Uganda except that E. coli was more frequent than S. aureus in that study. The bacterial profile from blood cultures in the present study is comparable to that documented by Dagnew M, et al. [10] from Gonder University Hospital. In that study Coagulase

negative staphylococci was dominant followed by S. aureus and Klebsiella Spp. Which is similar to the present rate of isolation except that the biotypes of Klebsiella and coagulase negative staphylococci (CoNS) were not specified in that study.

Acinetobacter baumanni were isolated at comparable rate to *Klebsiella pneumoniae* from urine samples and from pus and discharges. *Acinetobacter baumanii* were more frequently isolated compared to *Acinetobacter lwofii*. The frequency of isolation of *Acinetobacter spp* in the present study is comparable to previously reported from Ethiopia Eyasu T, et al. [26]. *Acinetobacter baumannii* has been commonly considered as opportunistic bacterial pathogen primarily associated with hospital-acquired infections. Recently, however, the emergence of *Acinetobacter baumannii* as a pathogen has been noted both from Ethiopia and other countries [27-30]. Howard, et al. [28] associated increase in *Acinetobacter baumannii* incidence; largely with infected combat troops returning from conflict zones in Iraq, coupled with a dramatic increase in the incidence of multidrug-resistant (MDR) strains, have significantly raised the profile of this emerging opportunistic pathogen.

In the present study Coagulase negative staphylococci (CoNS) biotypes were most frequent from among the gram positive bacteria. *S. aureus* isolates were only 39/273(14 %) from all clinical samples in the present study. On the other hand *S. aureus* was the most frequently isolated in many other studies both in Ethiopia and elsewhere [9-12]. Most of the Coagulase negative *Staphylococcus (CoNS) species* were commonly reported worldwide as opportunistic pathogens [31]. Therefore the coagulase negative-staphylococci in the present study could be hospital acquired and may be causative agents of infections in patients who are immunosuppressed. Many reports [31,32] from elsewhere indicated that coagulase negative staphylococci have become problematic by being multidrug resistant in nosocomial infections. Evaluating major bacterial pathogens and their antimicrobial resistance patterns Azim, et al. [1] from children's Hospital in Teheran found that Coagulase negative staphylococci (CoNS) were most frequent from different clinical samples which is comparable to the present findings except that their sample size was larger and a retrospective data of three years period. Michalik M, et al. [31] reviewed works on CoNS and documented evidence that CoNS are responsible for a variety of infections that differ in localization, manifestation or course of infection. However, these bacteria are opportunistic pathogens that are present in the skin and mucous membranes of healthy individuals and become true pathogens mostly for predisposed patients, i.e. immunocompromised individuals, patients with catheters, prosthetic implants, dialysis, and oncological diseases, and neonate.

Antimicrobial Resistance Profile

The present study showed that most isolates of *E.coli* from urine samples were multiply resistant to ampicillin, amoxicillin, trimethoprim-sulphametoaxzole, levofloxacillin and ampicillin-sulbactam. Some strains of *E.coli* which were predominant isolates from urine have shown resistance from14 up to 18 different antimicrobial drugs tested in the present study but mostly susceptible to amikacin and gentamicin. A Similar pattern has been documented elsewhere from Ethiopia and other parts of the world [11,23]. Previous workers studied susceptibility of uropathogens from Hiwot Fana Hospital Eastern Ethiopia observed that *Staphylococcus spp.* and *E. coli* were the most frequent isolates and these were resistant to more than one drugs.

Similarly in the present study, *S.aureus* isolated from pus was resistant to multiple antimicrobials, oxcillin, amoxicillin, and tetracycline. Reports from Gonder Referral Hospital North Western Ethiopia Dyno S, et al. [32] documented a similar pattern of resistance of *S. aureus* to the same antimicrobial drugs. On the other hand, in the present study *S. aureus* isolates were susceptible to linezolid, synercid, gentamicin and vancomyicin which agrees with the previous reports Mohammed A, et al. [33] except that linezolid and synercid were not included in their susceptibility tests. In the present study, diverse coagulase negative (CoNS) staphylococci were isolated from pus and discharge samples. Among these *S. auricularis* and *S. epidermidis* were dominant and were resistant to amoxicillin, ampicillin, oxacillin and penicillin. Most 4/4(100%) *S. auricularis* and 2/3 (67%) *S. epidermidis* were susceptible to both daptomycin and vancomycin. Although the results in the present study fits with previous reports Dyno S, et al. [32,33], the sample sizes in the previous studies were larger and mostly disc diffusion method was employed to test antimicrobial susceptibility whereas minimum inhibitory concentration (MIC) method was used in the present study. The most frequent gram positive bacterial isolates from blood culture were coagulase negative staphylococci (CoNS) 26/43 (60%) followed by *S.aureus* 15/43(34%). The present result agrees with reports from Mekele Hospital North Ethiopia and from Addis Ababa Regional Laboratory and from Jimma Hospital South west Ethiopia . The CoNS were multiply resistant to more than two antimicrobial drugs. They were resistant to amoxicillin, imipenem, and to ampicillin. The multidrug resistance of gram positive bacterial from blood culture was also reported by various researchers from Ethiopia and elsewhere. Similarly *E.coli* from blood culture showed resistance up to seven different antimicrobials. Although there is variation in sample size, method of testing susceptibility and facilities, the bacterial isolates and antimicrobial resistance patterns of isolates from blood culture in the present study are similar to those reported by previous workers [34-39].

Cumulative antibiogram is a periodic profile of antimicrobial susceptibilities of various organisms isolated from patients within an institution or can be developed to track patterns of resistance in broader geographic areas using data from multiple institutions. Antibiogram is commonly utilized to monitor recent antimicrobial susceptibility patterns in order to guide empirical antimicrobial therapy selection Truong WR, et al. [40]. It is an essential resource for institutions to track changes in antimicrobial resistance and to guide empirical antimicrobial therapy [36].

Cumulative antimicrobial susceptibility of major bacterial isolates from all clinical samples is given in the present study. *E. coli* isolates were most susceptible 51/54(94%) to amikacin, followed by ertapenem, meropenem 39/54(72%) and gentamicin 38/53(70%). Similarly *Klebsiella isolates* were most susceptible to amikacin and ertapenem whereas Acinetobacter *species* were most susceptible to ciprofloxacin and amikacin. *S. aureus* from among the gram positive isolates 27/39(69%) and 26/39(67%) were susceptible to levofloxacillin and gentamicin respectively. On the other hand CoNS were susceptible 47(52%), 45(50%), 44(49%) respectively to vancomycin, daptomycin and gentamycin

Limitations

In the present Study the sample sizes were relatively small, and demographic data are not included, however the results indicated that there is a wide spread of multiple antimicrobial resistant bacterial strains in the studied hospitals and other health institutions.

Conclusion and Recommendation

Antimicrobial resistance (AMR) poses a major threat to human health around the world. In the present study most frequently isolated bacterial pathogens from urine, blood cultures, pus and discharge samples were *E.coli, Klebsiella pneumonia* and *Acinetobacter baumanii* from among gram negative bacteria and coagulase negative staphylococci (CoNS) followed by *S. aureus* from among gram positive bacteria. These bacterial isolates from different clinical samples were multiply resistant to routinely used antibiotics. Thus it can be concluded that infections with bacteria resistant to multiple antimicrobials are major problem in the studied setting. On the other hand, the most frequent Gram negative bacteria, *E.coli* and *Klebsiella pneumonia* are most susceptible to amikacin and ertapenem, but Acinetobacter baumanii isolates were most susceptible to ciprofloxacin and amikacin and gram positive isolates, *S. aureus* were most susceptible to levofloxacillin and gentamicin. Similarly, coagulase negative staphylococci (CoNS) were most susceptible to vancomycin, daptomycin and gentamicin. Therefore Clinicians should practice rational choice of antibiotics and treatment should be guided by antimicrobial susceptibility test. In the absence of antimicrobial susceptibility test, we suggest that the above mentioned drugs are the most appropriate antibiotics for empirical treatment in the study hospitals and health institutions. Furthermore critical measures need to be taken to curb the increasing spread of AMR bacteria.

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