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Original Article

Identification and Antimicrobial Susceptibility Test of Bacterial Pathogens Using Microscan Panel Method, Bethzatha Hospital, Ethiopia

Zeleke W Tenssay* and Ashebir Gebre

Bethezatha Hospital, Addis Ababa, Ethiopia

***Corresponding author**

Zeleke W Tenssay, Bethezatha Hospital, Addis Ababa, Ethiopia, Email: woldetenssayzeleke@gmail.com

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Keywords

- Clinical specimens; Microscan Panel; Bacterial isolates; antimicrobial agents; antimicrobial susceptibility; multidrug resistance

Abstract

Introduction: 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.

Materials and methods: [Different clinical specimens (urine, blood, pus and discharges from different sites) from various wards of Bethezatha Hospital and other Health Institutions were cultured for isolation and identification of bacterial pathogens and antimicrobial susceptibility test. Identification and antimicrobial susceptibility tests were done using Micro Scan identification Panel methods. The panels were read by Micro Scan Auto Scan 4 reader after incubating for 14 to 18 hours at 35°c.

Results and discussion: A total of 995 clinical specimens were cultured in Microbiology Laboratory from May 2021 to February 2022. The most frequent specimens were, urine 89 (32%), blood 77(28%), pus and discharges from different body sites 65(23%). Out of these, 275(27%) yielded different bacterial pathogens. The most dominant bacterial isolates from among gram negatives bacteria included, *E. coli, Acinetobacter, and Klebsiella spp.,* 52(19%) , 32(12%) , 26 (10%) respectively; and from the gram positive bacteria, *Staphylococcus aureus* 41(15%) ,coagulase negative staphylococcus species and other gram positive cocci were isolated from 79(29%). The bacterial isolates in the present study were among the leading pathogens that are associated with antimicrobial resistance. Multidrug resistance were most frequent among the isolates. Out of the 275 isolates, 222 (80.7%) were resistant to two or more antimicrobial agents tested; and of these 161(59%) were resistant to five or more antimicrobials.

Conclusion: Although the sample size in the present study was relatively small, the results indicated that there is a wide spread of antimicrobial resistant bacterial strains in the studied hospitals and other health institutions. Therefore, critical measures need to be taken to curb the increasing spread of AMR strains in the studied areas if we are to control infections caused by AMR bacteria.

INTRODUCTION

Across the globe, the emergence of antimicrobial resistance (AMR) is threatening the effective and successful treatment of infectious diseases. In Ethiopia, although limited there have been studies on bacterial isolates and antimicrobial susceptibility for decades from different parts of the country [1-3]. The records on antimicrobial resistance reflect that bacterial isolates from clinical specimens have increased multidrug resistance to commonly available antimicrobial agents prescribed in the country [2-4]. Some of the reasons that contribute for the increasing resistance of bacterial isolates from clinical specimens include the improper utilization of antimicrobial agents, the use of fake and counterfeit medicines, poor prescribing habits and non-compliance to prescribed treatments. Ethiopia has realised the problem 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 [5,6]. Nevertheless, due to different limiting factors it has not attained the desired goal in this area so far. One of the control measures of antimicrobial resistance is to know the susceptibility of pathogenic bacteria from clinical specimens and treat patients accordingly. Therefore, it is necessary to emphasise on the importance of performing antimicrobial susceptibility tests and continuous monitoring of drugs to control spread of antimicrobial resistance (AMR).

Bethzatha Hospital has an Advanced Laboratory that receives different clinical specimens from its wards and other health institutions from around Addis Ababa for diagnostic purposes. Microbiology Laboratory is one of the divisions of the diagnostic Laboratory and has been performing culture and antimicrobial susceptibility tests. The present attempt is to summarise the retrospective data from May 2021 to February 2022 and make a communication to the relevant bodies.

Objective

To summarize bacterial isolates and antimicrobial susceptibility of the isolates from clinical specimens and communicate current trends of antimicrobial resistance (AMR).

MATERIALS AND METHODS

Different clinical specimens (urine, blood, pus and discharges from different sites) received from various wards of Bethezatha Hospital and other Health Institutions were cultured for isolation and identification of bacterial pathogens and testing antimicrobial susceptibility. The culture 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. 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 organisms dried overnight positive COMBO. Microscan dried overnight COMBO Panel is Panel containing both dried biochemical reagents and antimicrobials. In Microscan COMBO Panels, susceptibility to 28 different antimicrobials was tested by the minimum inhibitory concentration methods, with break points reference to CLSI (Clinical and Laboratory Standard Institute) guideline. In MicroScan Panel identification methods, 3-4 pure bacterial colonies were picked by means of 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). As per manufacturer's instruction some slow growing *Streptococcus species* were inoculated using the turbidity standard method.in which a 0.5 Mcfurland solution was prepared in 3ml of inoculum water of which 100 µL was pipetted into 25 ml of inoculation water; 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 panel; and for gram positive COMBO Panel only arginine and urea containing wells were overlaid with the mineral oil.

Some reagents recommended by the manufacturer were added to the panels after incubation for 14 to 18 hours at 35°c aerobically. The panels were read by MicroScan AutoScan 4 automated reader (Beckman Coulter, Brea, CA, USA).

RESULTS AND DISCUSSION

A total of 995 clinical specimens were received by the bacteriology division, Central Bethzatha Advanced Laboratory from May 2021 to February 2022. The most frequent specimens were, urine 89 (32%), blood 77(28%), pus and discharges from different body sites 65(23%), and body fluid including cerebrospinal fluid 45(16%). Out of these, 275(27%) yielded different bacterial pathogens. The Microscan automated reader gives the identification for each bacterial biotypes 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 this automated system. However consideration of the current spread of nosocomial infections and opportunistic agents with referencing to literature and personal experience in the area were used to report the data.

The most dominant bacterial isolates from gram negatives included, *E. coli, Acinetobacter, and Klebsiella spp.,* 52(19%), 32(12%), 26 (10%) respectively; and from the gram positive *Staphylococcus aureus* 41(15%) and coagulase negative staphylococcus species and other gram positive cocci were isolated from 79(29%). The frequency of isolation of *E. coli* from different clinical specimens was comparable to other studies from Ethiopia [2,3]. The reoccurring of Klebsiellla spp. in the present study is also comparable to other reports from elsewhere in Ethiopia [2-4]. *Acinetobacter species* were the third most common isolates from all clinical specimens and *Acinetobacter baumanii* were the commonest from the group (not shown on the Tables). The frequent isolation of *Acinetobacter spp*. in the present study was not recorded in other reports elsewhere from Ethiopia [2,3], however the increasing incidence of *Acinetobacter spp*. in nosocomial infection has been reported both from Ethiopia and other countries [7-10]. *Acinetobacter baumannii* is an opportunistic bacterial pathogen primarily associated with hospital-acquired infections [9, 10]. Howard *et al.* [9] 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 the most frequent 79(29%) isolates of the gram positive bacteria were coagulase-negative species of *Staphylococci*; only 41(15%) was coagulase positive (*S. aureus* or related species). In the present study, coagulase-negative Staphylococci were the predominant isolate from among the gram positive bacteria. Most of these gram positive *Staphylococcus species* are commonly reported worldwide as opportunistic pathogens [10,11]. On the other hand *S. aureus* was the most frequent isolate in many other studies both in Ethiopia and elsewhere [2-4,12,13]. Most of these coagulase negative-staphylococci could be opportunistic pathogens and hence may be causative agents of some patients who may be immunosuppressed [12-14]. So with regard to the coagulase negative staphylococci, it may be up to the clinician to evaluate the microbiological report in relation to the patient status and the hospital management.

The bacterial isolates in the present study were considered as leading pathogens associated with antimicrobial resistance globally [15]. Multidrug resistance were most frequent in the present study. Out of the 275 isolates 222 (80.7%) were resistant to two or more antimicrobial agents tested (Table 2). Of these 161(59%) were resistant to five or more antimicrobials (Table 2). For instance, 35(85%) *E. coli* strains isolated from urine were resistance to two or more antimicrobial agents tested. Similarly 6/7 (85%) of the *Klebsiella* strains isolated from urine were found to be resistant to two or more antimicrobial agents. Previous works on antimicrobial susceptibility of bacterial isolates from both clinical and environmental samples in Ethiopia showed that

Table 1: Bacterial Isolates from Different Clinical Sample.

Types Of Sample	Number Of Samples	%	DOMINANT BACTERIAL PATHOGENS											
			GRAM NEGATIVE								GRAM POSITIVE			
			E. coli		Klebsiella spp		Acinetobacter		Other Gram Negative Bacteria		S. aureus / similar spp. (coagulase positive)		Other Staphylococci	
			NUMBER	% FROM TOTAL	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%	NUMBER	%
urine	89	32.4	41	15	7	2.5	7	2.5	13	4.7	18	6.5	2	0.7
blood	77	28	2	0.7	13	4.7	4	1.5	6	2.2	15	5.5	43	15.6
Pus	65	23.6	7	2.5	2	0.7	8	2.9	10	3.6	19	6.9	12	4.4
Body fluid	19	6.9	2	0.7	2	0.7	8	2.9	1	0.4	1	0.4	7	2.5
CSF	13	4.7	-		2	0.7	1	0.4	6	2.2	1	0.4	3	1.1
Swabs from skin and other sites	7	2.5	-	-	1	0.4	-		1	0.4	2	0.7	3	1.1
Sputum	5	1.8	-		1	0.4					1	0.4	3	1.1
TOTAL	275													

Table 2: Multidrug Resistance Patterns.

RESISTANCE TO TWO ANIMICROBIALS	RESISTANCE TO THREE ANTIMICROBIALS	RESISTANCETO FOUR ANTIMICROBIALS
A/S,AM,_	A/S,CP,TSM	A/S,AM, AZ,TSM
A/S,TSM	AM,AZ, COL,	AK, AMC, A/S, AZT
AK,AZ,	AM,AZ,TSM	AM, CZ, CFM,TSM
AM,COL	AM,CD,LE,	AM, CIP,LE, TOB
AZ, FM	AM,CP,COL,	AM, CIP,LE, TSM
AZ,COL,	AMC, IP, LE,	AM,,CIP,LE,TSM
AZ,OC	AMC,AM,CFX	AM,AZ,FM,FSA
CD, LZL	AMC,AM,CFX	AMC,AZ,CL, OC
CD,FM	AZ,ER,FM	AMC,AZ,CM,DO
CFX, FM	CIP,LE,TSM	AMC,CL,,GM, LE,
CZ,COL,	CIP,LE,TSM	AMC. AZ, NF, OC,
FM, RF		AZ, CD,ER, TET
FM,MFN		AZ, COL, EP, MRP
LZL,SYA,		AZ,CD,ER,TET
OC,AMC		AZ,CZ,COL,EP,
TET,LZ		CIP, COL, LE, TSM
		CZ, CIP, LE, TSM
		LE,LZL,MFN,TET

Table 3: Antibiogram.

	DOMINANT ISOLATES	TOTAL	AK	EP	MRP	GM	AMC	TOB	CFX	AZK	A/S	TSM	LE	CFT	AM	CTX	CIP	CD	OC	VAN	DO	DAP
GRAM NEGATIVE	E. coli	53	51 (96)	39 (72)	3 9 (72)	38 (71.7)	31 (58.5)	30 (56.6)	24 (45.3)	19 (35.8)	17 (32)	17 (32)	14 (26.4)	12 (22.6)	12 (22.6)	11 (20.8)	9 (17)					
	Klebsiella spp.	32	24 (75)	17 (53)	12 (37.5)	13 (40.60)	12 (37.5)	14 (43.8)	6 (18.8)	3 (9.4)	7 (21.8)	11 (34.4)	16 (50)	4 (12.5)	3 (9.4)	6 (18.8)	8 (25)					
	Acinetobacter Spp.	26	17 (65)	-	13 (50)	13 (50)	1	13 (50)	-	-	9 (34.6)	8 (30.8)	12 (46)	5 (19)	-	3 (11.5)	15 (57.7)					
GRAM POSITIVE	S. aureus	41	9 (22)	-	-	26 (63)	14 (34)	1	-	14 (34)	2	16 (39)	27 (65)	10 (24)	1		14 (34)	12 (29)	15 (36.5)	16 (39)	9 (22)	12 (29)
	Other staphylococci	75	2(2.7)	-	-	44(58.6)	14(18.7)	-	-	8(10.7)	-	38(50.7)	35(46.7)	15(20)	5(6.6)	-	32(42.7)	21(28)	15(20)	47(62)	3(4)	45(60)

multidrug resistance to commonly available antimicrobial agents is widely distributed in Ethiopia [13,14,16-18]. Antimicrobial resistance (AMR) poses a major threat to human health around the world [15-17]. Naghavi [15] have estimated the effect of AMR on incidence, deaths, hospital length of stay, and health-care costs for specific pathogen–drug combinations in selected locations. That study presented the most comprehensive estimates of AMR burden to date.

Antibiogram is an essential resource for institutions to track changes in antimicrobial resistance and to guide empirical antimicrobial therapy. So with this consideration antibiogram of the major bacterial isolates in the present study is depicted (Table 3). *E. coli* was most susceptible (51/53) to Amikacin, followed by both Erthropenem, Meropenem (39/53) and Gentamycin (38/53). The least effective antimicrobial agents against *E. coli* strains in the present study were Ampicillin (22.6%), Cephotaxime (20. 8%) and Ciprofloxacin (17%) in that order. Similar decreases were observed in susceptibility of *Klebsiella species to* cephotaxime (18.8%), Azithromycin and Ampicillin (9.4%). On the other hand no strains of *Acinetobacter* were found to be susceptible to Ampicillin. Similarly, *Klebsiella* isolates were most susceptible to Amikacin and Erthropenem but more strains (16/32) of *Klebsiella spp.* were susceptible to Levofloxacillin than to Gentamicin(13/32). On the other hand more, 15/26 (58%) of the *Acinetobacter species* were susceptible to Ciprofloxacin than to Meropenem and Gentamicin (13/26). From the gram positive isolates *S. aureus* 27(65%) and 26(63%) were susceptible to Levofloxacillin and Gentamicin respectively. Similarly, only two out 41 *S. aureus* isolates were susceptible to Ampicillin sub-lactam and only one strain was susceptible to both Ampicillin and Tobramycin. None of the *S. aureus* isolates were found to be susceptible to Erthapenam, Meropenem and Cefuroxime.

CONCLUSION

In conclusion although the sample size in the present study was relatively small, the results indicated that there is a wide spread of antimicrobial resistant bacterial strains in the studied hospitals and other health institutions. Therefore, critical measures need to be taken to curb the increasing spread of AMR strains in the studied areas if we are to control infections caused by AMR bacteria.

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