

Bacterial Profiles and Antimicrobial Susceptibility Pattern of Isolates from Inanimate Hospital Environments at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia

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Shemse Sebre^{1,2}
Woldaregay Erku Abegaz¹
Aminu Seman^{1,2}
Tewachew Awoke³
Zelalem Desalegn¹
Wude Mihret²
Adane Mihret^{1,2}
Tamrat Abebe¹

¹Department of Microbiology, Immunology, and Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; ²Armauer Hansen Research Institute, Addis Ababa, Ethiopia; ³Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia

Introduction: Microbial contamination of the hospital environment plays an important role in the spread of healthcare-associated infections (HCAIs). This study was conducted to determine bacterial contamination, bacterial profiles, and antimicrobial susceptibility pattern of bacterial isolates from environmental surfaces and medical equipment.

Methods: A cross-sectional study was conducted at Tikur Anbessa Specialized Hospital (TASH) from June to September 2018. A total of 164 inanimate surfaces located at intensive care units (ICUs) and operation theaters (OTs) were swabbed. All isolates were identified by using routine bacterial culture, Gram staining, and a panel of biochemical tests. For each identified bacteria, antibiogram profiles were determined by the Kirby–Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Results: Out of the 164 swabbed samples, 141 (86%) were positive for bacterial growth. The predominant bacteria identified from OTs and ICUs were *Staphylococci aureus* (23% vs 11.5%), *Acinetobacter baumannii* (3.8% vs 17.5%) and coagulase-negative *Staphylococcus* (CoNS) (12.6% vs 2.7%) respectively. Linens were the most contaminated materials among items studied at the hospital (14.8%). Gram-positive bacteria (GPB) had significantly high resistance levels to penicillin (92.8%), cefoxitin (83.5%), and erythromycin (53.6%). On the other hand, Gram-negative bacteria (GNB) revealed the highest resistance levels to ampicillin (97.5%), ceftazidime (91.3%), ceftriaxone (91.3%), and aztreonam (90%). However, a low resistance level was recorded for amikacin (25%) followed by Ciprofloxacin (37.5%). Of the 63 *S. aureus* isolates, 54 (85.7%) were methicillin-resistant *S. aureus* (MRSA).

Conclusion: The inanimate surfaces and commonly touched medical equipment within OTs and ICUs are reservoirs of potentially pathogenic bacteria that could predispose critically ill patients to acquire HCAIs. The proportions of the antimicrobial resistance profile of the isolates are much higher from studied clean inanimate environments.

Keywords: multidrug-resistant, swab method, operation theaters, inanimate environments, intensive care unit, healthcare-associated infections

Correspondence: Shemse Sebre
Department of Microbiology,
Immunology, and Parasitology, School of
Medicine, College of Health Sciences,
Addis Ababa University, P. Box: 9086,
Addis Ababa, Ethiopia
Tel +251921778095
Email shemse.sebre@aau.edu.et

Introduction

The hospital environment represents a new ecological place for medically important nosocomial pathogens, antibiotic-resistant microorganisms, and reservoirs of resistance genes, which have been common, found on various surfaces within hospitals (eg medical equipment, housekeeping surfaces, workplaces and lobby (furniture)).^{1,2}

Studies investigating hospital environments reported that pathogens were ubiquitous in all hospital units but the interest was usually focused on intensive care and operation unit, especially due to the vulnerability of patients in these units.³ There is also high antibiotic usage and invasive procedure from these units.¹

Bacterial cross-contamination plays an important role in healthcare-associated infections (HCAIs) and resistant strain dissemination.^{1,4} The majority of the HCAIs are believed to be transmitted directly from patient to patient, but increasing evidence demonstrates that also the medical personnel, as well as the clinical environment (ie, surfaces and equipment), often are a source of infections.⁵ Hospital design and hygienic practices have been largely directed at controlling nosocomial pathogens and resistant strains contaminating air, hands, equipment, and surfaces.⁶ A better understanding of how bacterial cross-contamination occurs can provide the basis for the development of evidence-based preventive measures.⁴

The emergence of multi-drug resistant (MDR) strains in a hospital environment; particularly in developing countries, is an increasing problem that is an obstacle for the management of HCAIs.^{7–10} In Ethiopia, studies reported a high prevalence of HCAIs mainly due to MDR pathogens including the country's largest tertiary referral Hospitals,^{11–13} which warrants the critical need for a reassessment of the role played by the inanimate environment in the transmission of nosocomial infections.^{6,14}

Studies on the bacterial contamination of the ward of the hospital environments in Ethiopia reported high bacterial load and multidrug-resistant (MDR) strains.^{9,10,15,16} However, few data exist on the bacterial contamination of the hospital environment in the studied hospital. Therefore, this study aimed to determine bacterial contamination, detect potential pathogenic bacteria, and to determine the antimicrobial susceptibility patterns from inanimate hospital environments in the environments of Operation Theaters (OTs) and Intensive Care Units (ICUs) at Tikur Anbessa Specialized Teaching Hospital in Addis Ababa, Ethiopia.

Materials and Methods

Study Setting, Study Period, and Sampling Locations

A cross-sectional study was conducted at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia from June to September 2018. TASH is a tertiary hospital and

major referral center for other hospitals in Ethiopia. TASH has 800 beds and provides care for approximately 370,000–400,000 patients per year. The samples were collected from four intensive care units including Surgical, Pediatric, Medical, and Medical-Surgical units. A total of seven operating theaters were examined including Emergency, Neurology, Endo-Renal, Obstetrics and gynecology, Pediatrics, Cardio-Vascular, and Gastrointestinal tract (GIT) units.

Surfaces Sampling

The detection of bacteria in ICUs and OTs were performed by using the swab method from surfaces and medical devices. No prior notice was given to ward staff before the collection of environmental samples. Disinfection was always done at the start of the day by using hypochlorite solutions. In the operation theaters cleaning was also made before, between, and after any surgical procedures. All samples were collected every morning after the cleaning was completed. Moreover, samples in OTs were collected before the start of operations. Sampling sites around a bed in each ICUs and OTs were chosen based on the frequency with which the surfaces were touched. Sterile swabs were moistened in Brain Heart Infusion (BHI) and then, was used to swab (i) commonly touched medical equipment including beds, monitors, operation room-light, linens, ventilators, oxygen supply, anesthesia machine, suction buttons, and Laparoscopy (ii) workstation, including keyboards, computer mice; (iii) environments including floors, wall, and corridors; (iv) Lobby (furniture) including a chair, table, lockers, and trowels; (v) Sinks; (vi) hospital textiles including bed linen based on methods described previously.^{17–20}

Microbiology Analysis

Each swab sample was pre-enriched in sterile BHI and incubated at 37°C for 24 hours. A loop full of the turbid broth was then sub-cultured on blood agar (Oxoid, UK), Mannitol salt agar (MSA), MacConkey agar, and Chromagar TM Strep B base plates (Chromagar microbiology, France). Differential and selective characteristics for each agar medium were recorded for the initial screening of suspected potential pathogens. Furthermore, specific colony color (mauve color) on Chromagar TM Strep B was considered for Group B *Streptococci* (GBS) while yellow colony color on MSA was considered for *S. aureus*.

Gram-negative bacteria were further identified by Gram stain and standard biochemical tests like Triple

Sugar Iron Agar (TSI), urea, citrate, Sulfide Indole Motility (SIM) medium, growth in Lysine Iron Agar (LIA), Mannitol, malonate, and oxidase test. On the other hand, Gram-positive bacteria were further identified by Gram stain, optochin, bacitracin, CAMP test, and different biochemical tests such as catalase, coagulase, bile esculin, and salt tolerance test described based on the handbook of Clinical Microbiology Procedures ([S1 Tables 1 and 2](#)).²¹

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the isolates was performed using 21 antibiotics (Oxoid, UK) based on the Kirby–Bauer disk diffusion method on Mueller-Hinton agar (MHA) (Oxoid, UK) and Mueller-Hinton with blood agar (Oxoid, UK) for *Streptococci* spp and *Enterococcus* spp.²² An inoculum for each isolate was prepared by emulsifying colonies from an overnight pure culture in sterile normal saline (0.85%) in test tubes with the turbidity adjusted to 0.5 McFarland standards. The bacterial suspension was uniformly streaked on MHA plates using sterile swabs and left for 3 minutes before the introduction of the antibiotics.

For Gram-negative bacteria the following antibiotics were used (in µg/disk): ampicillin (10), amoxicillin and clavulanic acid (10/10), ceftriaxone (30), cefotaxime (30), ceftazidime (30), amikacin (30), gentamicin (10), ciprofloxacin (5), sulfamethoxazole-trimethoprim (1.25/23.75), cefoxitin (30), cefuroxime (30), cefepime (30), piperacillin-tazobactam (100/10), meropenem (10) and aztreonam (30) based on Clinical Laboratory Standards Institute (CLSI).²²

On the other hand, for Gram-positive bacteria antibiotics (in µg/disk) selected for susceptibility testing included penicillin (10 units), gentamicin (10), erythromycin (15), ciprofloxacin (5), doxycycline (30), vancomycin (30), cefoxitin (30), sulfamethoxazole-trimethoprim (1.25/23.75), clindamycin (2) and chloramphenicol (30). The plates were incubated at 35 °C for 24 h, and the diameters of the zone of inhibition were measured with Vernier caliper, and results were reported as susceptible (S) or resistant (R), according to CLSI guidelines.²² Isolates showing an intermediate level of susceptibility were classified as resistant.

Quality Assurance

To ensure the quality of the result from different assays, internal quality assurance systems were in place for all

laboratory procedures and double-checking of the result was done. All the methods to be used were validated as fit for the purpose before use in the study. Standard operating procedures (SOPs) were used for the specific purpose of all laboratory procedures. Quality control strains of *Enterococcus faecalis* ATCC[®] 29212, *S. aureus* ATCC[®] 25923, *E. coli* ATCC[®] 2592, *K. pneumoniae* ATCC[®] 1705 and *K. pneumoniae* ATCC[®] 1706 were used to confirm the result of antibiotics, media and to assess the quality of the general laboratory procedure.²²

Statistical Analysis

Data analysis was performed using the Stata version 14 software program (Stata Corporation, Lakeway Drive, College Station, Texas), and descriptive statistics (percentages or frequency) were calculated ([S1 Table 3](#)). A difference was considered statistically significant for P-value ≤ 0.05.

Ethics Approval

The study protocol was approved by the Department of Microbiology, Immunology and Parasitology Research Ethics Review Committee (DRERC), College of Health Sciences, Addis Ababa University (Ref. no. DRERC/17/18/02-G). Before sample collection, written approval was obtained from the administrative unit of Tikur Anbessa Specialized Hospital.

Results

Culture Results

During the four months of study, a total of 164 environmental swabs were collected in the studied OTs (n=99) and ICUs (n=65) of the hospital. Of these swab samples, 141 (86%) were positive for bacterial growth, from which a total of 183 bacterial isolates were identified. Multi-bacterial contamination was detected in 26.8% of the samples, mainly found on the surfaces of ventilators, beds, and linens.

Frequency of Bacterial Isolates

Out of the 183 bacterial isolates, 103 (56.3%) were Gram-positive bacteria (GPB) and the rest Gram-negative bacteria (GNB). Among the GPB *S. aureus* (34.4%), CoNS (15.3%), and *Bacillus* spp (3.3%) were the dominant isolates. Among the GNB *Acinetobacter baumannii* (21.3%), *Pseudomonas aeruginosa* (7.7%), and *E. coli* (4.9%) were the dominant isolates. Overall, *S. aureus* was the most frequently isolated bacteria (34.4%) followed by *Acinetobacter baumannii* (21.3%) and CoNS (15.3%) ([Table 1](#)).

Table 1 The Frequency of Isolated Bacteria at TASH, 2018

Isolates	N (%)
Gram negative	80(43.7)
<i>Acinetobacter baumannii</i>	39(21.3)
<i>Pseudomonas aeruginosa</i>	14(7.7)
<i>Escherichia coli</i>	9(4.9)
<i>Serratia marcescens</i>	4(2.2)
<i>Klebsiella pneumoniae</i>	6(3.3)
<i>Klebsiella oxytoca</i>	4(2.2)
Others*	4(2.2)
Gram positive	103(56.3)
<i>Staphylococci aureus</i>	63(34.4)
CoNS	28(15.3)
<i>Bacillus</i> spp	6(3.3)
<i>Streptococcus agalactiae</i>	3(1.6)
<i>Enterococcus</i> spp	3(1.6)

Notes: *Others, *Enterobacter cloacae*, *Shigella* spp, *Klebsiella rhinoscleromatis*.

Abbreviation: CoNS, coagulase-negative staphylococci.

Distribution of Bacterial Isolates Between ICUs and OTs

Most of the potential bacterial pathogens were isolated from Intensive care units (ICUs), 50.3% (92/183). Significant differences between Gram-positive and Gram-negative bacteria were observed between wards in OTs (39.9% vs 9.8%) and ICUs (16.4% vs 33.9%) respectively ($p=0.000$). The ICUs were mainly contaminated with GNB, 67.4% (62/92), of which the predominant ones being *A. baumannii* accounting for 34.8% (32/92) followed by *S. aureus* with 22.8% (21/92) isolation rate. Most of the bacteria in ICUs were isolated from Medical-Surgical unit (16.4%, 30/183). The major pathogens in this ICU were *S. aureus* from GPB and *Acinetobacter baumannii* from GNB, each with an isolation rate of (33.3%, 10/16). The Operation Theaters (OTs) were mainly contaminated by GPB, 80.2% (73/91). The major pathogens in the theatre were *S. aureus*, 46.2% (42/91), and CoNS, 25.3% (23/91). Endo-Renal theatre was mostly contaminated with *S. aureus* with a rate as high as 31.3% (5/16) (Table 2).

Distribution of Bacterial Pathogens Over Different Surfaces

The highest bacterial contaminated samples were taken from bed linens followed by environmental surfaces and beds. Linens were mostly contaminated with *Klebsiella* spp., (54.5%, 6/11), followed by *A. baumannii*, (15.4%, 6/39). Beds were mainly contaminated with *S. aureus*

(12.7%, 8/63). Sinks were mainly colonized by *S. aureus* (9.5%, 6/63), *P. aeruginosa* (7.1%, 1/14), and *A. baumannii* (5.1%, 2/39). *Klebsiella* spp is mainly contaminated ventilators (27.3%, 3/11) (Table 3).

Antibiogram Profile for Gram-Positive Isolates

The proportions of antimicrobial resistance among GPB were high for penicillin (92.8%), cefoxitin (83.5%), and erythromycin (53.6%). A low level of resistance was recorded for clindamycin (10.4%) and gentamicin (16.5%). Using cefoxitin disk as a surrogate marker, 54 (85.7%) of *S. aureus* isolates were defined as methicillin-resistant *S. aureus* (MRSA). A high resistance level was also recorded to a penicillin (93.7%). Vancomycin resistance was demonstrated by (1/3, 33.3%) of *Enterococcus* spp (Table 4).

Antibiogram Profile for Gram-Negative Isolates

Most of the GNB exhibited significantly high resistance to most of the tested antibiotics; for example, ampicillin (97.5%), ceftazidime (91.3%), ceftriaxone (91.3%) and aztreonam (90%), amoxicillin and clavulanic acid (85%), cefotaxime (83.8%), and cefoxitin (76.3%). Similarly, significant resistance level was also recorded for cefepime (75%), sulfamethoxazole-trimethoprim (71.3%), piperacillin-tazobactam (68.7%), and meropenem (56.3%). Low-level resistance was recorded for amikacin (25%), ciprofloxacin (37.5%), and gentamicin (46.3%). *A. baumannii* showed the highest resistance level to almost all tested antibiotics including penicillin, cephalosporins, and carbapenems and monobactam groups of antibiotics including ampicillin (100%), aztreonam (100%), ceftazidime (100%), amoxicillin, and clavulanic acid (100%), ceftriaxone (97.4%) and cefotaxime (92.3%). Low resistance level by *A. baumannii* was recorded to amikacin (35.9%) (Table 5).

Discussions

Numerous studies have employed molecular typing to determine the clonal relationship between inanimate surface and clinical strains, suggesting that the source of nosocomial infections was linked to the hospital environments, health care workers (HCWs) hands as well as clinical specimens from admitted patients.^{14,23,24} It has also been reported that patients admitted to rooms previously occupied by individuals infected or colonized

Table 2 Distribution of Potentially Pathogenic Bacteria Between ICUs and OTs at TASH, 2018

Bacteria	ICUs (N= 92)				OTs (N=91)						
	Surgical n (%)	Pediatric n (%)	Medical n (%)	Medical-Surgical n (%)	Emergency n (%)	Neurology n (%)	Endo-Renal n (%)	Gyn-obs n (%)	Pediatric n (%)	Cardo-Vs n (%)	GIT n (%)
<i>S. aureus</i>	4(25)	4(16.7)	3(13.6)	10(33.3)	8(66.7)	7(53.8)	5(31.3)	6(60)	4(33.3)	6(42.9)	6(42.9)
CoNS	1(6.3)	0(0)	3(13.6)	1(3.3)	4(33.3)	2(15.4)	0(0)	3(30)	6(50)	6(42.9)	2(14.3)
<i>Bacillus spp</i>	0(0)	0(0)	0(0)	0(0)	0(0)	2(15.4)	0(0)	1(10)	0(0)	0(0)	3(21.4)
<i>Enterococcus spp</i>	0(0)	1(4.2)	1(4.5)	0(0)	0(0)	0(0)	0(0)	0(0)	1(8.3)	0(0)	0(0)
GBS	0(0)	0(0)	0(0)	2(6.7)	0(0)	1(7.7)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>A. baumannii</i>	6(37.5)	6(25)	10(45.5)	10(33.3)	0(0)	0(0)	4(25)	0(0)	1(8.3)	1(7.1)	1(7.1)
<i>P. aeruginosa</i>	2(12.5)	5(20.8)	2(9.1)	0(0)	0(0)	1(7.7)	2(12.5)	0(0)	0(0)	0(0)	2(14.3)
<i>Klebsiella spp</i>	1(6.3)	4(16.7)	2(9.1)	2(6.7)	0(0)	0(0)	2(12.5)	0(0)	0(0)	0(0)	0(0)
<i>E. coli</i>	1(6.3)	4(16.7)	1(4.5)	3(10)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>S. marcescens</i>	1(6.3)	0(0)	0(0)	0(0)	0(0)	0(0)	2(12.5)	0(0)	0(0)	1(7.1)	0(0)
Others *	0(0)	0(0)	0(0)	2(6.7)	0(0)	0(0)	1(6.3)	0(0)	0(0)	0(0)	0(0)
Total, N (%)	16 (8.7)	24(13.1)	22(12)	30(16.4)	12(6.6)	13(7.1)	16(8.7)	10(5.5)	12(6.6)	14(7.7)	14(7.7)

Notes: Others* (*Shigella spp*, *E. cloacae*); *Klebsiella spp* (*K. pneumoniae*, *K. oxytoca*, and *K. rhinoscleromatis*).

Abbreviations: GIT, gastro-intestinal tract unit; Cardo-Vs, cardiovascular unit; Gyn-obs, gynaecology obstetrics; GBS, Group B Streptococcus (*Streptococcus agalactiae*); CoNS, coagulase-negative staphylococci; OTs, operation theaters; ICUs, intensive care units.

Table 3 Distribution of Bacteria Over Different Surfaces in ICU and OTs at TASH, Addis Ababa, Ethiopia, 2018

Sampling Points	Bacteria, n (%)										
	<i>S. aureus</i>	CoNS	<i>Bacillus spp</i>	<i>Enterococcus spp</i>	GBS	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>Klebsiella spp</i>	<i>E. coli</i>	<i>S. marcescens</i>	Others A
Anastasia machine	5(7.9)	2(7.1)	1(16.7)	0(0)	0(0)	1(2.6)	1(7.1)	0(0)	0(0)	0(0)	0(0)
Bed	8(12.7)	2(7.1)	0(0)	0(0)	1(33.3)	8(20.5)	1(7.1)	1(9.1)	2(22.2)	0(0)	0(0)
Environmental surface*	6(9.5)	6(21.4)	3(50)	1(33.3)	0(0)	3(7.7)	1(7.1)	1(9.1)	0(0)	1(25)	0(0)
Monitor	5(7.9)	3(10.7)	0(0)	0(0)	1(33.3)	4(10.3)	1(7.1)	0(0)	1(11.1)	0(0)	1(33.3)
Sink	6(9.5)	0(0)	0(0)	0(0)	0(0)	2(5.1)	1(7.1)	0(0)	0(0)	0(0)	1(33.3)
Suction Machine	7(11.1)	3(10.7)	0(0)	1(33.3)	0(0)	5(12.8)	3(21.4)	0(0)	1(11.1)	0(0)	0(0)
Linens	5(7.9)	4(14.3)	0(0)	0(0)	1(33.3)	6(15.4)	3(21.4)	6(54.5)	2(22.2)	0(0)	0(0)
Lobby (furniture)	5(7.9)	3(10.7)	0(0)	0(0)	0(0)	5(12.8)	0(0)	0(0)	1(11.1)	1(25)	0(0)
Ventilator	1(1.6)	1(3.6)	0(0)	0(0)	0(0)	3(7.7)	1(7.1)	3(27.3)	2(22.2)	0(0)	0(0)
Work station	6(9.5)	1(3.6)	2(33.3)	0(0)	0(0)	2(5.1)	0(0)	0(0)	0(0)	2(50)	0(0)
Others B	9(14.3)	3(10.7)	0(0)	1(33.3)	0(0)	0(0)	2(14.3)	0(0)	0(0)	0(0)	1(33.3)
Total	n=63	n=28	n=6	n=3	n=3	n=39	n=14	n=11	n=9	n=4	n=3

Notes: Others^A (*Shigella spp*, *Enterobacter cloacae*); Others^B (Laparoscopy, Operation room (OR)-Light, oxygen cylinder, Trowels); *Environmental surfaces (Doorknob, Floor, Corridor and Wall).

Abbreviations: GBS, Group B Streptococcus; CoNS, coagulase-negative Staphylococci.

Table 4 Antimicrobial Susceptibility Pattern of Gram-Positive Bacteria at TASH, 2018

Isolates	Ptn	Antimicrobial Agents N (%)									
		GEN	CIP	CHL	SXT	VAN	ERY	DA	DOX	FOX	PEN
CoNS	R	5(17.9)	10(35.7)	6(21.4)	11(39.3)	NT	16(57.1)	1(3.6)	15(53.6)	22(78.6)	26(92.9)
	S	23(82.1)	18(64.3)	22(78.6)	17(60.7)	NT	12(42.9)	27(96.4)	13(46.4)	6(21.4)	2(7.1)
<i>Enterococcus spp</i>	R	NT	1(33.3)	1(33.3)	NT	1(33.3)	2(67.7)	2(66.7)	1(33.3)	NT	2(66.7)
	S	NT	2(66.7)	2(67.7)	NT	2(66.7)	1(33.3)	1(33.3)	2(66.7)	NT	1(33.3)
GBS	R	NT	NT	2(66.7)	NT	2(66.7)	3(100)	0(0)	1(33.3)	NT	3(100)
	S	NT	NT	1(33.3)	NT	1(33.3)	0(0)	3(100)	2(66.7)	NT	0(0)
<i>S. aureus</i>	R	10(15.9)	12(19)	15(23.8)	30(47.6)	NT	31(49.2)	7(11.3)	24(38.1)	54(85.7)	59(93.7)
	S	53(84.1)	51(81)	48(76.2)	33(52.4)	NT	32(50.8)	55(88.7)	39(61.9)	9(14.3)	4(6.3)
Total	R	15(16.5)	23(24.5)	23(24)	41(45)	3(50)	52(53.6)	10(10.4)	41(42.3)	76(83.5)	90(92.8)
	S	76(83.5)	71(75.5)	73(76)	50(55)	3(50)	45(46.4)	86(89.6)	56(57.7)	15(16.5)	7(7.2)

Abbreviations: N, number of tested strains; R, resistant; S, sensitive; Ptn, pattern; FOX, cefoxitin; GEN, gentamicin; CIP, ciprofloxacin; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin; ERY, erythromycin; DA, clindamycin; DOX, doxycycline; PEN, penicillin; NT, not tested; GBS, Group B Streptococcus (*Streptococcus agalactiae*); CoNS, coagulase-negative Staphylococci.

with MDR strain are up to a three-fold higher risk of acquiring HCAs from contaminated environmental surfaces or equipment if terminal cleaning is not effective.^{24–26} The occurrence of antimicrobial resistance (AMR) is also increasing and resulting in higher morbidity and mortality associated with HCAs.^{23,27}

In the present study, out of 164 environmental samples from swabs, 141 (86%) were positive for bacterial contamination which has similarly been reported from studies like Zimbabwe (86.2%)¹⁴ and Morocco (96.3%).²⁸ In contrast to our result, studies conducted from Gaza Strip (24.7%),²⁹ Sudan (29.7%),³⁰ Uganda (44.2%),³¹ Nigeria (39.4%),³² and Bahir Dar, Northwest Ethiopia (39.6%)¹⁶ reported far lower surface contamination rates. Differences in hand hygiene, ventilation system, sterilization, and disinfection techniques could account for these discrepancies.^{1,33,34}

Higher levels of bacterial contamination observed in our study could be attributed primarily to the use of ineffective disinfectants during surface cleaning, and inadequate uses of standard precautions such as hand hygiene and contact precautions, as well as the migration of the organisms through airflow.^{14,20,35,36} This situation is prominently linked to hospitals that show an unwillingness to put funds into contamination control such as the ventilation systems, those that lack information about the level of contamination and ineffectiveness of commonly used disinfectants in their hospital, and those with inappropriate waste controls.

The results of our study showed substantial contamination of inanimate environments by varied groups of

bacteria, including both Gram-positive (56.3%) and Gram-negative (43.7%). Similar reports were documented by several authors from other studies in Ethiopia and abroad such as Gondar, Ethiopia (60.5% vs 39.5%),³⁷ Northwest, Ethiopia (81.6% vs 18.4%),¹⁶ Iran (60.7% vs 39.3%)³⁸ and Nigeria (52.2% vs 47.8%).³⁹ The dominance of GPB could be explained by the fact that these bacteria, being devoid of the lipid-dominant desiccation-prone outer membrane, have a natural ability to retain their viability on abiotic hospital environments for several days to months.^{34,38}

However, contrary to our findings studies conducted in Zimbabwe,¹⁴ Gaza Strip,²⁹ and Morocco²⁸ documented Gram-negative bacteria as the predominant environmental isolates. These variations may be due to different sampling times, the presence of already colonized and/or infected patients, the use of different sampling techniques and culture methodologies, and variation in sampling sites (eg, OTs vs ICUs).^{40–43} In fact, in agreement with the latter reasoning, more GNB (67.4%; 62/92) than Gram-positive ones were obtained from ICUs inanimate environments even our findings.

Overall, *S. aureus* was the most frequently isolated bacteria (34.4%) followed by *A. baumannii* (21.3%) and CoNS (15.3%) across the wards which is consistent with findings from different studies from Ethiopia and abroad.^{32,39,44} *S. aureus* constitute part of the normal human flora, inhabiting the skin, mucous membranes⁴⁵ and regularly shed onto the hospital environment by patients and medical personnel, whereupon they persist.¹⁴ These isolates were also indicators of inadequate clinical

Table 5 Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria at TASH, 2018

Isolates	Ptn	Antimicrobial Agent's n (%)													SXT
		AMP	AZM	CTX	CRO	CTZ	FOX	FEP	AMC	TZP	MRP	AK	GEN	CIP	
<i>Acinetobacter baumannii</i>	R	39(100)	39(100)	36(92.3)	38(97.4)	39(100)	37(94.9)	34(87.2)	39(100)	34(87.2)	29(74.4)	14(35.9)	27(69.2)	18(46.2)	31(79.5)
	S	0(0)	0(0)	3(7.7)	1(2.6)	0(0)	2(5.1)	5(12.8)	0(0)	0(0)	10(25.6)	25(64.1)	12(30.8)	21(53.8)	8(20.5)
<i>Escherichia coli</i>	R	9(100)	6(66.7)	7(77.8)	7(77.8)	7(77.8)	4(44.4)	7(77.7)	5(55.6)	5(55.6)	3(33.3)	2(22.2)	4(44.4)	4(44.4)	7(77.8)
	S	0(0)	3(33.3)	2(22.2)	2(22.2)	2(22.2)	5(55.6)	2(33.3)	4(44.4)	4(44.4)	6(66.7)	7(77.8)	5(55.6)	5(55.6)	2(22.2)
<i>Enterobacter cloacae</i>	R	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)	0(0)	1(50)	2(100)	2(100)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	2(100)	1(50)	0(0)	0(0)
<i>Klebsiella oxytoca</i>	R	4(100)	3(75)	3(75)	4(100)	3(75)	3(75)	3(75)	3(75)	3(75)	1(25)	1(25)	1(25)	2(50)	2(50)
	S	0(0)	1(25)	1(25)	0(0)	1(25)	1(25)	1(25)	1(25)	3(75)	3(75)	3(75)	3(75)	2(50)	2(50)
<i>Klebsiella pneumoniae</i>	R	5(83.3)	4(66.7)	4(66.7)	6(100)	5(83.3)	3(50)	5(83.3)	6(100)	4(66.7)	4(66.7)	3(50)	2(33.3)	2(33.3)	5(83.3)
	S	1(16.7)	2(33.3)	2(33.3)	0(0)	1(16.7)	3(50)	1(16.7)	0(0)	2(33.3)	2(33.3)	3(50)	4(66.7)	4(66.7)	1(16.7)
<i>Klebsiella rhinoscleromatis</i>	R	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>Pseudomonas aeruginosa</i>	R	13(92.9)	13(92.9)	12(85.7)	12(85.7)	11(78.6)	11(78.6)	5(35.7)	8(57.1)	6(42.9)	6(42.9)	0(0)	2(14.3)	2(14.3)	8(57.1)
	S	1(7.1)	1(7.1)	2(14.3)	2(14.3)	3(21.4)	3(21.4)	9(64.3)	6(42.9)	8(57.1)	8(57.1)	14(100)	12(85.7)	12(85.7)	6(42.9)
<i>Serratia marcescens</i>	R	4(100)	3(75)	1(25)	2(50)	4(100)	1(25)	3(75)	3(75)	2(50)	1(25)	0(0)	0(0)	0(0)	1(25)
	S	0(0)	1(25)	3(75)	2(50)	0(0)	3(75)	1(25)	1(25)	2(50)	3(75)	4(100)	4(100)	4(100)	3(75)
<i>Shigella spp</i>	R	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)
Total	R	78(97.5)	72(90)	67(83.8)	73(91.3)	73(91.3)	61(76.3)	60(75)	68(85)	55(68.7)	45(56.3)	20(25)	37(46.3)	30(37.5)	57(71.3)
	S	2(2.5)	8(10)	13(16.2)	7(8.7)	7(8.7)	19(23.7)	20(25)	12(15)	12(15)	35(43.7)	60(75)	43(53.7)	50(62.5)	23(28.7)

Abbreviations: N, number of tested strains; R, resistant; S, sensitive; %, percentage; Ptn, pattern; AMP, ampicillin; AZT, aztreonam; CTX, ceftaxime; CRO, ceftriaxone; CTZ, ceftazidime; FOX, ceftioxi; FEP, cefepime; AMC, amoxicillin and clavulanic acid; CHL, chloramphenicol; MRP, meropenem; AK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; SXT, sulfamethoxazole + trimethoprim.

surface hygiene.^{17,30,46} Moreover, these bacteria were also resistant to common disinfectant methods and hence spread easily in the environment, which enables them to colonize and infect the patients receiving health care service.^{29,38}

Among the different surfaces and inanimate objects examined, the highest bacterial contaminated samples were taken from bed linens, environmental surfaces, and beds, similar to the observations from other studies elsewhere.^{3,32,38,41} Bed linens and beds were mainly contaminated by *A. baumannii* (15.4% and 20.5%), CoNS (14.3% and 7.1%), and *S. aureus* (7.9% and 12.7%), respectively. Comparable results were obtained on beds and linens samples from studies conducted in Iran³⁸ and Nigeria.³² The sources of such contamination could be cross-contamination from a patient's flora, health care workers' hands, contaminated storage carts, or due to contamination during the washing process especially that of bed linens.^{38,40,42}

In our study, sinks were mainly colonized by *S. aureus* (9.5%, 6/63), *P. aeruginosa* (7.1%, 1/14), and *A. baumannii* (5.1%, 2/39), which is in line with several reports that hospital-associated outbreaks in critical care wards occur largely due to the opportunistic pathogen.^{14,32,47} This could be linked to the fact that the moist hospital environments, particularly sinks, are conducive for the persistence of these bacteria, which are known to have the ability to form biofilms in water, sinks, toilets, showers, and drains.^{48,49} Moreover, acquisition of multiple virulence determinants and intrinsic resistance to commonly used antibiotics and disinfectants by these pathogens may result in maintaining their viability and hence persistence under such harsh environments.^{48,50}

Bloodstream infection and ventilator-associated pneumonia especially in the ICUs are usually linked to device contamination such as central venous catheters, urinary catheters, and ventilators.⁵¹ In our study, ventilators were frequently contaminated by *Klebsiella* spp (27.3%, 3/11), which was also reported from a study conducted in Iran (54.4%, 6/11).³⁸ The Source of contamination of ventilators by *K. pneumoniae* might be from the aspiration of secretions from the oropharynx of colonized patients, where staff hands may act as the transmission vehicle.^{52,53}

In regards to the antimicrobial resistance profile of the isolates, our results showed high proportions of drug resistance, where most of the GNB were highly resistant to most of the tested antibiotics such as ampicillin (97.5%), ceftazidime (91.3%), ceftriaxone (91.3%), aztreonam (90%), amoxicillin and clavulanic acid (85%), cefotaxime (83.8%), and

cefepime (76.3%), which is in line with similar resistance rates from other studies conducted elsewhere like Gaza in Palestine,²⁹ Morocco³ and Sudan.³⁰ Increased resistance to β -lactams antibiotics is due to the selective pressure exerted by the antibiotics.⁵⁴ Because these tested antimicrobials represent the antibiotics most frequently used in practice, serious problems can be encountered while prescribing those antibiotics.³ One way of fighting such a rise of resistance should include establishing guidelines for prescribing antibiotics¹⁶ based on locally generated antimicrobial resistance data such as the findings from this study.

On the other hand, a low resistance level was recorded to non-beta-lactam antimicrobials such as amikacin (25%) and ciprofloxacin (37.5%) which has similarly been reported from Sudan for amikacin (23.5%) and ciprofloxacin (42.7%).³⁰ Still lower resistance rate was documented for these two antibiotics in Palestine for amikacin (6.1%) and ciprofloxacin (27.3%),²⁹ possibly an area where they may not routinely be prescribed for community and/or hospital-acquired infections.

Not surprisingly, GPB demonstrated elevated resistance to penicillin (92.8%), cefoxitin (83.5%), and erythromycin (53.6%). Similarly, a high resistance level was also reported from Ethiopia by a Meta-analysis study for penicillin and erythromycin with a pooled resistance level of 99.1% and 97.2%, respectively.⁵⁵ Moreover, a similar resistance level was also reported from Uganda for penicillin (93%).³¹ Of the 63 *S. aureus* isolates obtained in this study, 54 (85.7%) were MRSA, which is close to the rate reported from Zimbabwe (100%),¹⁴ although much higher than the rate from Uganda (52%).⁵⁶

Conclusion

In this study, bacterial samples were sought for and isolated only from the environmental surfaces and medical equipment; not from patients and hands of health professionals. *S. aureus*, *Acinetobacter baumannii*, and CoNS form the majority of the environmental contaminants most likely to cause HAIs. The proportions of the antimicrobial resistance profile of the isolates are much higher from studied clean inanimate environments. Our results may be indicative evidence that bacterial environmental contamination is possibly contributing to HAIs and MDR strain dissemination in the hospital environment. We recommended that special attention to infection control policies; antimicrobial resistance screening, good clinical practice, and cleaning techniques are needed to reduce the potential risk of pathogenic bacteria and resistant strain

transmission among hospital staff and patients. Furthermore, large-scale investigations are needed to assess the clonal relationship between the inanimate surface and clinical strains.

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Disclosure

The authors declare that they have no conflicts of interest for this work.

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