Original Article

Magnitude and drug resistance profile of Extended Spectrum β-Lactamase (ESBL) producing gram-negative bacteria from different inanimate objects at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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Abstract

Background: Infections caused by gram-negative bacteria are causing morbidity and mortality worldwide. The production of Extended-Spectrum β-Lactamases (ESBLs) is an important mechanism that is responsible for resistance to the third-generation cephalosporin.

Aim: The purpose of this study was to determine the magnitude and drug resistance profile of ESBL producing gram-negative bacteria isolated from various inanimate objects at Tikur Anbessa Specialized Hospital (TASH).

Methods: Laboratory based study was conducted on stored isolates from January to March 2019. The samples were taken from different inanimate objects (Intensive care unit (ICU) tables, ICU sinks, ICU IV stands, ICU beds, Incubators, ICU pediatrics trolley, oxygen regulators, Operation room (OR) tables, OR beds, OR computers, OR doors, lift buttons, x-ray chairs, and some other items) in Tikur Anbessa Specialized Hospital(TASH) and 216 isolates were used for further analysis. Biochemical tests for identification and antimicrobial susceptibility test were done by disc diffusion method. Screening of ESBLs was done using ESBL CHROME agar and confirmed with a combined disk diffusion test. The data were analyzed using SPSS software version -20 and descriptive statistical tests including frequency and percentage were calculated.

Results: In this study out of 216 gram negative bacteria, 15.3% of them were found to be ESBL producers based on the confirmatory test (combined disk method) from the various inanimate objects of TASH. *Klebsiella ozaenae, Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella rhinoscleromatis, Citrobacter spp, Escherichia coli, Serriatia spp* and *Acinetobacter spp* were ESBL producing gram-negative bacteria and found to be 100% resistant to ceftazidime and ceftriaxone.

Conclusion: It is worrisome to detect ESBL producing gram-negative bacteria from the inanimate objects of TASH, calling for systematic screening of inanimate objects for ESBL and other multidrug-resistant bacteria in the hospital. Furthermore, strengthening the infection prevention practice is vital to halt the transmission of these microorganisms. [*Ethiop. J. Health Dev.* 2023; 37(1) 000-000]

Keywords: Extended spectrum β -lactamase, Inanimate objects, Gram negative, Ethiopia

Introduction

The hospital environment and inanimate objects can be colonized by a diverse group of microbial agents. The organisms from objects of frequent contact in the hospital can be transmitted to healthcare workers and patients (1). The emergence of resistance to antimicrobial agents is a global public health problem , especially in pathogens that cause nosocomial infections which contribute to morbidity, mortality and increased healthcare costs resulting from treatment failures (2).

β-Lactams are a group of antibiotics acting on the cell wall of a bacterial cell. These include penicillins, cephalosporins, carbapenems, and monobactams. These bind to and inhibit the carboxypeptidases and transpeptidases. These are the cell wall synthesizing enzymes. As a result, there is a weakening of the cell wall structure which results in cell lysis (3), whereas Beta-lactamases are enzymes produced by the bacteria that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds. ESBLs are one group of β –lactamases which are able to hydrolyze and cause resistance against a wide range of β- lactam antibiotics, such as, third-generation cephalosporins and monobactams (4,5).

ESBL can cause resistance to the cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and monobactams (aztreonam). However, it does not hydrolyze the cephamycins (cefoxitin and cefotetan). The carbapenems (imipenem, meropenem) and their hydrolytic activity can be inhibited by β-lactamase inhibitors like clavulanic acid and tazobactam (3,4,6,7). In addition to this, ESBL producing bacteria also cause resistance to other classes of antibiotics such as aminoglycosides, cotrimoxazole, tetracycline, and fluoroquinolones (1). The common infections with ESBL-producing organisms include all the infections caused by gram-negative organisms such as urinary tract infections, wound infection, peritonitis, cholangitis, intra-abdominal abscess, pneumonia, and catheter-associated bloodstream infections. Resistance to multiple antibiotics makes these infections difficult to treat and results in poor outcomes for patients. ESBL-producing bacteria are a particular problem for patients in critical care units (8,9). Among *Enterobacteriaceae*, ESBLs are found mainly in

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Klebsiella spp and *Escherichia coli*, as well as in other genera, such as *Citrobacter, Enterobacter, Morganella, Proteus, Providencia, Salmonella, Serratia,* and *Pseudomonas (*6,10,11,12 and 13*).*

ESBL producing bacteria are spread through inadequately decontaminated hands of staff and indirectly via the environment. They are capable of prolonged survival on wet surfaces and have been found colonizing taps and sink drains in the wards (14).

Determining ESBL producing gram-negative bacteria is important to understand the epidemiology of these bacteria in the hospital environments, as well as to design and implement hospital infection prevention strategies. However, reports about ESBL producing gram-negative bacteria from inanimate objects at Tikur Anbessa Specialized Hospital (TASH) are limited. Hence, this study was carried out to assess and determine the magnitude and drug resistance profile of Extended Spectrum β-Lactamase producing gram-negative bacteria from various inanimate objects at Tikur Anbessa Specialized Hospital.

Methods

A laboratory-based study was conducted from January to March 2019, at (TASH, Addis Ababa, Ethiopia. TASH was built in 1933EC and it is the largest referral hospital in the country and currently TASH is the main teaching center for both clinical and preclinical training for various disciplines.

The current study was done on stored isolates from an ongoing Ph.D. project (Title: Burden of methicillin resistance *Staphylococcus aureus* and associated factors at TASH evidence from colonization of patients, health care workers, administrative staff and selected inanimate objects Addis Ababa, Ethiopia (where, the second author of this submission is the principal investigator of the PhD project). Further identification of the stored isolates including ESBL detection, and antibiotic susceptibility testing were conducted. The bacteria were isolated from different inanimate objects, including ICU tables, ICU sinks, ICU intravenous fluid (IV) stands, ICU beds, Incubators, ICU pediatrics trolley, oxygen regulators, OR tables, OR beds, OR computers, OR doors, lift buttons and x-ray machine. All major wards of TASH such as delivery rooms, intensive care units, operation rooms, medical, surgical, obstetrics and gynecology, pediatric and Emergency departments were included.

Culture and Identification

A total of 208 swabs samples were collected purposively from the various inanimate objects (two swabs per one inanimate objects for 8 consecutive weeks on weekly bases and 216 gram negative bacteria were isolated using MacConkey agar (Oxoid Ltd, BD) and stored at negative 20 degree centigrade in refrigerator. We also analyzed air samples from ICUs and operation rooms using settle plate methods .All these isolates were further sub-cultured on MacConkey agar (Oxoid Ltd, BD) and CHROMagar ESBL (CHROMagar, Paris, France) and incubated at 37 °C for 24–48 hours and the growth was inspected for colony morphology. Colonies were also sub cultured on nutrient agar to obtain pure colony and the bacteria were identified to the species level by using different biochemical tests.

Antibiotic Susceptibility Testing

The antimicrobial susceptibility pattern of the isolates was determined by the Kirby–Bauer disc diffusion technique on Muller Hinton agar (Oxoid Ltd, BD). The standard inoculum which was adjusted using 0.5 McFarland standards was swabbed on the plate, antibiotics discs were dispensed and incubated at 37°C for 24 hours as per 2018 CLSI guidelines (16). The susceptibility of the isolates to third-generation cephalosporins, ceftazidime, cefotaxime, ceftriaxone, each 30 µg/disc and other antibiotics such as ciprofloxacin(5 µg), piperacillin-tazobactam (100/10µg), amikacin (30 µg), gentamycin (10 µg), meropenem (30 µg), ampicillin (10 ug), chloramphenicol (30), Trimethoprim-sulfamethoxazole (1.25/23.75), cefoxitin(30ug), cefotetan(30ug), Cefepime (30ug), cefuroxime (30ug), Amoxacilliin clavulanate (20/10ug), were determined. The zone of inhibition was measured to the nearest millimeter and isolate was reported as sensitive, intermediate, or resistant according to the CLSI standard tables for each antibiotic.

Extended-Spectrum Beta-lactamase Detection

The Chromogenic agar medium (CHROMID^{TM} ESBL) was used for rapid screening of extended-spectrum beta-lactamase-producing gram negative-bacteria (ESBL). HiCrome ESBL supplement, containing antibiotics such as ceftazidime, cefotaxime, ceftriaxone, aztreonam, and fluconazole, is used to inhibit other contaminating microorganisms and non-ESBL producers. It provides results in 18-24 hours, and those colonies which produce ESBL were confirmed by using combined disk test (14).

Combined Disk Test (CDT)

A confirmatory test was performed for the detection of ESBL by the combined-disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, according to the 2018 CLSI guideline (16). In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated by using sterile cotton swab on the surface of a Mueller Hinton Agar plate. The cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart on the agar. Similarly, the ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg / 10 µg) disks were placed 20 mm apart. After incubating overnight at 37°C, a ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone was considered as ESBL producer (16).

Quality Control and Data Quality Assurance

Klebsiella pneumoniae ATCC 700603, (ESBL-positive), *Escherichia coli* ATCC 25922 (ESBL negative), were used for the quality control of the ESBL testing methods, and *Pseudomonas aeruginosa* ATCC 27853 was also used for the quality control of the Kirby-Bauer disk diffusion methods and antibiotic

disc potency. Culture Media were prepared based on the manufactures instruction then the sterility of culture media was checked by incubating 5% of the batch at 35–37 °C overnight and observing bacterial growth. Stored isolates were checked for their appropriate storage and cross-matched with their labeling on the logbook. Before entry to the statistical tool for analysis, results were recorded appropriately on the logbook, and data was cleaned and checked for completion.

Data entry and Analysis

The obtained data were entered and analyzed using SPSS software (version 20), ESBL carriage with ward type and source of swabs were descriptively analyzed mainly using frequency and percentage. Finally, the results were presented in words, graphs, and tables.

Ethical approval

Ethical approval was obtained for using stored isolates from the Department of Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University (Ref.No MLS/01/2019). We used stored isolates from an on-going PhD research project, which has Institutional and National ethical approval.

Results

Magnitude and antimicrobial susceptibility patterns of gram-negative bacterial isolates

A total of 216 gram-negative bacteria were identified from the various inanimate objects found in different wards of TASH. Among these, *Klebsiella spp*, *Acinetobacter spp* and *Citrobacter spp* were the three most dominant gram-negative bacteria with magnitude 31.9 % (69/216); 17.6 % (38/216) and 12 % (26/216) respectively (Table 1). Out of the 69 *Klebsiella spp*, 26 of them were from neonatal ICU and 16 from the medical wards and out of 38 *Acinetobacter spp*, 17 isolates were from medical wards, 9 from neonatal ICU and 8 isolates were from Adult ICU. The majority of the gram negative isolates were come from the medical wards with a magnitude of 29.6 % (64/216) followed by neonatal ICU, 25.9 % (56/216), adult ICU,15.7 % (34/216) and Delivery ward accounting 6.9% (15/216). The predominant bacteria in the medical ward were *Acinetobacter spp, Klebsiella spp, Serratia spp* and *E.coli* accounting 26.6 % (17/64), 25 % (16/64), 10.9 % (7/64), and 9.4% (6/64) respectively.

Antimicrobial susceptibility test was done for all isolated gram-negative bacteria and the highest sensitivity was recorded against amikacin (69.4%), gentamicin (63%), and ciprofloxacin (61.6%) respectively. However, the majority of bacteria showed high resistance to ceftriaxone (88.9%), cefuroxime (87.2%), and ampicillin (86.7%). *Klebsiella spp*, the predominant bacteria identified in the current study were most sensitive to ciprofloxacin (59.4%), amikacin (53.6%), piperacillin-tazobactam (50.7%), chloramphenicol (49.3%), and gentamicin (46.4%) respectively. *Acinetobacter spp* was the second most isolated bacteria and showed high resistance against ceftriaxone (100%), ceftazidime, and cefotaxime each accounting for 81.6%, on the other hand, it showed better sensitivity for other antibiotics such as amikacin (76.3%), gentamicin (68.4%), and tobramycin (63.1%) (Table 1).

Table 1:-Antimicrobial susceptibility patterns of gram- negative bacteria from hospital environment at TASH, Addis Ababa, Ethiopia, 2019.

Species	AST	List of antibiotics (N, %)																
		AMP	**AUG**	**CFP**	**CTX**	**CTR**	**CTT**	**CXT**	**CRX**	**CAZ**	**MEM**	**GEN**	**AMK**	**CIP**	**COT**	**CHL**	**PTZ**	**TOB**
Klebsiella_spp n=69	**S**	5 (7.2)	13 (8.8)	15 (21.7)	11 (15.9)	5 (7.4)	21 30.4)	24 (34.8)	6 (8.7)	8 (11.6)	26 (37.7)	32 (46.4)	37 (53.6)	41 (59.4)	17 (24.6)	34 (49.3)	35 (50.7)	26 (37.7)
	R	64 (92.7)	56 (81.2)	54 (78.3)	58 (84.1)	63 (92.6)	48 (69.6)	45 (65.2)	63 (91.3)	61 (88.4)	43 (62.3)	37 (53.6)	32 (46.3)	28 (40.6)	52 (75.4)	35 (50.7)	34 (49.3)	43 (62.3)
Entrobacter _spp n=9	**S**	2 (22.2)	5 (55.6)	6 (66.7)	5 (55.6)	3 (33.3)	5 (55.6)	4 (44.4)	2 (22.2)	4 (44.4)	5 (55.6)	8 (88.9)	8 (88.9)	7 (77.8)	6 (66.7)	6 (66.7)	7 (77.8)	8 (88.9)
	R	7 (77.8)	4 (44.4)	3 (33.3)	4 (44.4)	6 (66.7)	4 (44.4)	5 (55.6)	7 (77.8)	5 (55.6)	4 (44.4)	1 (11.1)	1 (11.1)	2 (22.2)	3 (33.3)	3 (33.3)	2 (22.2)	1 (11.1)
Proteus _spp n=19	**S**	3 (15.8)	8 (42.1)	6 (31.6)	9 (47.4)	3 (15.8)	1 (5.3)	1 (5.3)	3 (15.8)	6 (31.6)	3 (15.8)	13 (68.4)	13 (68.4)	15 (78.9)	7 (36.8)	14 (73.7)	9 47.7)	17 (89.5)
	R	16 (84.2)	11 (57.9)	13 (68.4)	10 (52.6)	16 (84.2)	18 (94.7)	18 (94.7)	16 (84.2)	13 (68.4)	16 (84.2)	6 (31.6)	6 (31.6)	4 (21.1)	12 (63.2)	5 (26.3)	10 (52.6)	2 (10.5)
Providencia_spp n=3	**S**	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.7)	1 (33.3)	1 (33.3)	0(0.0)	2 (66.7)	0(0.0)	2 (66.7)	3 (100)	1 (33.3)	0(0.0)	1 (33.3)	2 66.7)	2 (66.7)
	R	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	2 (66.7)	2 (66.7)	3 (100)	1 (33.3)	3 (100)	1 (33.3)	0(0.0)	2 (66.7)	3 (100)	2 (66.7)	1 (33.3)	1 (33.3)
Citrobacter_spp n=26	**S**	4 (15.4)	9 (34.6)	5 (19.2)	7 (26.9)	2 (7.7)	2 (7.7)	3 (11.5)	3 (11.5)	4 (15.4)	8 (30.8)	16 (61.5)	18 (69.2)	13 (50.0)	11 (42.3)	11 (42.3)	11 (42.3)	18 (69.2)
	R	22 (84.6)	17 (65.4)	21 (80.8)	19 (73.1)	24 (92.3)	24 (92.3)	23 (88.5)	23 (88.5)	22 (84.6)	18 (69.2)	10 (38.5)	8 (30.8)	13 (50.0)	15 (57.7)	15 (57.7)	15 (57.7)	8 (30.8)
E.coli n=14	**S**	3 (21.4)	6 (42.9)	9 (64.3)	5 (35.7)	3 (21.4)	8 (57.1)	9 (64.3)	5 (35.7)	4 (28.6)	13 (92.4)	12 (85.7)	13 (92.9)	9 (64.3)	7 (50.0)	9 (64.3)	10 71.4)	9 (64.3)

	R	11 (78.6)	8 (57.1)	5 (35.7)	9 (64.3)	11 (78.6)	6 (42.9)	5 (35.7)	9 (64.3)	10 (71.4)	1 (7.1)	2 (14.3)	1 (7.1)	5 (35.7)	7 (50.0)	5 (35.7)	4 (28.6)	5 (35.7)
Morganella morganii n=2	**S**	1 (50.0)	1 (50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
	R	1 (50.0)	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)
Shigella_spp n=8	**S**	4 (50.0)	4 (50.0	4 50.0)	3 (37.5)	0(0.0)	4 (50.0)	3 (37.5)	1 (12.5)	4 (50.0)	5 (62.5)	6 (75.0)	7 (87.5)	6 (75.0)	1 (12.5)	7 (87.5)	7 (87.5)	4 (50.0)
	R	4 (50.0)	4 (50.0	4 50.0)	5 (62.5)	8 (100)	4 (50.0)	5 (62.5)	7 (87.5)	4 (50.0)	3 (37.7)	2 (25.0)	1 (12.5)	2 (25.0)	7 (87.5)	1 (12.5)	1 (12.5)	4 (50.0)
Salomnella_spp n=5	**S**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (20.0)	1 (20.0)	5 (19.2)	0(0.0)	0(0.0)	3 (60.0)	3 (60.0)	3 (60.0)	1 (20.0)	0(0.0)	1 (20.0)	0 (0.0)
	R	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	4 (80.0)	4 (80)	21 (80.8)	5 (100)	5 (100)	2 (40.0)	2 (40.0)	2 (40.0)	4 (80.0)	5 (100)	4 (80.0)	5 (100)
Arizona n=3	**S**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1 (33.3)	0(0.0)	3 (100)	0(0.0)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	0(0.0)	1 (33.3)	2 (66.7)	1 (33.3)
	R	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	2 (66.7)	3 (100)	0(0.0)	3 (100)	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)	1 (33.3)	2 (66.7)
Serriatia spp n=15	**S**	0(0.0)	8 (57.1)	10 (66.7)	6 (40.0)	4 (28.6)	4 (28.6)	3 (21.4)	2 (14.3)	9 (60.0)	12 (80.0)	14 (93.3)	15 (100)	12 (80.0)	6 (40.0)	5 (35.7)	10 (66.7)	15 (100)
	R	15 (100)	7 (42.9)	3 (33.7)	9 (60.0)	10 (71.4)	10 (71.4)	11 (78.6)	12 (85.7)	6 (40.0)	3 (20.0)	1 (6.7)	0(0.0)	3 (20.0)	9 (60.0)	9 (64.3)	5 (33.3)	0(0.0)
Pseudomonas_spp n=5	**S**	NA*	NA*	2 (40.0)	NA*	NA*	NA*	NA*	NA*	1 (20.0)	1 (20.0)	2 (40.0)	2 (40.0)	2 (40.0)	NA*	NA*	2 (40.0)	2 (40.0)

	R			3 (60.0)						4 (80.0)	4 (80.0)	3 (60.0)	3 (60.0)	3 (60.0)			3 (60.0)	3 (60.0)
Acinetobacter_spp n=38	**S**	NA*	NA*	13 (34.2)	7 (18.4)	0(0.0)	NA*	NA*	NA*	7 (18.4)	19 (50.0	26 (68.4)	29 (76.3)	22 (57.9	15 (39.5)	NA*	23 (60.5)	24 (63.2)
	R			25 (65.8	31 (81.6	27 (100				31 (81.6)	19 (50.0	12 (31.6)	9 (23.7)	16 (42.1)	23 (60.5		15 (39.5)	14 (36.8)
Total	S	23 (13.4)	55 (32.0)	71 (32.9)	54 (25.6)	22 (11.1)	48 (27.9)	49 (28.5)	22 (12.8)	49 (22.7)	93 (43.0)	136 (63.0)	150 (69.4)	133 (61.6)	72 (34.1)	90 (51.1)	120 (55.6)	127 (58.8)
	R	149 (86.7)	117 (68)	145 (67.1)	157 (74.4)	176 (88.9)	124 (72.1)	123 (71.5)	150 (87.2)	167 (77.3)	123 (57.0)	80 (37.0)	66 (30.6)	83 (38.4)	139 (65.8)	86 (48.9)	96 (44.5)	89 (41.2)

Abbreviations: *AMP ampicillin, AUG amoxicillin with clavunic acid ,CFP cefpime, CTX cefotaxime, CTR ceftriaxone,CTT cefotetan, CXT cefoxitin, CRX cefuroxime, CAZ ceftazidime , MEM meropenem, mGEN gentamycin, AMK amikacine, CIP ciprofoxacin, COT sulfamethoxazole + trimethoprim (cotrimoxazole), CHL chloramphenicol, PTZ piperacillin -tazobactam, TOB tobramycin, S sensitive, , R resistance, NA*, Not applicable*

Magnitude of ESBL-producing gram-negative bacteria among different sampling sites and inanimate objects. In this study out of 216 gram negative isolates, 33 of them were found to be ESBL producers making magnitude of 15.3% based on the combined disk method (Figure 1). The predominant ESBL producing species were *Klebsiella ozaenae* accounting for 24.2% (n=8) followed by *Acinetobacter spp* 21.2% (n=7) and *Escherichia coli* 18.2 % (n=6). ESBL production was also seen in *Klebsiella oxytoca* in 12.1% (n=4**),** *Klebsiella pneumoniae* 9.1% (n=3), *Klebsiella rhinoscleromatis* 6.1% (n=2) and *Serriatia spp* 6.1% (n=2). Out of a total of 69 isolates of *Klebsiella spp*, 17(24.6%) were positive for ESBL (Table 2). In the current study among *Klebsiella spp*, ESBL production was very common among *Klebsiella ozaenae and Klebsiella oxytoca*, that is from 21 isolates, 8 (38.1%) and from 10 isolates, 4(40%) were ESBL positive for *K.ozaenae* and *K.oxtyoca* respectively. However, from *Klebsiella rhinoscleromatis* isolates, only 2 out of 18 isolates were ESBL-producers, representing only 11.1% (Table 2).

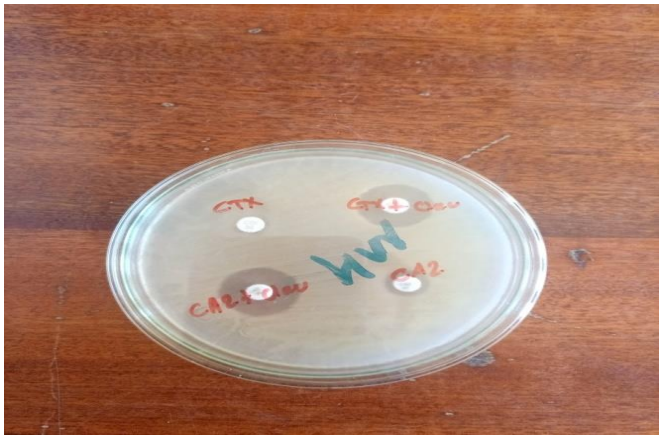

Fig. 1:- Enhancement zones of inhibition by >5mm of discs containing ceftazidime+clavulanic acid and cefotaxime+clavulanic acid, at TASH, Addis Ababa Ethiopia, 2019.

Among the inanimate objects, the highest number of ESBL producing gram-negative bacteria were found from chairs 15.2 % (5/33), the pooled sample of computers and office telephones 15.2 % (5/33), sinks 12.1 % (4/33), tables , information desks and desktop computer each accounted 9.1 % (3/33) and less than 7 % were found from monitors, incubators, and beds. However, no ESBL producing bacteria were isolated from oxygen regulators, doors, lift buttons and air samples (Fig.2).

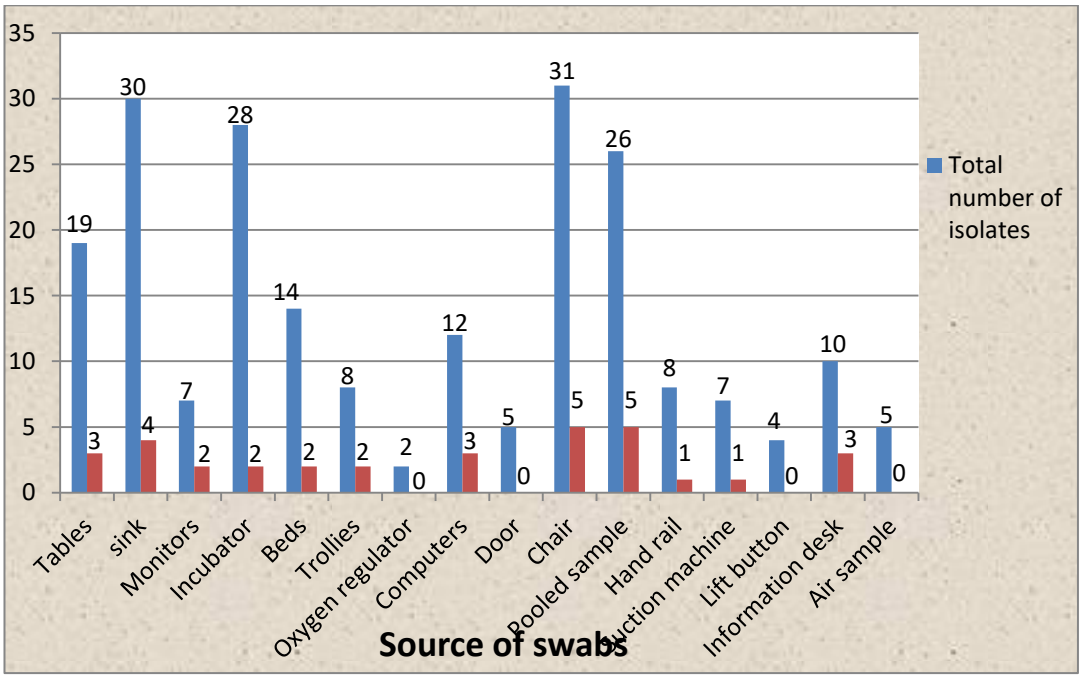

Fig.2:- ESBL -producing gram-negative bacteria with the source of the samples at TASH, Addis Ababa Ethiopia, 2019.

Out of the total 216 isolates, 15.3 % (33/216) of them were ESBL producing gram-negative bacteria. Compared to other hospital wards the highest ESBL producing bacteria were isolated from medical wards and adult ICU which accounts for 48.5 % (16/33) and 21.2 % (7/33) respectively (Fig.3).

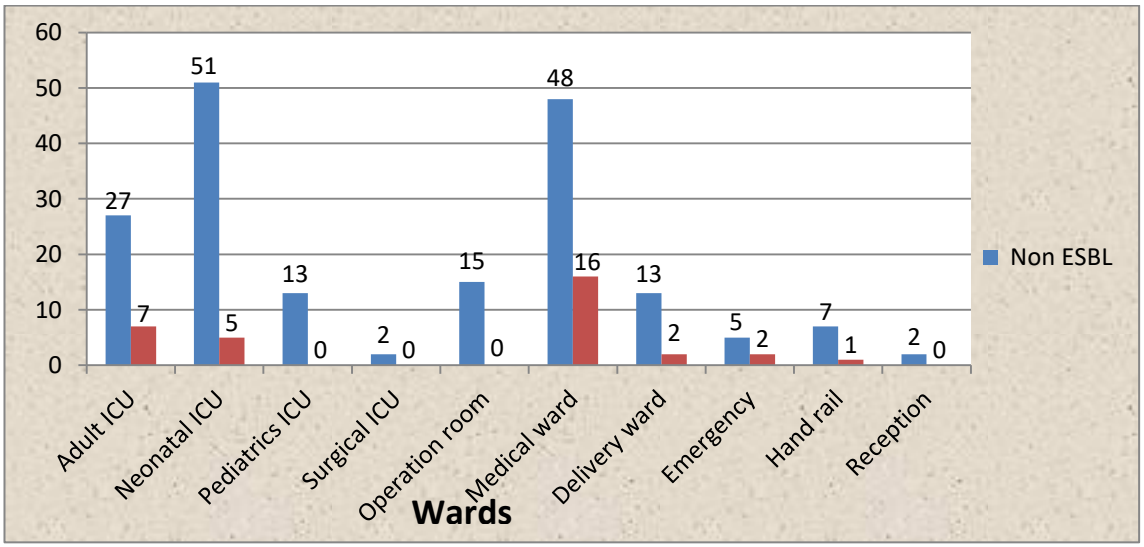

Fig. 3 ESBL- producing gram-bacteria stratified by ward specialty of TASH, Addis Ababa Ethiopia, 2019.

The medical ward was the most common site for ESBL producers and the predominant isolates in this ward were *Acinetobacter spp* followed by *E.coli*, *K.ozaenae*, and *K.oxytoca*. The majority of *K.ozaenae* was isolated from adult ICU 37.5 % (3) and medical ward 25.0 % (2). Among *Acinetobacter spp* 85.7 % *(*6/7) of them were isolated from the medical ward and the rest from neonatal ICU.

Antimicrobial susceptibility patterns of ESBL producing gram-negative bacteria

Antibiotic susceptibility test for all the 216 gram-negative bacteria was performed. In this study, all ESBL producing gram-negative bacteria were found to be 100% resistant to ceftazidime and ceftriaxone. A significant resistance rate was also observed for ampicillin (92.3%), amoxicillin-clavulanic acid (80.8%), cefuroxime (80.8%), amikacin (80.1%), and cotrimoxazole (66.6%). However, the lowest level of resistance was reported for chloramphenicol (37.0%), cefoxitin (41.5%), tobramycin (48.5%), piperacillin tazobactam (51.5%) and ciprofloxacin (57.6%) (Table 3).

Table 2: Profile of bacterial isolates which produce ESBL at TASH, Addis Ababa, Ethiopia, 2019.

ESBL status	**Bacterial isolates, No (%)**									
	Klebsiella_spp (N=69)				*Citrobacter _spp* n=26	*E.coli _spp* n=14	*Serriatia _spp* n=15	*Acineto-bacter_* spp n=38	Other gram negative bacteria	Total (%)
	K.ozaenae n=21	*K. rhinoscleromatis* n=18	*K. pneumoniae* n=20	*K. oxytoca* n=10						
Positive	8 (24.2)	2(6.1)	3(9.1)	4(12.1)	1(3.0)	6(18.2)	2(6.1)	7(21.2)	0(0.0%)	33 (15.3)
Negative	13 (7.1)	16(8.7)	17(9.3)	6(3.3)	25(13.7)	8(4.4)	13(7.1)	31(16.9)	183(84.7)	183 (84.7)

ESBL producing *Klebsiella ozaenae* had a resistance rate of 25.0% to meropenem, and 37.5% to amikacin. The most active antibiotic against *Klebsiella pneumoniae* was amikacin, with a100% susceptibility, followed by 66.7% susceptibility each for gentamicin, ciprofloxacin, and chloramphenicol. About 71.4% of *Acinetobacter spp* were resistant to cefotaxime and 100% susceptible to amikacin and meropenem (Table 3).

Table 3:- Antimicrobial susceptibility patterns of ESBL- producing gram-negative bacteria from the hospital environment at TASH, Addis Ababa, Ethiopia, 2019.

Bacterial isolates	**Pattern**	**List of antibiotics (N, %)**																
		AMP	**AUG**	**CFP**	**CTX**	**CTR**	**CTT**	**CXT**	**CRX**	**CAZ**	**MEM**	**GEN**	**AMK**	**CIP**	**COT**	**CHL**	**PTZ**	**TOB**
K.ozaenae (n=8)	**S**	1 (12.5)	1 (12.5)	2 (25.0)	1 (12.5)	0 (0.0)	3 (37.5)	5 (62.5)	1 (12.5)	0 (0.0)	6 (75.0)	2 (25.0)	5 (62.5)	4 (50.0)	2 (25.0)	5 (62.5)	3 (37.5)	2 (25.0)
	R	7 (87.5)	7 (87.5)	6 (75.5)	7 (87.5)	8 (100)	5 (62.5)	3 (37.5)	7 (87.5)	8 (100)	2 (25.0)	6 (75.0)	3 (37.5)	4 (50.0)	6 (75.0)	3 (37.5)	5 (62.5)	6 (75.0)
K.pneumoniae (n=3)	**S**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	1 (33.3)	0 (0.0)	1 (33.3	2 (66.7)	3 (100)	2 (66.7)	0 (0.0)	2 (66.7)	1 (33.3)	1 (33.3)
	R	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.0)	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)	1 (33.3)	0 (0.0)	1 (33.3)	3 (100)	1 (33.3)	2 (66.7)	2 (66.7)
K.oxytoca (n=4)	**S**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	3 (75.1)	0 (0.0)	0 (0.0)	4 (100)	1 (25.0)	2 (50.0)	2 (50.0)	0 (0.0)	2 (50.0)	1 (25.0)	0 (0.0)
	R	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	2 (50.0)	1 (25.0)	4 (100)	4 (100)	0 (0.0)	3 (75.0)	2 (50.0)	2 (50.0)	4 (100)	2 (50.0)	3 (75.0)	4 (100)
K.rhinosclero matis (n=2)	**S**	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	0 (0.0)	2 (100)	2 (100)	1 (50.0)
	R	2 (100)	2 (100)	2 (100)	1 (50.0)	2 (100)	2 (100)	1 (50.0)	1 (50.0)	2 (100)	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	2 (100)	0 (0.0)	0 (0.0)	1 (50.0)
Citrobacter_ Spp (n=1)	**S**	0 (0.0)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.0)	1 (100)	1 (100)	1 (100)
	R	1 (100)	0 (0.0)	0 (0.0)	1 (100)	1 (0.0)	1 (100)	0 (0.0)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)
Escherichia coli(n=6)	**S**	1 (16.7)	2 (33.3)	2 (33.3)	1 (16.7)	0 (0.0)	4 (66.7)	4 (66.7)	2 (33.3)	0 (0.0)	6 (100	6 (100)	6 (100)	2 (33.3)	4 (66.7)	5 (83.3)	3 (50.0)	3 (50.0)
	R	5 (83.3)	4 (66.7)	4 (66.7)	5 (83.3)	6 (100)	2 (33.3)	2 (33.3)	4 (66.7)	6 (100)	0 (0.0)	0 (0.0)	0 (0.0)	4 (66.7)	2 (33.3)	1 (16.7)	3 (50.0)	3 (50.0)
Serraitia_ Spp (n=2)	**S**	0(0.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	2 (100)	2 (100)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100)
	R	2 (100)	1 (50.0)	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)	2 (100)	2 (100)	0 (0.0)
Acinetobacter _spp	**S**	NA*	NA*	6 (85.7)	2 (28.6)	0 (0.0)	NA*	NA*	NA*	0 (0.0)	7 (100)	6 (85.7)	7 (100)	5 (71.4)	4 (57.1)	NA*	5 (71.4)	7 (100)

n=7	**R**			1 (14.3)	5 (71.4)	7 (100)				7 (100)	0 (0.0)	1 (14.3)	0 (0.0)	2 (28.6)	3 (42.9)		2 (28.6)	0 (0.0)
Total	**S**	2 (7.7)	5 (19.2)	12 (36.4)	5 (15.2)	0 (0.0)	11 (42.3)	15 (57.70	5 (19.2)	0 (0.0)	27 (81.8)	20 (60.6)	27 (81.8)	14 (42.4)	11 (33.3)	17 (63.0)	16 (48.5)	17 (51.5)
	R	24 (92.3)	21 (80.8	21 (63.6)	28 (84.8)	27 (100)	15 (57.7)	11 (41.5)	21 (80.8)	33 (100)	6 (18.2)	10 (39.4)	6 (18.2)	19 (57.6)	22 (66.6)	10 (37.0)	17 (51.5)	16 (48.5)

Abbreviations: *AMP ampicillin, AUG amoxicillin with clavunic acid ,CFP cefpime, CTX cefotaxime, CTR ceftriaxone,CTT cefotetan, CXT cefoxitin, CRX cefuroxime, CAZ ceftazidime , MEM meropenem, mGEN gentamycin, AMK amikacine, CIP ciprofoxacin, COT sulfamethoxazole + trimethoprim (cotrimoxazole), CHL chloramphenicol, PTZ piperacillin -tazobactam, TOB tobramycin, S sensitive, , R resistance, NA*, Not applicable*

ESBL producing *E. coli* showed the highest resistance to ceftazidime (100%) and ceftriaxone (100%), followed by cefotaxime (83.3%), also, it was 66.7% resistant to ciprofloxacin, cefepime, and amoxicillin with clavulanic acid. However, all isolates were 100% susceptible to meropenem, amikacin, and gentamycin. *Serriatia spp*, was highly resistant for many of the antibiotics tested and it was 100% resistant for cefotaxime, ceftriaxone, cefotetan, cefoxitin, cefuroxime, ceftazidime, chloramphenicol, and piperacillin- tazobactam. However, it was 100% susceptible to amikacin and ciprofloxacin (Table 3).

Discussion

Medical equipment and open surfaces are suitable for contamination with microorganisms and can be colonized by gram-negative bacteria which are the most common cause of hospital and community-acquired infections. The occurrence of bacterial pathogens in the hospital environment is associated with an increased incidence of nosocomial infections (15). The results of our study indicate the presence of ESBL-producing gram negative-bacteria from different inanimate objects in the hospital environment. ESBL-producing bacteria could cause serious clinical infection and treatment failure, decreased rate of clinical and microbiological responses, and be responsible for prolonged hospital stay (18).

In the current study, the most commonly isolated gram-negative bacteria were *Klebsiella spp* 69/216 (31.9%), followed by *Acinetobacter spp* 38/216 (17.6%), these findings are in agreement with other similar studies (14,19). On the other hand, studies have done in Upper Egypt (20) and Iran (21), reported *E.coli* and *Enterobacter aerogenes* as the dominant gram-negative bacteria.

In this study many of the gram-negative bacterial isolates showed high resistance to ceftriaxone (88.9%), followed by ampicillin (86.7%) and ceftazidime (77.3%). Significant levels of resistance were also recorded to amoxicillin with clavulanic acid (68.0%) and sulfamethoxazole-trimethoprim (65.8%)). This finding is supported by other studies conducted in Addis Ababa, Ethiopia where high levels of resistance to ampicillin (75.4%) and amoxicillin with clavulanic acid (64.0%) (22), were reported. Similarly, findings were reported from Gondar, Ethiopia, with resistance to ampicillin (84.6%) and sulfamethoxazole-trimethoprim (79.5%) (23). However higher resistance level was reported in Tanzania, for ampicillin (100%), amoxicillin with clavulanic acid (98.7%) and sulfamethoxazole-trimethoprim (95.2%) (24), and in Iran, with resistance to amoxicillin with clavulanic acid (91.4%) and sulfamethoxazole-trimethoprim (93.8%) (25).

In our study, of all antimicrobials agent tested amikacin (69.4%) and gentamicin (63%) were the most active antibiotics for the majority of the isolates, this is in line with the study conducted in India that reported amikacin and gentamicin were effective drugs with sensitivity level of 63.6% and 40.9% respectively (26).

The magnitude of ESBL producing gram-negative bacteria was 15.3 % (33/216) which indicates an alarming level of contamination which is similar to a previous study in Ethiopia (14.8%) (15). Our findings were lower than those studies conducted in Uganda (21.33%) (19), France (31%) (14), Upper Egypt (35.2%) (20) and Algeria (21.35%) (27), these variations might be due to the difference in the number of patients that attended each hospital, sample size, the methodology used and geographic differences among the study areas.

In the current study, the common ESBL producing gram-negative bacteria were *Klebsiella spp* (24.6%), followed by *Acinetobacter spp* (21.2%) and *E.coli* (18.2%). This is similar to a study conducted in Algeria, *Klebsiella spp* (28.45 %), *E. coli* (25.41%), France, *Klebsiella sp* (55.0%) (27) Nepal, *Acinetobacter* species (52.6%), and *E. coli* (46.6%) were reported as the dominant ESBL producing bacteria (28). This result may suggest that ESBL producing *Klebsiella* has a higher ability to survive in the environment than other bacteria.

In our study, 9.1% of ESBL producing gram-negative bacteria were *Klebsiella pneumoniae*, which is lower than a study conducted by Roux *et al* (87.8%) (14) and Affifi *et al* in Upper Egypt (56.2%) (25). Another study from Egypt had also reported a lower percentage (2%) (29). This difference might be associated with differences in the overall prevalence of gram negative bacteria among countries and hygiene and general infection prevention measures.

According to the current study, medical wards (48.5%) had the highest rate and delivery wards (6.1%) had the lowest rate of ESBL contamination level, differing from the findings of the study by Ayatollah *et al*, who reported the intensive care unit (ICU) with the highest contamination rate (33.1%) (21). This variation may be explained by several factors including the ward conditions and cleaning practices. Our results also indicate a higher level of contamination in the ICU and medical ward compared with the delivery ward, which further highlights the need to develop programs for the prevention of infections in these wards. Nevertheless, infections in the delivery ward should not be neglected as it can be a source of infection for susceptible groups like neonates.

The highest ESBL contamination was observed from chairs (15.2%) followed by pooled samples (15.2%) and Sinks (12.1%). However, in another study, incubators (20.45%) and floor areas (17.9%) had the highest incidents of contamination (27). These differences may be due to differences in the cleaning practices in the hospitals. It is generally assumed that gram-negative microorganisms require moist or damp sites for most survival. However, recent reports suggest that *E. coli* and *Klebsiella* spp may survive more than a year in dry surroundings (30,31).

In our findings, the resistance level was higher among ESBL-producers than non-ESBL producers and all isolates of ESBL-producing bacteria were 100% resistant to ceftazidime and ceftriaxone and this result is in line with a study done in Ethiopia (15), India, ceftazidime (100%), ceftriaxone(100%) (26), however higher than reports from Algeria, showed resistance rate for ceftazidime (37.83%), ceftriaxone (38.62%) (27), and Bangladesh resistance level for ceftazidime (81.2%), ceftriaxone (80.8%) (32).The resistance level of ESBL-producing bacteria for non-β-lactam antibiotics is lower than to β-lactam antibiotics. Of all antimicrobials, tested the carbapenems (meropenem)(81.8%) and amikacin (81.8%) had the highest activity against the ESBL producing organisms followed by gentamicin (60.6%) which is in line with other studies (33,15).

Our findings show *Klebsiella Pneumoniae* with 33.3% resistant to gentamicin and ciprofloxacin which is lower than a study done by Afifi *et al*, gentamicin (84.4%) and ciprofloxacin (77.7%) (20). *E.coli* was found to be one of the common ESBL-producers and was 66.7% resistant to ciprofloxacin and amoxicillin-clavulanic acid. However it was 100% sensitive for meropenem, gentamicin, and amikacin. In contrary to our study, Kader *et al* found a lower resistance rate of ciprofloxacin (54%) and susceptibility of meropenem (93%) and amikacin (72.8%) (34). Although we have generated important information with regard to magnitude of ESBL producing bacteria among hospital inanimate objects, the study has some limitations; first we have collected our isolates from one hospital, so the results may not be generalizable to the entire city or country. Second, we did not link the level of contamination of inanimate objects with actual ESBL infection at the specified wards which could give more pronounced implications for infection prevention measures.

Conclusion and Recommendation

The study showed considerable contamination of hospital environments/ inanimate objects with ESBL-producing gram-negative bacteria. The finding of this study emphasizes the need for continuous surveillance of hospital environments to detect resistant microorganisms. Implementation of infection control measures to reduce the increasing burden of antibiotic resistance is of paramount importance. This is especially very crucial in susceptible groups of patients like neonates and patients in ICUs and operating rooms.

Competing Interests

The authors declare that they have no competing interests.

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Author's contribution

AA: Conception of the research idea, study design, analysis, and interpreted the result; and also wrote the manuscript.

KD: Conception of the research idea, study design , analysis, supervision during data collection and analysis, critical review of the research and the manuscript write up.

YW: Conception of the research idea, advising through critical review of the research and the manuscript write up.

All authors read and approved the final manuscript.

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