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Original Article

Phenotypic and Genotypic Detection of Extended-Spectrum β-Lactamase (ESBL) among Gram-Negative Bacteria Isolated from ICUs and Surgical Operating Rooms, Khartoum-Sudan

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ABSTRACT

Background: Extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria are an important cause of hospital environmental contamination and antimicrobial resistance. Critical care areas such as intensive care units (ICUs) and surgical operating rooms may act as reservoirs for resistant organisms and facilitate their spread in healthcare settings.

Methods: This descriptive cross-sectional study was conducted from April to August 2021 in seven hospitals in Khartoum State, Sudan. A total of 246 environmental swabs were collected from high-touch surfaces in ICUs and surgical operating rooms. Isolates were identified using standard microbiological methods, antimicrobial susceptibility was assessed by disk diffusion, ESBL production was confirmed phenotypically by modified double-disk synergy test, and *bla*TEM, *bla*SHV, and *bla*CTX-M genes were detected by touchdown multiplex polymerase chain reaction.

Results: Of the 246 swabs, 86 (35%) yielded bacterial growth, producing 99 isolates, including 50 Gram-negative bacteria. *Pseudomonas aeruginosa* was the most common isolate (30/50, 60%), followed by *Providencia* spp. and *Serratia* spp. (each 6/50, 12%). ESBL production was confirmed in 10/50 Gram-negative isolates (20%). ESBL-positive isolates were mainly *P. aeruginosa* (3), *Providencia* spp. (3), and *Serratia* spp. (2), with smaller numbers of *E. coli* and *Acinetobacter* spp. Resistance among ESBL producers was highest to cefotaxime, ceftazidime, and aztreonam. Molecular testing detected *bla*TEM in 3/10 ESBL-positive isolates, while *bla*SHV and *bla*CTX-M were not detected.

Conclusions: ICU and operating-room surfaces in Khartoum hospitals were contaminated with ESBL-producing Gram-negative bacteria, particularly *P. aeruginosa*, indicating that the hospital environment may serve as a reservoir for multidrug-resistant organisms. Strengthened environmental cleaning, routine ESBL surveillance, and improved antimicrobial stewardship are needed to reduce transmission risk.

Key words: ESBL, Gram-negative bacteria, ICU, antimicrobial resistance, *bla*TEM, Sudan, hospital environment

INTRODUCTION

The emergence of antimicrobial resistance (AMR) represents one of the most pressing public health challenges of the 21st century, significantly undermining the effectiveness of available therapeutic options and increasing morbidity, mortality, and healthcare costs worldwide. [1] Among the key drivers of AMR are extended-spectrum β-lactamases (ESBLs), a group of plasmid-mediated enzymes capable of hydrolyzing penicillins, third-generation cephalosporins, and monobactams, while remaining inhibited by β-lactamase inhibitors such as clavulanic acid. [2, 3]

Initially derived from TEM and SHV β-lactamases, ESBLs were first reported in Europe and the United States during the 1980s. In recent decades, CTX-M–type enzymes have emerged as the predominant ESBL variants globally, largely due to their association with mobile genetic elements that facilitate rapid horizontal gene transfer among bacterial populations. [4, 5] These enzymes are most commonly identified in members of the Enterobacteriaceae family, particularly *Escherichia coli* and *Klebsiella* species, although they have also been reported in non-fermenting Gram-negative organisms such as *Pseudomonas aeruginosa* and *Acinetobacter* species, albeit less frequently and with different resistance mechanisms contributing to β-lactam resistance. [6–8]

Hospital environments, particularly intensive care units (ICUs) and surgical operating rooms, are recognized as critical reservoirs for multidrug-resistant organisms. High-touch surfaces and moist environments—including medical equipment, wash basins, floors, and bed rails—can facilitate the persistence and transmission of resistant pathogens. [9, 10] Despite routine infection prevention and control measures, environmental contamination with ESBL-producing organisms continues to be reported, raising concerns about their role in healthcare-associated infections and cross-transmission. [11]

In low- and middle-income countries, including Sudan, the burden of AMR is compounded by limited surveillance systems, inconsistent infection control practices, and widespread empirical antibiotic use. However, data on the environmental distribution of ESBL-producing Gram-negative bacteria in critical care settings remain scarce. Understanding both the phenotypic prevalence and molecular characteristics of these organisms is essential for informing targeted infection control strategies and antimicrobial stewardship programs. [12]

Hypothesis

Hospital environmental surfaces in critical care settings serve as reservoirs for ESBL-producing Gram-negative bacteria harboring transferable resistance genes.

Objective

This study aimed to determine the prevalence, antimicrobial susceptibility patterns, and molecular characteristics (*bla*TEM, *bla*SHV, and *bla*CTX-M) of ESBL-producing Gram-negative bacteria isolated from environmental surfaces in ICUs and surgical operating rooms across multiple hospitals in Khartoum State, Sudan. Additionally, the study sought to evaluate the distribution of these organisms across different environmental sources and assess their association with ESBL production.

MATERIAL AND METHODS

Study Design, Setting, and Population

This descriptive, laboratory-based cross-sectional study was conducted between April and August 2021 at several hospitals in Khartoum State. Participating institutions included AL-Silah AL-Tibiyu Hospital, Al-Amal National Hospital, East Nile Hospital, Ribat National Hospital, Dar AL-Elaj Special Hospital, Ibrahim Malik Hospital, and Yastabshiroon Hospital. Environmental samples were collected exclusively from ICUs and surgical operating rooms within these hospitals; no samples related to patients were included. A list of the participating hospitals is provided in **Table 1**.

Sample Size calculation

$$n = \frac{Z^2 p(1-p)}{d^2}$$

The sample size was calculated using the formula for an unknown population proportion:

Where:

- $Z = 1.96$ (95% confidence level)
- $P = 0.20$ (estimated proportion of ESBL-producing Gram-negative bacteria in hospital environments, based on pilot data from similar settings)
- $q = 1 - P = 0.80$
- $d = 0.05$ (margin of error)

$$n = \frac{(1.96^2) \times 0.20 \times 0.80}{(0.05)^2} = 245.86 \approx 246$$

A total of 246 environmental swabs were therefore collected.

Sampling Strategy

Purposive sampling was employed to select high-touch surfaces and critical equipment within clinical areas.

Table 1: The hospital and the number of ICU units and the surgical operation room.

Name of hospital	Number of ICU units	Number of surgical operation rooms
Al-Silah Al-Tibiyu Hospital	2	2
National Ribat Hospital	1	-
Al-Amal National Hospital	1	-
Ibrahim Malik Hospital	-	4
Yastabshiroon Hospital	1	3
Dar AL-Elaj Specialized Hospital	1	1
East Nile Hospital	1	-

Environmental swabs were collected following routine cleaning and disinfection protocols and before patient entry to assess residual contamination. Sampling sites included beds, floors, door handles, wash basins, medical equipment (dialysis machines, suction devices, monitoring equipment), water coolers, Mayo tables, walls, soap dispensers (solid and liquid), tables, stools, and soap dilution bags.

Sample Collection

A total of 246 environmental swabs were collected using sterile cotton swabs pre-moistened with nutrient broth media. All sampling procedures were performed under aseptic conditions after confirming that areas had been properly sterilized and prepared for patient use.

Laboratory Methods

Primary Culture and Isolation

All samples were directly cultured on MacConkey agar and incubated at 37°C overnight for initial bacterial isolation. [13]

Bacterial Identification

Bacterial identification was performed using standard microbiological techniques, including:

- Gram staining
- Colonial morphology assessment on MacConkey agar
- Growth characteristics evaluation
- Biochemical testing

Antimicrobial Susceptibility Testing

Disc diffusion method (DDM) was performed on Mueller-Hinton agar using McFarland standard 0.5 for inoculum preparation. The following antibiotics were tested: meropenem (10 µg), cefepime (30 µg), ceftriaxone (30 µg), colistin (10 µg), and amoxicillin/clavulanic acid (30 µg). Antibiotic disks were placed 24 mm apart and 10 mm from plate edges. After 24-hour incubation at 37°C, inhibition zones were measured and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines. [14]

ESBLs Detection

Phenotypic Detection: Modified Double Disc Synergy Test (MDDST)

Mueller-Hinton agar was inoculated with standardized inoculum (corresponding to a 0.5 McFarland tube) using a sterile cotton swab. An Augmentin (20 µg amoxycillin and 10 µg of clavulanic acid [Amoxycillin/clavulanic acid (AMC)]) disc was placed in the center of the plate, and test discs of third-generation cephalosporins: ceftazidime 30 µg (CAZ), cefotaxime 30 µg (CTX), and aztreonam 30 µg (ATM) discs were placed at a distance of 15 mm from the Augmentin disc. The plate was incubated overnight at 37°C. ESBL production was considered positive if the zone of inhibition around the test discs increased towards the Augmentin disc or neither disc was inhibitory alone, but bacterial growth was inhibited where the two antibiotics diffused together. [14]

Molecular Detection of ESBL-Genes (TEM, SHV, and CTXM)

DNA Extraction

The boiling method by the Prime Thermal Cycler was used for DNA extraction. Two to three colony from bacteria culture was taken and transferred into clean sterile Eppendorf tube contain 160 µL of distilled water, centrifuged the tube at the centrifuge for 5 minutes at 10.000 rpm speed, discarded the supernatant, then add 200 µL of double distilled water to the pellet and put the tube in the heat block and boiling them for 10 to 15 minutes, freeze the tubes for 10 minutes at −20°C, repeated the boiling step for 2 minutes, repeat the freezing steps for 2 minutes, centrifuged the tube for 20 minutes at 10.000 rpm, then taken the supernatant into clean Eppendorf tube, keep the tube at −20°C for long term use. A NanoDrop spectrophotometer was used to quantify the DNA concentration in each sample.

Primers: As shown in **Table 2**.

Master mix: Bolis Biodyne, a German company.

Preparation of Reaction Mixtures for ESBL Genes

A 20 µL volume using the Bolis Biodyne, a German company, master mix 4 µL was dissolved by 10 µL of dissolved water, for each gene, 0.5 µL of forward primer, 0.5 µL reverse, and 5 µL of DNA extract was transferred into a polymerase chain reaction (PCR) tube for one sample.

Protocol Used for Amplification of TEM, SHV, and CTX Genes

The Touchdown Multiplex PCR was performed to simultaneously amplify TEM, SHV, and CTX-M using a thermocycler. Primers were designed with a similar melting temperature to allow efficient co-amplification primers under the following conditions:

Initial activation at 95°C for 3 to 5 minutes.

Down phase (10 cycles):

- Denaturation 95°C for 30 seconds
- Annealing 68 to 58°C (−1°C\cycles)
- Extensions 72°C for 45 seconds

Table 2: Primer sequences used for the amplification of the gene.

Primers name	DNA sequence (5 to 3)	Amplicon's size (bp)
*bla*TEM	5′-GTG CGG TAT TAT CCC GTG TT-3′ 5′-AAC TTT ATC CGC CTC CAT CC-3′	416
*bla*SHV	5′-GGA AAC GGA ACT GAATGAGG-3′ 5′-ATC CCG CAG ATA AAT CAC CA-3′	301
*bla*CTXM	5′-CGC TTT CCA ATG TGC AGT AC-3′ 5′-TCG CCG CTG CCG GTC TTA TC-3′	510

Amplification phase (25 cycles):

- Denaturation 95°C for 30 seconds
- Annealing 58°C for 30 seconds
- Extensions 72°C for 45 seconds

Final extension: 72°C for 5 minutes

Visualization of PCR Product

The gel casting tray was flooded with 10x TBE buffer near the gel cover surface, then 5 µL of PCR products of each sample was put into each well. Then, to the first well of the casting tray, 5 µL of DNA ladder (marker) was injected for each run. The gel electrophoresis apparatus (Primer, 120 V, 25 mA, 1:00 hour) was run at 120 V for 60 minutes. After that, the gel was removed by the gel holder and visualized by UV illumination. The gel was photographed using the Polaroid film.

Data Management and Statistical Analysis

Data were coded, entered into Microsoft Excel, and analyzed using SPSS, version 23.0 (IBM). Descriptive statistics (frequencies, percentages) were calculated. Associations between categorical variables (e.g., organism type and ESBL production, source of isolation, antibiotic resistance) were assessed using the chi-square test (or Fisher's exact test when expected cell counts were <5). A 95% confidence interval was applied, and a *P* value of <0.05 was considered statistically significant.

Quality Control and Methodological Considerations

To improve internal consistency, all samples were processed using the same culture media, incubation conditions, and phenotypic testing workflow throughout the study period. Standard microbiological procedures were followed for isolate recovery, identification, antimicrobial susceptibility testing, and PCR processing.

Ethical Consideration

Ethical approval was obtained from the Postgraduate College, Department of Microbiology, University of Medical Sciences and Technology, and from the Research Department of the Khartoum State Ministry of Health. Written permission was also obtained from the management of each participating hospital, ICU, and surgical operating room. The purpose and implications of the study were communicated to those in charge of each sampling area. All residual specimens were handled in accordance with institutional biosafety protocols and disposed of appropriately. COVID-19 safety precautions were observed throughout sample collection, including the use of face masks, gloves, and maintenance of a physical distance of at least 1.8 m (6 feet) from other individuals.

RESULTS

Bacterial Isolation and Overall Yield

A sum of 246 environmental swabs was gathered from various hospitals throughout Khartoum state over a 4-month span, from April to August. Of these, 86 swabs (35%) exhibited positive bacterial growth, while the other 160 swabs (65%) were sterile (no growth). Among the positive samples, 74 (86%) yielded a single bacterial organism, whereas 12 samples(14%) exhibited mixed growth, indicating the presence of more than one type of bacterium. In total, 99 bacterial isolates were recovered from the 86 positive swabs. Of these, 50 isolates (50.6 %) were identified as Gram-negative bacteria, while the remaining 49 (49.4 %) were Gram-positive bacteria.

Gram-Negative Organism

The most frequent bacterial strain was *Pseudomonas aeruginosa* (30). Other varieties of bacterial strains were isolated as well: *Providencia* spp. (6), *Serratia* spp. (6), *Escherichia coli* (3), *Acinetobacter* spp. (3), and, less frequently, *Proteus mirabilis* (1), *Klebsiella oxytoca* (1), as shown in **Table 3**.

Distribution of Bacterial Isolates According to Environmental Sources

Gram-negative bacteria were isolated from various areas within the ICU and surgical operating rooms. *Pseudomonas aeruginosa* is the most commonly isolated pathogen from beds, floors, wash basins, and machines. *Serratia* and *Providencia* species were also commonly detected, from water-related sources such as soap dilution bags, liquid soap, and water coolers. In contrast, *Klebsiella oxytoca* and *Proteus mirabilis* were less frequently isolated. Statistical analysis showed no significant difference in the distributions of isolates among the various environmental sources ($P = 0.532$), as shown in **Table 4**.

Antibiotic Susceptibility Patterns by DDM

Different antibiotics were used for testing Gram-negative bacterial isolates, including:

Meropenem 10 µg (MEM), Amoxicillin/clavulanic acid 30 µg (AMC), Ceftriaxone 30 µg (CRO), Cefepime 30 µg (FEP), Colistin 10 µg (CT). Out of 50 Gram-negative bacteria isolates, high resistance rates were observed against Amoxicillin/clavulanic acid in 29 isolates (58%), Ceftriaxone in 27 isolates (54%), Cefepime in 20 isolates (40%), Meropenem in 9 isolates (18%), and Colistin in 6 isolates (12%). A significant difference in susceptibility was observed only for Amoxiclav and Cefotaxime ($P < 0.05$), while no significant variation was detected for the other tested antibiotics ($P > 0.05$), as shown in **Table 5**.

Table 3: The frequencies and percentages of Gram-negative organisms.

Type of organism	Frequencies	Percentage %
Pseudomonas aeruginosa	30	60%
Serratia spp.	6	12%
Providencia spp.	6	12%
Escherichia coli	3	6%
Acinetobacter spp.	3	6%
Proteus mirabilis	1	2%
Klebsiella oxytoca	1	2%
Total	50	100%

Table 4: Distribution of bacterial isolates according to environmental sources.

Place	Total samples	*K. oxytoca*	*P. mirabilis*	*Acinetobacter spp.*	*E. coli*	*Providencia spp.*	*Serratia spp.*	*P. aeruginosa*
Bed	5	0	0	0	0	1	1	3
Floor	9	0	0	2	1	0	2	4
Wash basin	10	1	1	0	0	1	0	7
Solid soap	1	0	0	0	1	0	0	0
Liquid soap	4	0	0	0	0	1	0	3
Mayo table	2	0	0	0	0	0	0	2
Door knob	3	0	0	0	0	0	0	3
Wall	1	0	0	0	0	0	0	1
Scout	1	0	0	0	0	0	0	1
Soap dilution bag	3	0	0	1	0	2	0	0
Water cooler	3	0	0	0	0	0	2	1
Table	1	0	0	0	0	0	0	1
Machines	7	0	0	0	1	1	1	4
Total	50	1	1	3	3	6	6	30
***P* value**	0.532							

Table 5: Antibiotic activity against environmental isolates by disc diffusion method.

Organism	MEM 10 µg (R/I/S %) *P* value	AMC 30 µg (R/I/S %) *P* value	CRO 30 µg (R/I/S %) *P* value	FEP 30 µg (R/I/S %) *P* value	CT 10 µg (R/I/S %) *P* value
Klebsiella oxytoca	0/0/100 0.308	0/0/100 0.005	0/0/100 0.439	0/100/0 0.014	0/0/100 0.286
Proteus mirabilis	0/0/100 0.308	1/0/0 0.005	1/0/0 0.439	1/0/0 0.014	0/0/100 0.286
Acinetobacter species	1/0/2 0.308	2/1/0 0.005	2/0/1 0.439	2/1/0 0.014	1/0/2 0.286
Escherichia coli	1/0/2 0.308	2/1/0 0.005	3/0/0 0.439	3/0/0 0.014	0/0/3 0.286
Providencia species	3/0/3 0.308	6/0/0 0.005	5/0/1 0.439	4/2/0 0.014	1/0/5 0.286

Phenotypic Detection of ESBL by MDDST

ESBL production was phenotypically detected using the MDDST. This was performed by placing a disc of amoxicillin/clavulanate (20/10 µg) in the center of a Mueller-Hinton agar plate, with three cephalosporins: ceftazidime (30 µg), cefotaxime (30 µg), and aztreonam (30 µg) positioned 20 mm (center to center) away from the central disc. Out of 50 Gram-negative bacteria, 10 (20%) were confirmed as ESBL producers. The distribution of ESBL-producing isolates was as follows: *Pseudomonas aeruginosa,* 3 isolates (6%), *Providencia* species, 3 isolates (6%), *Serratia* species, 2 isolates (4%), *Escherichia coli,* 1 isolate (2%), and *Acinetobacter species,* 1 isolate (2%). Among ESBL-producing isolates, high resistance rates were observed against Cefotaxime in 9 isolates (90%), Ceftazidime in 7 isolates (70%), and Aztreonam in 6 isolates (60%), as shown in **Table 6**. The MDDST revealed statistically significant associations between antibiotic susceptibility patterns and environmental isolates for %, Ceftazidime (CAZ; $P = 0.025$) and Aztreonam (ATM; $P = 0.036$). In contrast, Cefotaxime (CTX) showed no significant associations ($P = 0.200$). **Table 6** describes antibiotic activity against environmental isolates by the MDDST.

Positive ESBL Detection by MDDST

Isolates were considered ESBL-positive by MDDST when there was a clear extension or enhancement (≥5 mm) of the inhibition zone of third-generation cephalosporin discs, toward the Amoxicillin/clavulanic acid disc was observed, as shown in **Figure 1**.

Negative ESBL Detection by MDDST

Isolates were considered ESBL-negative when no clear extension of the inhibition zone (≤5 mm) was observed between third-generation cephalosporin discs and the Amoxicillin/clavulanic acid disc, as shown in **Figure 2**.

Table 6: Antibiotic activity against environmental isolate by modified double disc synergy test.

Antibiotic	Response	Total	Klebsiella oxytoca	Proteus mirabilis	*Acinetobacter* spp.	E. coli	*Providencia* spp.	*Serratia* spp.	P. aeruginosa
CTX 30 µg (*P* = 0.200)	Resistant	11 (22%)	0 (0%)	0 (0%)	1 (33.3%)	1 (33.3%)	3 (50%)	3 (50%)	3 (10%)
	Intermediate	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (3.3%)
	Sensitive	38 (76%)	1 (100%)	1 (100%)	2 (66.7%)	2 (66.7%)	3 (50%)	3 (50%)	26 (86.7%)
	Total	50 (100%)	1 (100%)	1 (100%)	3 (100%)	3 (100%)	6 (100%)	6 (100%)	30 (100%)
CAZ 30 µg (*P* = 0.025)	Resistant	9 (18%)	0 (0%)	0 (0%)	1 (33.3%)	0 (0%)	3 (50%)	3 (50%)	2 (7.6%)
	Intermediate	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)	– (–)
	Sensitive	41 (82%)	1 (100%)	1 (100%)	2 (66.7%)	3 (100%)	3 (50%)	3 (50%)	28 (93.3%)
	Total	50 (100%)	1 (100%)	1 (100%)	3 (100%)	3 (100%)	6 (100%)	6 (100%)	30 (100%)
ATM 30 µg (*P* = 0.036)	Resistant	8 (16%)	0 (0%)	0 (0%)	1 (33.3%)	0 (0%)	2 (33.3%)	3 (50%)	2 (6.7%)
	Intermediate	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)	0 (0%)
	Sensitive	41 (82%)	1 (100%)	1 (100%)	2 (66.7%)	3 (100%)	3 (50%)	3 (50%)	28 (93.3%)
	Total	50 (100%)	1 (100%)	1 (100%)	3 (100%)	3 (100%)	6 (100%)	6 (100%)	30 (100%)

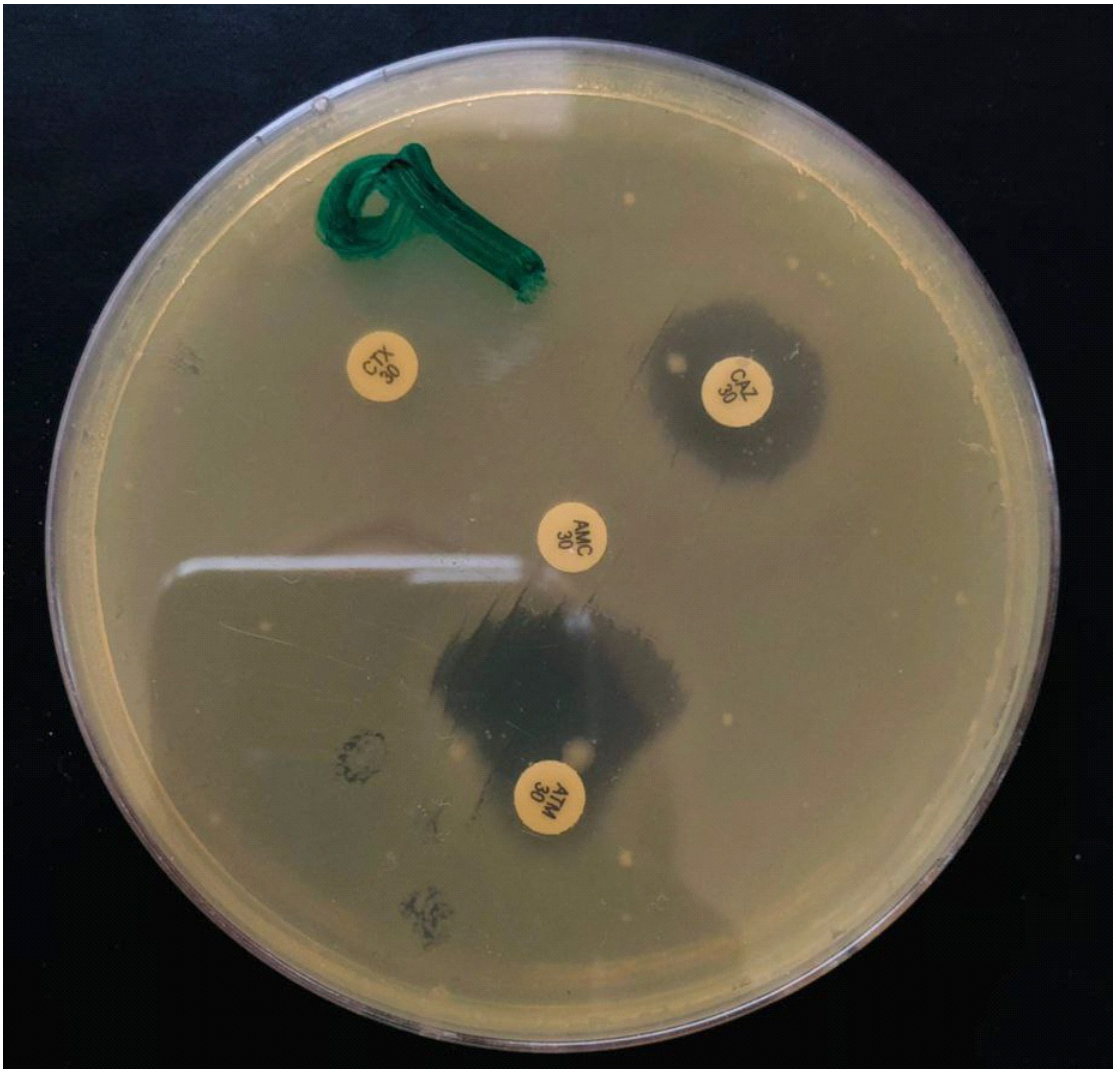

Figure 1: Positive ESBL detection by Modified Double Disc Synergy Test.

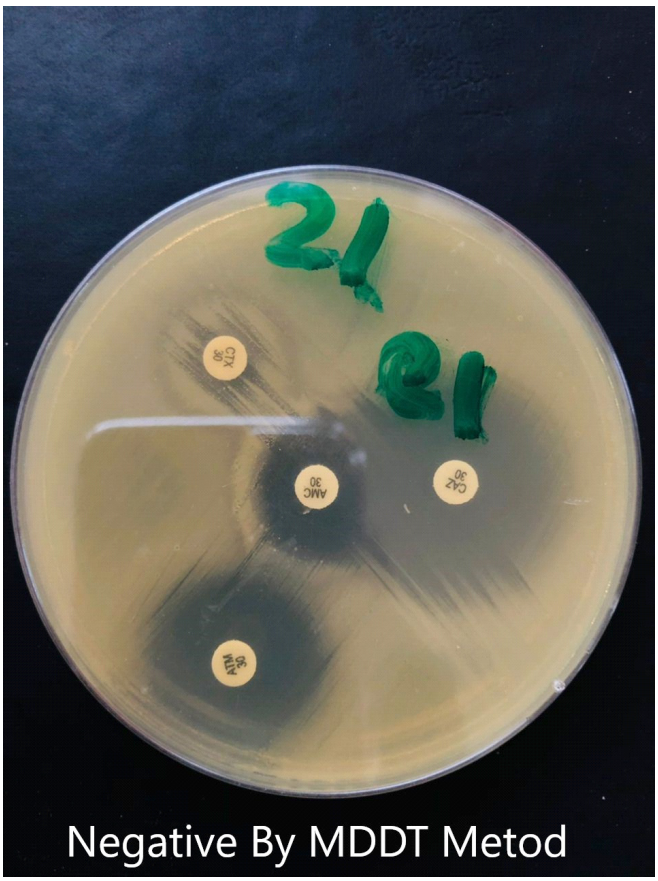

Figure 2: Negative ESBL detection by modified double disc synergy test (MDDST).

Distribution of Gram-Negative Bacteria Between the ICU and Surgical Operating Room

Distribution of 50 Gram-negative bacteria that were recovered from different hospitals. *Pseudomonas aeruginosa* was the most frequently isolated organism in both the ICU and surgical operating room, with an equal count of 15 isolates in each setting. This organism was recovered from a wide range of sources. *Serratia* and *Providencia* species 6 isolates ranked second from different sources, less frequently isolated organisms included *Escherichia coli* 3, *Acinetobacter* species 3 isolates, *Proteus mirabilis* 1 isolate, and *Klebsiella oxytoca*1 isolates this indicating lower environmental persistence compared to *Pseudomonas aeruginosa* and *Serratia* species.

The overall distribution of Gram-negative bacteria showed that 22 isolates (44%) were obtained from the ICU, while 28 isolates (56%) were recovered from the Surgical Operation room. Statistical analysis reveals no significant difference between the two locations (*P* value = 0.114). Detailed distributions are presented in **Tables 7** and **8**.

Distribution of ESBL in the ICU and Surgical Operating Room

Inanimate surfaces in both ICU and surgical operation rooms were found to be contaminated with ESBL-producing Gram-negative bacteria to varying degrees. The most frequently contaminated surfaces included the floor 5 (10%), Mayo-table 2 (4%), a wash basin 1 (1%), *a* machine 1 (1%), and a soap dilution bag 1 (1%), respectively. These findings suggest that moist and frequently touched surfaces serve as potential reservoirs for resistant organisms. Among the ESBL-producing *Pseudomonas aeruginosa* and *Providencia* species showed the highest prevalence, followed by *Serratia* species, as detailed are presented in **Table 9**.

Identification of ESBL Resistance genes in Gram-negative bacteria

The PCR method was used to identify all 50 Gram-negative bacterial isolates. All 50 Gram-negative bacterial isolates were tested for the presence of ESBL resistance genes TEM, SHV, and CTX utilizing the touchdown multiplex PCR method. Three isolates (6%; out of 10 phenotypically confirmed ESBL-producing isolates) were found to be positive for the TEM gene, comprising two species of *Providencia* and one species of *Serratia*. Of the remaining 47 isolates (94%) that were negative for all three genes, as shown in **Table 10**. These isolates exhibited resistance to all the antibiotics tested, except for one species of *Providencia*, which was susceptible solely to Colistin and resistant to all other antibiotics.

Detection of the TEM, SHV, and CTXM Gene Among Gram-Negative Isolates by PCR

PCR analysis revealed the presence of the TEM gene in many Gram-negative isolates, as indicated by clear amplification bands at the expected size. In contrast, no amplification was observed for the other tested ESBL genes, SHV and CTX, suggesting that TEM was the predominant gene among the isolates tested. These results are illustrated in the gel electrophoresis image, as shown in **Figure 3**.

DISCUSSION

In this study, Gram-negative bacteria accounted for 50.5% of all isolates recovered from environmental surfaces in ICUs and surgical operating rooms, closely aligning with findings from Laktib et al., who reported that Gram-negative organisms constituted 84.21% of hospital environmental isolates in Moroccan ICUs. [15] The predominance of Gram-negative bacteria in healthcare environments is concerning, given their intrinsic resistance mechanisms and capacity for horizontal gene transfer. [2]

Pseudomonas aeruginosa was the most frequently isolated organism, representing 60% of all Gram-negative isolates. This finding is consistent with Nwankwo, who reported *P. aeruginosa* as the predominant isolate (23.3%) from operating room fomites in Nigeria. [16] The high recovery rate of *P. aeruginosa* from moist environmental sources, including wash basins, floors, and medical equipment, highlights the organism's ability to survive in aqueous hospital environments and form biofilms on surfaces. [17]

Table 7: Frequency of bacterial growth in the ICU and surgical operation room.

Unit	Frequency	Percentage %
ICU	22	44%
Surgical operation room	28	56%
Total	50	100

Table 8: Distribution of Gram-negative bacteria between the ICU and the surgical operation room.

Organism	Total isolates	Surgical operation room	ICU	Hospitals (surgical)	Hospitals (ICU)	Environmental sources (surgical)	Environmental sources (ICU)
Pseudomonas aeruginosa	30	15	15	Dar Al-Elaj Specialized; Yastabshiroon	Al-Amal National; Dar Al-Elaj Specialized; Al-Silah Al-Tibiyu; National Ribat	Scout, Floor, Mayo table, Bed, Machine, Door knob, Liquid soap	Wash basin, Machine, Water cooler, Floor, Table, Bed, Wall, Door knob
Serratia species	4	2	2	Yastabshiroon	National Ribat	Machine, Bed	Water cooler
Providencia species	6	6	-	Ibrahim Malik	-	Bed, Machine, Liquid soap	-
Escherichia coli	3	3	-	Dar Al-Elaj Specialized	East Nile	Solid soap, Machine	Floor
Acinetobacter species	3	3	-	Ibrahim Malik	Dar Al-Elaj Specialized	Floor, Soap dilution bag	Floor
Proteus mirabilis	1	1	-	Yastabshiroon	-	Wash basin	-
Klebsiella oxytoca	1	-	1	-	Yastabshiroon	-	Wash basin
Total	50	28	22	-	-	-	-

Table 9: Distribution of ESBL in the ICU and the surgical operation room.

Organism	Total isolates	Hospital name	Sources (Surgical operation room)	Total isolates	Hospital name	Sources (ICU)	Percentage
Pseudomonas aeruginosa	3	Dar Al-Elaj Specialized	(1) Mayo table	1	Dar Al-Elaj Specialized	(1) floor	6%
Providencia species	3	Al-Amal National	(1) machine	0	-	-	6%
Serratia species	2	Dar Al-Elaj Specialized	(1) Mayo table	0	Yastabshiroon	(2) floor	4%
—	1	Yastabshiroon	(1) wash basin, (1) soap dilution bag	0	-	-	1%

Table 10: Detection of ESBL resistance genes among Gram-negative isolates (n = 50).

Category	Species	Number of isolates	%	ESBL phenotype	TEM gene	SHV gene	CTX gene	Antibiotic resistance
ESBL+ and TEM+	*Providencia* sp.	2	4%	Positive	+	-	-	Resistant to all (1 isolate susceptible only to Colistin)
ESBL+ and TEM+	*Serratia* sp.	1	2%	Positive	+	-	-	Resistant to all tested antibiotics
ESBL+ and TEM−	Other GNB	7	14%	Positive	-	-	-	Resistant to multiple antibiotics
ESBL−	Other GNB	40	80%	Negative	-	-	-	Variable resistance, none ESBL positive

10/50 isolates (20%) were ESBL-positive phenotypically. Among them, only 3 isolates (6%) carried the TEM gene (2 *Providencia*, 1 *Serratia*). All isolates were negative for SHV and CTX genes. TEM-positive isolates were multidrug-resistant, with one *Providencia* isolate susceptible only to Colistin.

The phenotypic prevalence of ESBL-producing Gram-negative bacteria in this study was 20% (10/50 isolates). This rate falls within the range of 3% to 33% reported in similar investigations of hospital environmental contamination. Comparative studies have documented ESBL contamination rates of 14.8% in Ethiopia, [18] 9% in Israel, [19] 3.1% in the United Kingdom, [11] and 33% in Pakistan. [20] The observed variation likely reflects differences in infection control practices, antibiotic stewardship programs, geographic distribution of resistance genes, and sampling methodologies across studies. [21]

Among the ESBL-producing isolates, high resistance rates were observed against ceftriaxone (100%), cefotaxime (80%), ceftazidime (70%), and aztreonam (60%). These findings parallel those of Daher et al., who reported resistance rates of 93.8% to ceftazidime, 87.5% to cefepime, and 86.7% to cefotaxime among ESBL-producing Enterobacteriaceae

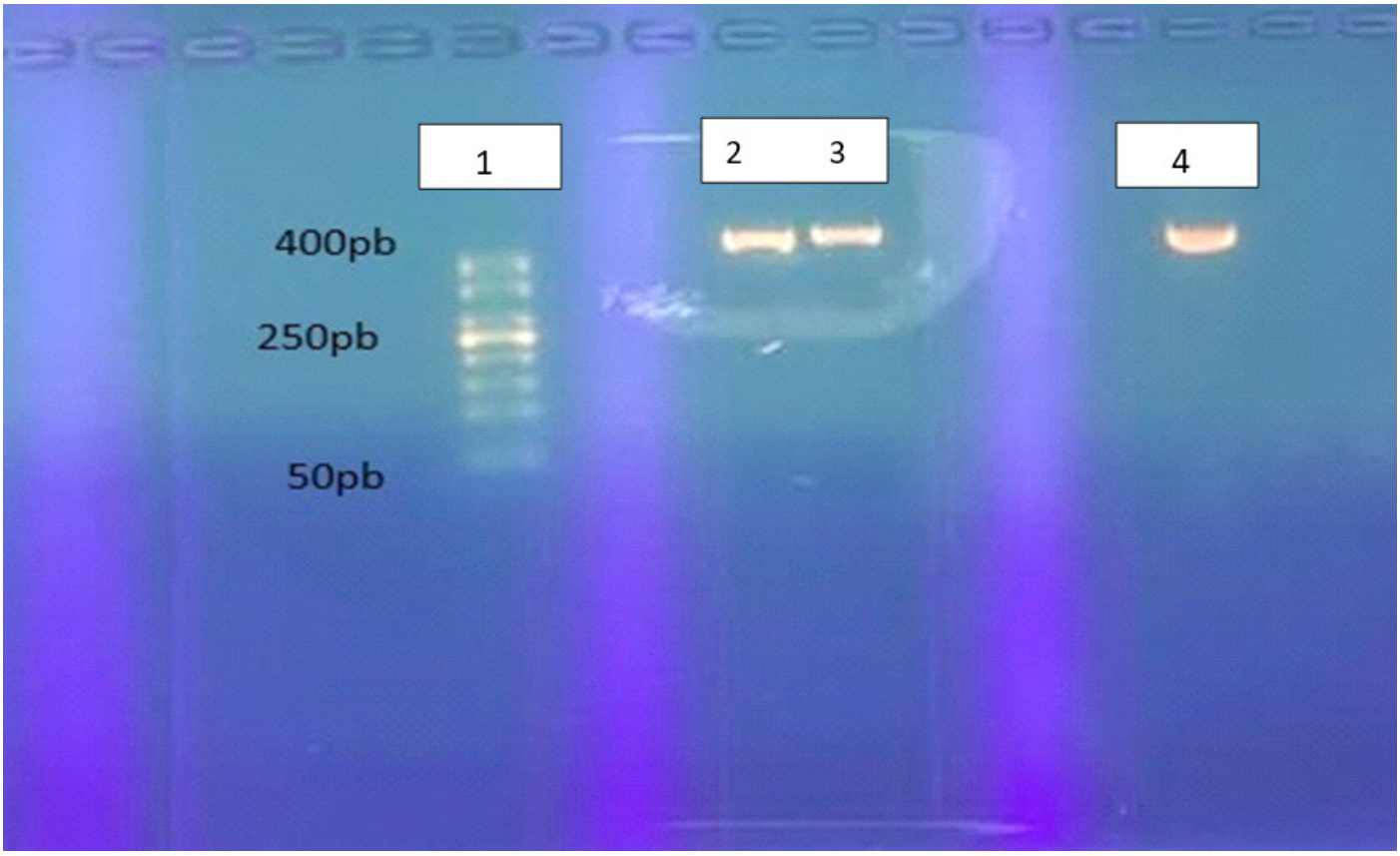

Figure 3: (1) PCR product of TEM genes (416 pb) run on ethidium bromide gel visualized under UV light. Lane 1: DNA ladder of 50 bp. Lane 2–4 showed a typical positive isolate for band size of 416 bp.

from hospital environments in Ethiopia. [22] Similarly, Engda et al. found that all ESBL-producing isolates demonstrated resistance to ceftriaxone, ceftazidime, and amoxicillin-clavulanic acid. [18] The consistently high resistance to third-generation cephalosporins among environmental isolates underscores the selective pressure exerted by widespread use of these agents in clinical settings. [4]

Providencia species and *Serratia* species demonstrated notable ESBL production in this study, with three isolates (6%) each. This contrasts with reports from other regions where *Klebsiella* species and *E. coli* predominate. Mane et al. reported ESBL prevalence of 28.45% among *K. pneumoniae* and 25.41% among *E. coli* [23] while Engda et al. found that *K. pneumoniae* (42.10%) and *E. coli* (35.09%) were the predominant ESBL producers. [18] The differences observed may be attributed to this study's exclusive focus on environmental sampling from critical care units, whereas prior investigations included both clinical isolates and samples from non-critical areas, where distinct microbial ecology patterns prevail. [24]

Molecular characterization revealed that only 3 of 10 phenotypically confirmed ESBL producers (30%) harbored the *bla*TEM gene, representing 6% of all Gram-negative isolates. No isolates carried *bla*SHV or *bla*CTX-M genes. This finding is partially consistent with Rivera-Jacinto and Rodríguez-Ulloa, who detected TEM in 11 of 15 ESBL-producing isolates (73.3%) from hospital environmental surfaces in Peru. [25] Zagui et al. reported β-lactamase genes in 35.6% of hospital wastewater isolates, with *bla*TEM being the most frequent (17.8%). [26] The absence of SHV and CTX-M genes in the present study is noteworthy, as CTX-M-type ESBLs have become dominant in many parts of Africa and the Middle East. [5] This discrepancy may reflect geographic variation in circulating gene pools, limitations in primer design (particularly failure to detect CTX-M-15 variants), or the small sample size of ESBL-positive isolates. [27]

The three TEM-positive isolates (two *Providencia* species and one *Serratia* species) exhibited multidrug resistance, with one isolate demonstrating susceptibility only to colistin. This pattern of co-resistance is well-documented, as ESBL-encoding plasmids frequently carry additional resistance determinants for aminoglycosides, fluoroquinolones, and sulphonamides. [28] Engda et al. similarly reported that all ESBL-producing Enterobacteriaceae in their study were resistant to multiple β-lactam antibiotics. [18]

Strengths and Limitations

Strengths include the multi-hospital design and combined phenotypic-genotypic approach in an underserved region. Limitations are: (1) PCR limited to TEM, SHV, CTX-M-1 group, missing other ESBL families and variants like CTX-M-15; (2) No confirmatory testing for AmpC or carbapenemases; (3) MDDST's known lower specificity for non-fermenters; (4) Single time-point sampling; (5) Lack of clinical isolate correlation to confirm transmission.

CONCLUSIONS

This study demonstrates that ICU and surgical operating room surfaces in Khartoum hospitals are contaminated with Gram-negative bacteria, including a substantial proportion of ESBL-producing isolates. The predominance of *Pseudomonas aeruginosa* and the detection of multidrug-resistant ESBL producers on high-touch and moist surfaces indicate that the hospital environment may serve as an important reservoir for resistant organisms.

The phenotypic and molecular findings together show that ESBL circulation in these settings is not only present but also biologically relevant, with *bla*TEM detected in a subset of ESBL-positive isolates. The absence of *bla*SHV and *bla*CTX-M in this study does not eliminate the possibility of other ESBL mechanisms, but it does suggest a locally distinct resistance pattern that deserves further investigation.

These results support the need for stronger infection prevention measures, including improved cleaning of wet surfaces, careful disinfection of shared equipment, and routine environmental surveillance for resistant organisms. They also reinforce the importance of antimicrobial stewardship, since limiting unnecessary antibiotic pressure is central to reducing selection and persistence of resistant bacteria in hospitals.

A direct implication of this work is that environmental monitoring should be integrated into infection control programs, especially in critical care and operative areas. Broader molecular testing and longitudinal studies will be necessary to clarify the full resistance profile and transmission potential of these isolates.

AUTHORS' CONTRIBUTION

Each author has made a substantial contribution to the present work in one or more areas, including conception, study design, conduct, data collection, analysis, and interpretation. All authors have given final approval of the version to be published, agreed on the journal to which the article has been submitted, and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

None.

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