JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Molecular characterization of carbapenemases production among environmental Gram-negative isolates at Addis Ababa, Ethiopia: first detection of NDM Producers in hospital environments

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Abstract

Introduction: The Gram-Negative bacteria, particularly carbapenem-resistant strains (CR-GNB), pose a global health threat due to high morbidity and mortality. Detecting carbapenemase-encoding genes is essential for understanding their spread in hospital environments. This study investigated environmental colonization by CR-GNB in Ethiopian hospitals, including genetic characterization of resistance genes.

Methodology: A cross-sectional study analyzed 103 environmental GNB isolates collected from inanimate surfaces at Tikur Anbessa Specialized Hospital (TASH) and ALERT Hospital (June–September 2021). Conventional microbiological methods identified the isolates, and antimicrobial susceptibility was tested using the Kirby-Bauer disk diffusion method. Carbapenemase production was screened using the Modified Hodge test (MHT) and combined disk test (CDT). Resistance genes (bla_{KPC}, bla_{NDM}, bla_{OXA-48}) were detected via PCR in isolates with reduced meropenem susceptibility.

Results: The predominant GNB were *Acinetobacter baumannii* (47%), *Pseudomonas aeruginosa* (33%), and *E. coli* (12%). Among 103 isolates, 62% showed reduced meropenem susceptibility. The most common CR-GNB was *Acinetobacter baumannii* (37.5%), followed by *E. coli* (18.8%) and *Klebsiella pneumoniae* (12.5%). Carbapenemase production was detected in 41.7% of isolates via PCR, with bla_{NDM} being the most common (43 isolates). Linens (26.4%) and beds (21.4%) had the highest contamination rates. Most carbapenemase-producing isolates were multidrug-resistant (MDR).

Conclusions: The presence of bla_{NDM} and bla_{KPC} genes highlights hospital surfaces as reservoirs for resistance genes, contributing to healthcare-associated infections. Routine surveillance and early detection of carbapenemase producers are crucial for infection control and antimicrobial resistance management.

Key words: Carbapenem-resistance; multidrug-resistant (MDR); β-lactamase, carbapenemase.

J Infect Dev Ctries 2025; 19(4):512-519. doi:10.3855/jidc.20561

(Received 07 July 2024 – Accepted 18 September 2024)

Introduction

Antimicrobial resistance (AMR) is based on the intensive and inappropriate use of antimicrobial agents, as well as coding for antimicrobial resistance genes in the three interfaces including human medicine, veterinary medicine, and environmental domains (inanimate surfaces). In this regard, Gram-negative bacteria (GNB) have grown to be a significant problem globally owing to their resistance to various antimicrobial agents in varies domains [1-3].

When treating health care-associated infections (HCAIs) that are multidrug resistant (MDR), carbapenems are typically the "last choice" of antimicrobial agents. However, recent findings of carbapenem resistance due to carbapenemase enzymes have warranted Health care professionals [4]. The mechanism of resistance to carbapenems occurs due to variations in outer membrane of GNB and/or by the horizontal gene transfer (HGT) encoding β-lactamase enzymes such as carbapenemases [2]. The World health organization (WHO) has identified a priority group of bacteria that include *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and members of the *Enterobacteriaceae* family [5]. These bacteria are known to produce transmissible enzymes for which new antimicrobial agents are desperately required [5-8]

Carbapenemases are a type of β-latamases with the ability to hydrolyze carbapenems can stop them from

accessing their target, the penicillin binding protein (PBP) [9]. Carbapenemases are classified into three distinct classes according to their structural similarities (A, B, and D) [10]. The class A [*Klebsiella pneumoniae* carbapenemase (*KPC*)], class B [(New Delhi Metallo-β-lactamase (*NDM*), Verona Integron-encoded Metallo-β-lactamase (*VIM*), *IMP*–type carbapenemases or Metalloβ-lactamases (MBLs)], and class D oxacillinases (*OXA-48*-like enzymes) are the most commonly detected carbapenem-hydrolyzing enzymes [7,11]. These enzymes are coded by bla_{NDM}, bla_{IMP}, bla_{KPC}, bla_{OXA}, and bla_{VIM} genes which are present in plasmids and chromosomes [11].

The detection of carbapenemases can be employed by using varies methods including disk diffusion methods and phenotypically confirmed by the Modified Hodge Test (MHT) and carbapenem inactivation method (CIM) or polymerase chain reaction (PCR) to detect the resistant encoding genes [12]. Comprehending the carbapenem resistance mechanisms enable to monitor the local dissemination and spread of AMR would allow to the selection of effective therapeutics for CR-GNB [7]. There has been little data available on the magnitude and dissemination of carbapenem resistance among GNB in the majority of sub-Sharan Africa, including Ethiopia [13,14]. Moreover, data on the CR-GNB dissemination from inanimate surfaces are currently lacking in study setting [15]. This may be due to the lack of guidelines and standards for detecting carbapenemases in most laboratories. To characterize the antimicrobial resistance mechanisms implicated, specific tests are required as ordinary susceptibility testing may not be accurate [6]. Therefore, rapid characterization of carbapenemase production is imperative role to implement effective prevention control actions to prevent its dissemination in the hospital environments [6].

Inanimate hospital surface colonization with carbapenemase-producing bacteria is important when patients are treated in hospitals with shared services [16]. In this regard, there is recently little data has been presented about the distribution of carbapenemase-producing GNB on hospital inanimate surfaces in Ethiopia. Therefore, the antimicrobial susceptibility patterns and carbapenem resistance encoding genes among GNB isolates from inanimate surfaces were assessed at the TASH and ALERT hospital in Addis Ababa, Ethiopia.

Methodology

Study sites

This study was employed at the Tikur Anbessa Specialized Hospital (TASH) and all African leprosy and tuberculosis rehabilitation and training centers (ALERT) in Addis Ababa, Ethiopia, from June to September 2021. TASH, the largest tertiary hospital in the Ethiopia occupied with 700 beds, was transferred to the School by Ministry of Health and has since become a university training hospital. TASH is currently the core teaching hospital for both undergraduate and post graduate training in varies disciplines. It is also a home where advanced clinical facilities are given to the entire nation. On the other hand, ALERT is recently occupied with 240 bed, give services in the following areas such as dermatology, ophthalmology, and surgery departments, also an orthopedic workshop, and a rehabilitation program.

Inanimate surface sampling and bacterial isolates

This cross-sectional study included hundred and three Gram-negative bacterial (GNB) isolates that were previously isolated from various inanimate hospital environments (surfaces and medical equipment) [17]. Sampling was performed in the intensive care units (ICUs) and Operation Theaters (OTs) of the two hospitals. The samples were collected on a single spot using sterile cotton tip swab moistened with sterile brain heart infusion broth (BHI) (Merck, Germany) from targeted surfaces, including beds, monitors, linens, ventilators, suction machines, lobbies (furniture), work stations, and sinks, as previously described [17,18]. The surface swab samples were incubated to enrich the samples for 18-24 hours at 37°C in BHI medium [19]. Then, a loop full of bacterial suspension is streaked to MacConkey agar and incubated for 24 h at 37°C. Bacteria identification was employed using conventional microbiological procedures, such as Gram stain and a panel of biochemical tests described in the Handbook of Clinical Microbiology Procedures [20].

Antimicrobial susceptibility testing (AST) of carbapenem resistant isolates

The AST of the identified bacteria to antimicrobials was performed using the disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guideline [21]. Briefly, AST was performed on Muller Hinton agar (Oxoid, UK) to the following 13 antimicrobial groups (in µg/disk): cephalosporins [ceftazidime (30), cefotaxime (30), ceftriaxone (30), and cefepime (30)], monobactam [aztreonam (30)], penicillin [ampicillin (10)],

aminoglycosides [amikacin (30) and gentamycin (10)], fluoroquinolones ciprofloxacin (5), chloramphenicol (10), and carbapenem [meropenem (10)]. *E. coli* ATCC 25922 was used as quality control indicators. Multidrug-resistance (MDR) is stated as a strain that show resistance to at least three antimicrobial agents belonging to three different classes [22]. Once a screening criterion, such as resistance to meropenem, was found, the presence of carbapenemase was further detected using an MHT and combined-disc test (CDT) [21].

Phenotypic detection of carbapenemase production

Isolates that showed meropenem resistance were further confirmed by MHT, according to CLSI guidelines [21]. Briefly, a lawn of pre-tested carbapenem-sensitive *E. coli* was inoculated onto Mueller–Hinton agar plates and allowed to dry for few minutes. Then a 10μg meropenem disc was put in the center of the test area. Test bacteria were seeded from the edge of the disc to the edge of the petri-dish. After overnight cultivation, the plates were checked for inward distortion of a zone of inhibition (clover leaf appearance) [21].

Furthermore, a combined-disc test was performed to test metallo-β-lactamase (MBL) production. Briefly, two antimicrobial agents impregnated with meropenem (10 μg) and 5 μL of 0.5 M EDTA (Sigma, Germany), and the other disc contained merely meropenem (10 μg) were put 20 mm at a distance on a Mueller Hinton agar plate streaked with each isolated bacterium. After 18-24 h of incubation, the medium showed rise of ≥ 7 mm in the zone of inhibition of the combination disks compared to the meropenem disks considered as MBL positive [21].

Genomic DNA preparation and Multiplex polymerase chain reaction (PCR)

The genomic DNA extraction was performed by heat treatment methods as previously stated (Li *et al.,* 2016). Briefly, three to five colonies of the isolates were extracted from a tryptic soy agar (Scharlau, Spain) plate and mixed in 100μL of DNase-free water in a sterile 1.5 mL microfuge tube to get a bacterial suspension equivalent to 1-2 × 10^9 CFU/mL. The bacterial suspension was submerged in a bath of boiling water for ten minutes. The lysed suspension was then centrifuged at 14000 rpm for five minutes. Then supernatant of extract (genomic DNA) was placed to a new sterile DNase-free microfuge tube by using DNase-free tips. The total genomic DNA extract was stored at -20 °C until used, and its quality and quantity were measured using a Nanodrop (Thermo Scientific, US) [23].

Carbapenemase positivity using the phenotypic-based method was further checked for the detection of carbapenemase-encoding genes (*bla*$_{NDM}$, *bla*$_{KPC}$ and *bla*$_{OXA-48}$) using multiplex PCR from all isolates with reduced meropenem susceptibility. Amplification was performed using a 48-well thermal cycler (Applied Biosystems at Life Technologies, Foster City, CA, USA). A final volume of 15μL amplification reaction [1μL of extracted DNA, 7.5 μL of 2x QIAGEN Multiplex PCR Master Mix Kit (QIAGEN), 1 μL of (0.2 μM) forward and reverse primers and 5.5 μL of nuclease-free water] were used. The Thermal cycles of PCR were conducted at 95 °C for 15 minutes, followed by 35 cycles of DNA denaturation at 94 °C for 30 s, annealing at 58 °C for 90 seconds specific for each primer, extension at 72˚C for 90 seconds, and followed by a final extension at 72°C for 10 minutes [24]. The PCR products were visualized on a 1.5% agarose gel stained with 0.5 × Tris-borate-EDTA (TBE) buffer using a GelDoc gel imaging system (Bio-Rad). Primer sequences and amplicon sizes are listed in Table 1

Permissions and ethical considerations

Before commencing the research, approval was obtained from the Department of Microbiology, Immunology, and Parasitology Research Ethics Review Committee (Protocol no DERC/17/18/02-G) and Armauer Hansen Research Institute (AHRI) (Protocol no AH02122/0012/18). Formal letters of permission for the collection of inanimate surface samples were obtained from the respective hospitals. This study was conducted in accordance with the Declaration of Helsinki guidelines.

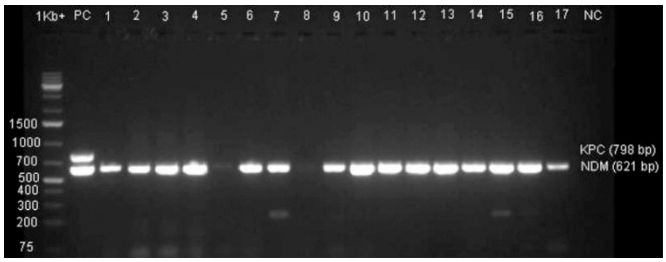
Quality control

To ensure the validity of the test reactions, PCR-grade water without DNA was used as a negative

Table 1. The primers used to amplify the selected carbapenemase genes at TASH and ALERT Hospitals, Addis Ababa Ethiopia, 2021.

Gene	Primer	Sequence (5′–3′)	Annealing Temp ˚C	Product size (bp)	Reference
***bla*$_{NDM}$**	**NDM-F**	GGTTTGGCGATCTGGTTTTC	65.5	621	[1]
	NDM-R	CGGAATGGCTCATCACGATC	67.8		
***bla*$_{KPC}$**	**KPC-F**	CGTCTAGTTCTGCTGTCTTG	57.8	798	
	KPC-R	CTTGTCATCCTTGTTAGGCG	62.2		
***bla*$_{OXA-48}$**	**OXA-48-F**	GCGTGGTTAAGGATGAACAC	65.6	438	
	OXA-48-R	CATCAAGTTCAACCCAACCG			

Figure 1. Agarose gel electrophoresis of carbapenemase gene PCR products showing samples from 1 to 17.



PC: Positive control (NDM and KPC); 1kb +: DNA ladder; 1-7: 9-17: positive samples; NC: Negative control; bp: base pair.

control for the PCR-amplified carbapenemase genes and a DNA template extracted from *E. coli* ATCC 25922 was used as a carbapenemase-negative strain. As positive control reactions template DNA extracted from a previously well characterized clinical carbapenemase-producing *K. pneumoniae* strain from a study conducted by Awoke *et al* [24] were used. All targeted DNA sequencing of PCR products and sequence amplicons were confirmed using a BLAST search at the National Center for Biotechnology Information (NCBI) (https: //blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analysis

The data were summarized, tabulated, and analyzed using Statistical Package for Social Sciences (SPSS) software version 25.

Results

Detection of carbapenem-resistant Environmental Isolates

In the present study, carbapenem resistance (CR) was detected in 62.1% (64/103) of the gram-negative isolates. CR was more frequently observed in *Acinetobacter baumannii* 24 (37.5%), *E. coli* 12 (18.8%), and *K. pneumoniae* 9 (14%). Carbapenemase production was detected in 44 (67.7%) and 7 (10.8%) of the CR isolates by CDT and MHT tests, respectively, as shown in (Table 2).

The distribution of Carbapenemase genes

PCR results showed that among the 64 CR isolates tested, 43 (67.2%) harbored carbapenemase genes. The most prevalent carbapenemase genes was *bla*$_{NDM}$ 42 (97.7%) (Figure 1). One *E. coli* isolates produced only KPC. Meanwhile, one isolates co-produced KPC and NDM while no OXA-48 were detected. The highest prevalence of *bla*$_{NDM}$ was observed in *A. baumannii* (18, 41.7%). Twenty-one CR isolates were PCR-negative, regardless of the phenotypic confirmatory method used. Overall, *bla*$_{NDM}$ was detected in *Acinetobacter baumannii* isolates (41.8%) and *E. coli* isolates (16.3%), whereas *bla*$_{KPC}$ was detected in *Acinetobacter baumannii* 1 (2.3%) and *E. coli* 1 (2.3%), respectively. The distribution of carbapenemase genes among various carbapenemase producers is shown in (Table 2).

Table 3. Distribution of carbapenemase gene-producing GNB isolated from the hospital environments at TASH and ALERT hospital, 2021.

Variables	Carbapenemase gene, n (%)
Hospitals	
TASH	29 (67.4)
ALERT	14 (32.6)
Ward type	
ICUs	29 (67.4)
OTs	14 (32.6)
Sampling points	
Sink	2 (4.7)
Bed	9 (21.4)
Linens	11 (26.2)
Suction Machine	1 (2.3)
Environmental surface	4 (9.5)
Ventilator	6 (14.3)
Lobby (furniture)	4 (9.5)
Oxygen cylinder	2 (4.7)
door knob	2 (4.7)
Telephone key	1 (2.3)
Environmental surface (Floor, Corroder, and Wall)	

Table 2. The distribution of GNB and carbapenem-resistant GNB at TASH and ALERT hospital, 2021.

Isolates	CR, n (%)	Phenotypic Carbapenemase, n (%)		No. (%) of carbapenemase gene type	
		MHT positive	CDT (MBL Positive)	NDM	KPC
E. coli (n = 12)	12 (18.8)	1 (14.2)	9 (20.5)	7 (16.3)	1
Citrobacter diverus (n = 1)	1 (1.6)	-	1 (2.3)	1 (2.3)	-
Klebsiella rhinoscleromatis (n = 1)	1 (1.6)	-	1 (2.3)	1 (2.3)	-
K. pneumoniae (n = 8)	8 (12.5)	4 (57.1)	8 (18.2)	8 (18.6)	-
Klebsiella oxytoca (n = 5)	-	-	-	-	-
Serratia spp (n = 9)	9 (14)	1 (14.2)	6 (13.6)	5 (11.6)	-
Acinetobacter baumannii (n = 48)	24 (37.5)	-	12 (27.3)	18 (41.7)	1
Enterobacter aerogenes (n = 2)	2 (3.1)	-	2 (4.5)	-	-
Pseudomonas aeruginosa (n = 16)	4 (6.3)	1 (14.2)	3 (6.8)	2 (4.7)	-
Shigella spp (n = 1)	1 (1.6)	-	1 (2.3)	1 (2.3)	-
Total (N = 103)	64 (62.1)	7 (6.8)	43 (41.7)	43 (67.2)	2

MHT: modified Hodge test; CDT: combined disk test; MBL: carbapenem-resistant.

Distribution of carbapenemase encoding genes among the various inanimate surfaces

Most of the carbapenemase-encoding genes were collected from TASH 29(67.4%) and were mainly identified in ICUs 19(65.5%). Regarding the inanimate surfaces, the highest carbapenemase-encoding genes were detected in linens 11 (26.4%), followed by bed 9 (21.4%). The linens and ventilators were colonized mainly with *Klebsiella pneumoniae* (50% vs. 33.3%) and *E. coli* (30% vs. 20%), respectively (Table 3).

Antimicrobial susceptibility profiles of carbapenemase-producing isolates

The resistance rates of carbapenemase-producing isolates for each tested antimicrobial agent are presented (Figure 2), and high resistance rates were observed in the penicillin group, such as ampicillin (97.7%); cephalosporin group, such as cefuroxime (100%), ceftriaxone (95.4%), and piperacillin tazobactam (90.7%); carbapenem group; and meropenem (100%). In contrast, low levels of resistance were observed in the aminoglycoside group, such as amikacin (25.6%), and the quinoline group, such as ciprofloxacin (20.9%), as shown in Supplementary Table 2.

Multidrug-resistance patterns of carbapenemase-producing isolates

The 13 tested antimicrobial agents were classified into 10 categories according to Magiorakos *et al*. [22] Based on this category, almost all carbapenemase-producing isolates were classified as MDR as depicted in (Table 4).

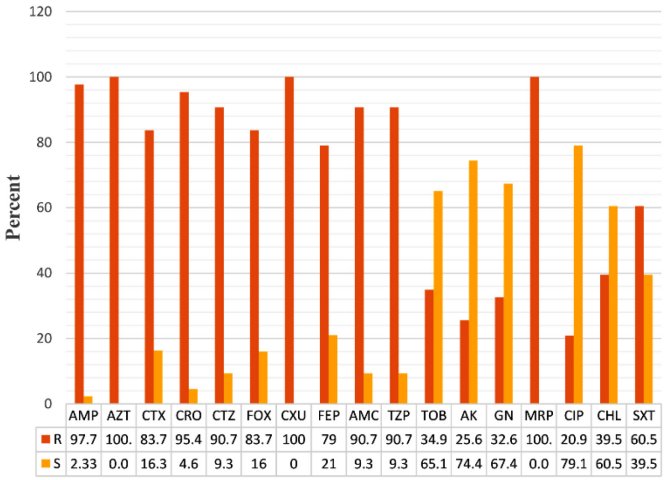
Discussion

Inanimate hospital surfaces have often been described as sources of outbreaks of multidrug-resistant (MDR) bacteria especially CR-GNB [2]. In the present study, 62.1% of the GNB isolates were carbapenem-resistant, which is similar to studies conducted in Egypt (62.1%) [11]. Our results were slightly higher than those in Tanzania (35%) from clinical isolate [4] and Algeria (48%) from inanimate surfaces [2]. Our results were lower than those of studies conducted on clinical isolates in Ethiopia by Seman *et al* [25] and Awoke *et al* [24] (17.3% and 29.6%, respectively). In the present study, the highest carbapenem resistance rate was observed in *Acinetobacter baumannii.*

This study found that CR was more commonly detected in linens and beds, and similar findings [2,26-28]. It is generally presumed that GNB needs moist places for improved durability [29] Moreover, higher levels of CR-GNB reported in our study could be related mostly to the use of ineffective disinfectants during cleaning of inanimate surfaces, and inadequate applications of basic precautions such as proper hand hygiene and contact precautions [17].

The detection of carbapenemase production by *Enterobacteriaceae* were mainly determined using MHT, Carbapenem Inactivation Method (CIM), and modified Carbapenem Inactivation Method (mCIM).

Figure 2. Antimicrobial susceptibility profile of carbapenem resistance isolates.



R: resistant; S: sensitive; AMP: ampicillin; AZT: aztreonam; CTX: cefotaxime; CRO: ceftriaxone; CTZ: ceftazidime; FOX: cefoxitin; CXU: cefuroxime; FEP: cefepime; AMC: amoxicillin and clavulanic acid; TZP: piperacillin-tazobactam; TOB: Tobramycin; AK: Amikacin; GEN: Gentamicin; MRP: Meropenem; CIP: ciprofloxacin; CHL: Chloramphenicol; SXT Sulfamethoxazole + trimethoprim

Table 4. Multidrug-resistance patterns of carbapenemase-producing isolates in the environments inanimate surface at TASH and ALERT hospital, 2021.

Isolates	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	MDR > 3 N (%)
Acinetobacter baumannii (n = 18)	-	-	-	-	-	1	5	1	4	3	4	18 (100)
E. coli (n = 8)	-	-	-	-	-	-	1	3	1	1	2	8 (100)
Klebsiella pneumoniae (n = 8)	-	-	-	-	-	2	1	1	2	1	1	8 (100)
Pseudomonas aeruginosa (n = 2)	-	-	-	-	-	-	1	1	-	-	-	2 (100)
Serratia spp (n = 5)	-	-	-	-	-	3	1	-	-	1	-	5 (100)
Klebsiella rhinoscleromatis (n = 1)	-	-	-	-	-	-	1	-	-	-	-	1 (100)
Shigella spp (n = 1)	-	-	-	-	-	-	-	1	-	-	-	1 (100)

R0: sensitive for all classes of antimicrobials, R1: resistant for one class of antimicrobials, R2: resistant for two classes of antimicrobials, R3: resistant for three classes of antimicrobials, etc., MDR = multidrug-resistant

The forementioned methods were less sensitive for the detection of carbapenemases producing *A. baumannii* acquiring to the weak expression of *NDM*-type carbapenemases [30]. This was also proven in this study as the MHT method has detected only (10.8%) of carbapenemase-producing isolates, which is in line with several studies and it is poor in detecting of *NDM* producers [31] while the combined disk test (CDT) detected (67.7%) carbapenem-resistant isolates as carbapenemase producers and good for detection of *NDM* producers [8,32]. This is because most of the resistance genes detected by molecular assays were phenotypically positive by CDT method, suggesting that these isolates possessed genes that may have been expressed [13]. Hence, the sensitivity of MHT methods were low for *NDM - 1* -producing isolates or isolates expressing weak enzyme productions [33].

The PCR results showed that among the 64 isolates tested, 43(67.2%) produced the carbapenemase gene, which is slightly higher than the data in Tanzania (35%) for clinical isolates [4]. The most prevalent carbapenemase gene detected in our study was bla_{NDM} (95.5%). This was also consistent with numerous studies conducted in various nations where MBLs, like *NDM-1*, are currently a source of great concern due to their capacity to confer a high level of resistance as well as the fact that their genes contain highly mobile genetic elements (MGEs) that aid in their spread among various bacterial species and genera [34].

Similarly, studies showed in many countries in Africa the genetic determinants of carbapenem resistance from clinical isolates were bla_{IMP}, $bla_{\text{VIM-1,}}$ $bla_{\text{SPM-l}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-23}}$ $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$ and bla_{KPC} [24,25,34].

The current study also showed the presence of *NDM-1*-type carbapenemases in *A. baumannii* which is in agreement with a study from Algeria [2]. At this time, *Acinetobacter baumannii* have been raised globally, and due to the limited availability of treatments, there have been multiple reports of nosocomial infections brought on by MDR *A. baumannii*, which represent a serious risk to hospitalized patients [16,30]. This study also revealed the presence of isolates producing carbapenemases in inanimate surfaces as main hotspot for the dissimilation and spread of resistance genes, which can be transmitted horizontally to other microbes from different sources [2] that require a one health approach [30].

Regarding the antimicrobial susceptibility of carbapenemase producers, low proportion of resistance were observed for amikacin (25.6%) and chloramphenicol (39.5%), which is in close agreement with a previous study, amikacin (20.9%) and chloramphenicol (39.3%) [24]. This may be because amikacin is not commonly prescribed to treat infectious diseases in the selected hospitals. A high resistance rate was detected for beta-lactam antimicrobials such as ampicillin (97.7%), cefuroxime (100%), ceftriaxone (95.4%), and piperacillin-tazobactam (90.7%), which is comparable to prior reports [24,25]. There are extremely few choices for treating widespread multidrug-resistant pathogens due to the long-standing use of Beta-lactam drugs as empirical therapies [11]. This is because many broad-spectrum antibiotics can be hydrolyzed or rendered inactive by isolates that produce carbapenemase; hence, a rise in their occurrence would seriously impair these antibiotics' capacity to treat infections acquired in hospitals and the community [35].

Conclusions and Recommendations

This study, revealed a high rate of carbapenem-resistance genes among GNB isolates from inanimate surfaces from the respective hospitals. Most of the carbapenemase producing isolates were detected from the bed linens. Since our hospital's surroundings and surrounding patient area are hotspots for resistant genes, we recommend regular testing for carbapenem resistance, stringent sanitation and regular controlling of the quality of disinfectant applied in the hospital environments. In addition, it would be beneficial to investigate additional antimicrobial drugs such as tigecycline and colistin as potential alternatives for treating these isolates. It is necessary to carry out more research to ascertain the molecular epidemiology and evolutionary history of strains that produce carbapenemase. Due to a lack of sufficient funding, the study did not examine the clonality of the isolates and the gene sequence.

Acknowledgements

Addis Ababa University (AAU) and the Armauer Hansen study Institute (AHRI) are acknowledged by the authors for funding this study endeavor.

Ethics

Before commencing the research, approval was obtained from the Department of Microbiology, Immunology, and Parasitology Research Ethics Review Committee (Reference no DERC/17/18/02-G) and Armauer Hansen Research Institute (AHRI) (Reference no AH02122/0012/18). Formal letters of permission for the collection of inanimate surface samples were obtained from the clinical directorates of the

two hospitals. This study was commenced in accordance with the Declaration of Helsinki guidelines.

Funding
This study was financially supported by AAU and the AHRI (Reference no AH02122/0012/18).

Availability of data and materials
The datasets supporting the conclusions of this manuscript are included within the manuscript and its supplementary files (Supplementary Table 1 and 2).

Authors' contributions
All authors worked together to complete this project. Conceptualization of the manuscript: SS, TA, AM. Formal analysis: SS. Investigation: SS, TW, AS. Methodology: SS, AM, TA, WE. Resources: AM and WM. Writing–original draft: SS. Writing, review & editing: SS, WE, WM, TA, AM, ZD, TW, AS. Each author reviewed and gave their approval for the published version.

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Conflict of interests
No conflict of interests is declared.

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Annex – Supplementary Items

Supplementary Table 1. Carbapenem-resistant GNB from inanimate surfaces at TASH and ALERT Hospital, 2021.

Code	Isolates	Sample type	Hospital	Ward	WARD	Phenotypic test		Carbapenemase genes		
						MHT	CDT	NDM	KPC	OXA-48
03TMS	*Acinetobacter baumannii*	linens	TASH	Medical-Surgical	ICU		Pos	NDM	KPC	
11TOER	*Acinetobacter baumannii*	Bed	TASH	Endo-Renal	OT		Pos	NDM		
04GI	*Acinetobacter baumannii*	Suction Machine	TASH	GIT	OT		Pos	NDM		
08TON	*Acinetobacter baumannii*	Floor	TASH	Neurology	OT		Pos	NDM		
13TMS	*Acinetobacter baumannii*	Bed	TASH	Medical-Surgical	ICU		Pos	NDM		
12TP	*Klebsiella pneumoniae*	linens	TASH	Pedatric	ICU	Pos	Pos	NDM		
12TOER	*Shigella spp*	Oxygen cylinder	TASH	Endo-Renal	OT		Pos	NDM		
06TOER	*Serratia spp*	Floor	TASH	Endo-Renal	OT		Pos	NDM		
14TM	*E. coli*	lobby (furniture)	TASH	Medical	ICU		Pos	NDM		
12PO	*Acinetobacter baumannii*	Corroder	TASH	Pediatrics	OT	Pos	Pos	NDM		
13TMS	*E. coli*	Bed	TASH	Medical-Surgical	ICU		Pos	NDM		
04TMS	*Klebsiella pneumoniae*	Bed	TASH	Medical-Surgical	ICU		Pos	NDM		
02TM	*Klebsiella pneumoniae*	Ventilator	TASH	Medical	ICU	Pos	Pos	NDM		
04TMS	*Acinetobacter baumannii*	Bed	TASH	Medical-Surgical	ICU		Pos	NDM		
13TP	*Acinetobacter baumannii*	linens	TASH	Pedatric	ICU		Pos	NDM		
16TP	*Acinetobacter baumannii*	ventilator	TASH	Pedatric	ICU	Pos	Pos	NDM		
04TP	*Acinetobacter baumannii*	linens	TASH	Pedatric	ICU		Pos	NDM		
02GI	*Acinetobacter baumannii*	Bed	TASH	GIT	OT		Pos	NDM		
12TOER	*Pseudomonas aeruginosa*	oxygen cylinder	TASH	Endo-Renal	OT		Pos	NDM		
13TP	*Klebsiella pneumoniae*	linens	TASH	Pedatric	ICU	Pos	Pos	NDM		
05TM	*Acinetobacter baumannii*	Bed	TASH	Medical	ICU	Pos	Pos	NDM		
05TMS	*E. coli*	ventilator	TASH	Medical-Surgical	ICU		Pos	NDM		
05TP	*E. coli*	Monitor	TASH	Pedatric	ICU		NEG		KPC	
12TP	*Pseudomonas aeruginosa*	linens	TASH	Pedatric	ICU		Pos	NDM		
07TMS	*Acinetobacter baumannii*	linens	TASH	Medical-Surgical	ICU		Pos	NDM		
14TS	*E. coli*	linens	TASH	Surgical	ICU		Pos	NDM		
07TOER	*Klebsiella rhinoscleromatis*	Wall	TASH	Endo-Renal	OT		Pos	NDM		
05TS	*Klebsiella pneumoniae*	ventilator	TASH	Surgical	ICU	Pos	Pos	NDM		
08TOER	*Acinetobacter baumannii*	lobby (furniture)	TASH	Endo-Renal	OT		Pos	NDM		
6ATI	*Serratia spp*	Ventilator	ALERT	Trauma ICU	ICU		Pos	NDM		
12ATI	*E. coli*	linens	ALERT	Trauma ICU	ICU		Pos	NDM		
14ATI	*Acinetobacter baumannii*	Ventilator	ALERT	Trauma ICU	ICU		Pos	NDM		
16ATI	*Acinetobacter baumannii*	Lobby (furniture)	ALERT	Trauma ICU	ICU		Pos	NDM		
16ATI	*Klebsiella pneumoniae*	lobby (furniture)	ALERT	Trauma ICU	ICU		Pos	NDM		
20ATI	*Acinetobacter baumannii*	telpone	ALERT	Trauma ICU	ICU		Pos	NDM		
22ATI	*E. coli*	Bed-ICU	ALERT	Trauma ICU	ICU		Pos	NDM		
27ATI	*Acinetobacter baumannii*	Bed-ICU	ALERT	Trauma ICU	ICU		Pos	NDM		
32ATI	*Klebsiella pneumoniae*	linens	ALERT	Trauma ICU	ICU		Pos	NDM		
32ATI	*Klebsiella pneumoniae*	linens	ALERT	Trauma ICU	ICU		Pos	NDM		
28AMO	*Serratia spp*	door knob	ALERT	Major-OR	OT		Pos	NDM		
41AMO	*Serratia spp*	door knob	ALERT	Major-OR	OT	Pos	Pos	NDM		
44AMO	*Citrobactor diverus*	Sink	ALERT	Major-OR	OT		Pos	NDM		
45AMO	*Serratia spp*	Sink	ALERT	Major-OR	OT		Pos	NDM		

Supplementary Table 2. Antimicrobial susceptibility profiles of carbapenemase-producing isolates at TASH and ALERT Hospital, 2021.

Isolates	AMP	AZT	CTX	CRO	CAZ	FOX	CXU	FEP	AMC	TZP	MRP	TOB	AK	GN	CIP	CHL	SXT
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R
Acinetobacter baumannii	S	R	R	R	R	R	R	S	R	R	R	S	S	S	S	R	S
Acinetobacter baumannii	R	R	R	R	R	R	R	S	R	R	R	S	S	S	S	R	R
Acinetobacter baumannii	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S	R	R
Klebsiella pneumoniae	R	R	S	R	R	R	R	S	R	S	R	S	S	S	S	S	S
Shigella spp	R	R	R	R	R	R	R	S	R	R	R	S	S	S	S	S	R
Serratia spp	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S
E. coli	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S
Acinetobacter baumannii	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	S	R
E. coli	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S
Acinetobacter baumannii	R	R	R	R	R	R	R	S	R	R	R	S	S	S	S	S	S
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R
Klebsiella pneumoniae	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	S	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E. coli	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
E. coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E. coli	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	R
Klebsiella rhino	R	R	R	R	R	S	R	R	R	R	R	S	S	S	S	S	S
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S	R	R
Serratia spp	R	R	R	R	R	S	R	R	R	R	R	R	R	S	S	R	R
E. coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S
Klebsiella pneumoniae	R	R	R	R	S	S	R	R	R	R	R	R	R	R	S	R	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S	S
E. coli	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R
Acinetobacter baumannii	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S
Klebsiella pneumoniae	R	R	S	R	S	S	R	S	S	R	R	S	S	S	S	S	S
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S
Serratia spp	R	R	S	R	S	S	R	S	S	R	R	S	S	S	S	S	S
Serratia spp	R	R	S	S	S	R	R	R	R	S	R	S	S	S	S	S	S
Citrobactor diverus	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	S
Serratia spp	R	R	S	S	R	R	R	S	R	S	R	S	S	S	S	S	S

R: resistant; S: sensitive; AMP: ampicillin; AZT: aztreonam; CTX: cefotaxime; CRO: ceftriaxone; CTZ: ceftazidime; FOX: cefoxitin; FEP: cefepime; AMC: amoxicillin and clavulanic acid; CHL: chloramphenicol; MRP: meropenem; AK: amikacin; GEN: gentamicin; CIP: Ciprofloxacin; SXT: sulfamethoxazole + trimethoprim.