Detection and Characterization of Carbapenem resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan

Hana S. Elbadawi
University of Khartoum
Kamal M. Elhag
University of Khartoum
Elsheikh Mahgoub
University of Khartoum
Hisham N Altayb
University of Khartoum
Francine Ntoumi
FCRM
Linzy Elton
University College London Institute for Global Health
Timothy D McHugh
University College London Institute for Global Health
Mohamed Osman
York University
John Tembo
University of Zambia
Giuseppe Ippolito
Institut mikrobiologii imeni S N Vinogradskogo RAN
Alimuddin Zumla
University College London Institute for Global Health
Muzamil Mahdi Abdel Hamid (mahdi@iend.org)
University of Khartoum https://orcid.org/0000-0002-6157-4388

Research

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Abstract

Background:

Antimicrobial resistance (AMR) poses a threat to global health security. Whilst over the past decade, there has been an increase in reports of nosocomial infections globally caused by carbapenem resistant Gram-negative bacilli (GNB), data from Africa have been scanty. We performed a study of carbapenem resistance genes among GNB isolated from patients treated in hospitals in Khartoum state, Sudan.

Methods:

A cross-sectional study was conducted at Soba University Hospital (SUH) and Institute of Endemic Diseases, University of Khartoum for the period October 2016 to February 2017. A total of 206 GNB isolates from different clinical specimens were analyzed for carbapenem resistance genes using phenotypic tests and affirmed by genes detection. Multiplex PCR was performed for each strain to detect the carbapenemase genes, including the *bla*NDM, *bla*VIM, *blaIMP*, *bla*KPC, and *bla*OXA-48. In addition to *bla*CTXM, *bla*TEM and *bla*SHV. DNA sequencing and bioinformatics analysis were used to detect genes subtypes.

Findings:

Of 206 isolates, 171 (83%) were confirmed resistant phenotypically and 121 (58.7%) isolates were positive for the presence of one or more carbapenemase gene. New Delhi metallo-β-lactamase (NDM) types were the most predominant genes, *blaNDM* 107(88.4%). Others included *blaIMP* 7 (5.7%), *blaOXA-48* 5(4.1%), *blaVIM* 2 (1.6%) and *blaKPC* 0 (0%). Co- resistance genes with NDM producing GNB were detected in 87 (81.3%) of all *bla*NDM positive isolates. A significant association between phenotypic and genotypic resistance was observed (P- value < 0.001). NDM-1 was the most sub type was observed in 75 isolates (70 %), other subtypes were NDM- 5 and NDM-6. Infections due to Carbapenem resistant GNB are increasing at SUH, with the *bla*NDM being the prevalent genes among clinical isolates and belong to the Indian lineage.

Conclusions:

The frequency of carbapenemase producing bacilli was found to be improperly high in Khartoum hospitals. NDM was found to be the most prevalent carbapenemase gene among clinical isolates. Close surveillance across all hospitals in Sudan is required. The relative distribution of Carbapenemase genes among GNB in nosocomial infections in Africa needs to be defined.

Introduction

The prevalence and distribution of antimicrobial resistant bacterial infections in the nosocomial setting in Africa is poorly defined (1, 2). Carbapenems are important broad-spectrum β-lactam antibiotics widely prescribed for the curing of multidrug-resistant Gram negative bacilli in systemic infections.

Carbapenems have been considered as a robust antibiotic to treat the extended spectrum β-lactamases (ESBLs) in the past ten years (3). ESBLs are one of the most common β-lactamases encoding resistance genes distributed among Gram negative bacilli through plasmids and transposons (4) and the novel β-lactamases with direct carbapenem-hydrolyzing activity has contributed to an increased prevalence of carbapenem resistant *Enterobacteriaceae* (CRE), which is causing therapeutic failure worldwide (5). Carbapenemase enzymes include New Delhi Metallo-beta-lactamase (blaNDM), veron integron metallo-beta-lactamases (blaVIM), imipenemase (blaIMP), *Klebsiella pneumoniae* carbapenemases (blaKPC), and oxacillinase-48 (blaOXA-48) (6). These enzymes are encoded by what is termed carbapenem resistance determining genes (CRDG), which hydrolyze β-lactam drugs including carbapenems and other β-lactam agents (7). Moreover resistance to carbapenem can occur by other mechanisms including overproduction of ESBL or AmpC enzyme in combination with porin mutations by reduced outer membrane permeability and activation of multidrug efflux pumps in response to antibiotic exposure (8). Carbapenem resistance genes enhance the mechanism of antibiotic resistance among *Enterobacteriaceae* and non-lactose fermenting Gram-negative bacilli in consequence of the selective pressure assessed by inadequate use of carbapenem and third generation cephalosporins (9). Moreover plasmids coding for carbapenemases may carry co-resistance genes for other β-lactam and non β-lactam antibiotics (7).

Detection of carbapenemase producing isolates by clinical Microbiology laboratory is essential to provide appropriate therapy and update therapeutic guide-lines for the clinicians. Furthermore the use of molecular analysis to detect resistance genes provides evidence on clinically observed treatment failure. Whilst over the past decade, there has been an increase in reports of nosocomial infections globally caused by carbapenem resistant Gram-negative bacilli (GNB), data from Africa have been scanty and antimicrobial stewardship is not optimally practiced. We performed a study of carbapenem resistance genes among GNB isolated from patients treated in hospitals in Soba University Hospital Khartoum state.

Methods

Study design and clinical Isolates

A cross-sectional laboratory based study was conducted at the Microbiology department in Soba University Hospital and institute of Endemic Diseases, University of Khartoum; involving Gram negative clinical bacterial isolates, suspected as carbapenemase producing based on carbapenem sensitivity testing zone inhibition (zone size less than 20 mm). These were isolated from cultures of varios clinical specimens: blood, urine, wound swabs, sputum, tips of catheters, and other body fluids, between 1st October 2016 and 25th February 2017 from hospitalized patients in Soba University Hospital. Quality control strains [*E. coli* (ATCC #25922) and *P. aeruginosa* (ATCC #27853)] used in antimicrobial susceptibility testing and the biochemical test had been used for primary identification (10). Molecular identification (PCR) was used for all study isolates to confirm the biochemical identification using species specific primers for *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and

Acinetobacter *baumannii.* A universal primer (16SrRNA) was used for identification of unknown species by biochemical tests. **Table.1**. All isolates were stored in 20% glycerol at -20 °C until use.

Subculturing and Disk Diffusion Susceptibility Testing

Isolates were subcultured on blood agar (BA) and then subjected to susceptibility testing to the following antimicrobials: amoxycillin clavulanate (AMC) (30 µg); cefuroxime (CXM)(30 µg); cephalexin (CL)(30 µg); ceftriaxone (CRO)(30 µg); ceftazidime (CAZ) (30 µg); cefotaxime (CTX)(30 µg); meropenem (MEM) (10 µg); imipenem (IPM) (10 µg); amikacin (AK)(30 µg); gentamicin (Gen)(10 µg); ciprofloxacin (CIP) (5 µg); trimethoprim-sulfamethoxazole (SXT)(25 µg); temocillin (TEM)(30 C); azteroname (AZT)(30 µg). The Kirby Bauer (disk diffusion) was performed; each isolate was swabbed on to Muller-Hinton agar and the antibiotic discs were placed on top, incubated at 37° C for 18–24 hours and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (11).

Phenotypic screening and confirmatory test for carbapenemase

Bacterial isolates were screened for carbapenemase production according to CLSI guidelines (CLSI, 2017). In this method, carbapenem, meropenem (MEM) and imipenem (IPM) discs (10 g, each) (Mast Diagnostic, UK) were used. Isolates that showed a zone of inhibition ≤ 20 mm in diameter for meropenem were considered as suspected carbapenemase producers. Phenotypic confirmatory test for carbapenemases production were applied by boronic acid synergy test for class A β-lactamases, the EDTA synergy for Metallo-β-lactamase and the Modified Hodge Test (MHT) for detecting KPC and OXA-48 producers (12).

Detection of Carbapenemase encoding genes

PCR was carried out using a thermal cycler and the following primers (Macrogen, Korea), *bla* VIM, *bla IMP, bla NDM, bla NDM*-1, *bla KPC, bla OXA-48, bla*TEM, *bla* SHV and *bla* CTX-M genes **Table 2**. The reaction was carried out in a total reaction volume of 25 µl (5 µl Master mix of Maxime RT premix kit) (8). The purity and integrity of each PCR product was evaluated and the specific amplified product was detected by comparing with standard DNA ladder.

DNA Sequencing

The PCR product of *bla* NDM genes and 16srRNA were purified and Sanger sequencing was performed by Macrogen Company (*Seoul, Korea*).

Bioinformatics Analysis

First of all we ensure the ambiguous sites are correctly called and determined the overall quality of the sequences proofed the nucleotides chromatogram by using Finch TV software version 1.4.0 (http://www.geospiza.com/Products/finchtv.shtml). Then nucleotides sequences of the NDM genes achieved were searched for sequence similarity using nucleotide BLAST (13) (http:

//blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment for highly similar sequences, were retrieved from NCBI using the MEGA version 7 software (14). Phylogenetic tree of *bla* NDM genes and their evolutionary relationship with those obtained from database were conducted using MEGA version 7 (14).

Statistical analysis

Data were analyzed using SPSS software version 20.0. Cross tabulation was used to present the relationships between data of antimicrobial sensitivity, phenotypic tests and resistant genes detection among the study isolates, qualitative data were performed through χ2test, and significance was set at $p \leq 0.05$.

Results

Demographic distribution

The demographic characteristics of patients under study and the frequency of GNB isolates according to age groups are shown in (**Figure.**1). The most of the isolates were from neonates less than one year old in 42.5% followed by adult group between 13–80 years 38.3% and 19.5% for age group between 1–12 years. Males 53.4% (110/206) were predominant among admitted wards patients and females 46.6% (96/206).

Antimicrobial susceptibility

The antibiotic resistance pattern is shown in (Fig. 2). Out of 206 isolates tested, the highest percentage of resistance 98%, 93.5% were found in ampicillin and cephalexin respectively, followed by amoxicillin clavulanic acid 90%, cefotaxime 89.7%, ceftriaxone 88.4%, ceftazidime 84.2% and aztreonam 66%, temocillin 64%, Sulfamethoxasole-trimethoprim 78.4% and nitrofurantoin 75.2%. The resistance rate was also higher in ciprofloxacin 83.1%, gentamicin 85% and amikacin 70%. High resistant rate associated with meropenem 63.1% and imipenem 61.6%.

Prevalence of carbapenemase producing Gram-negative bacilli based on phenotypic tests

Carbapenemase activity was detected in 171 (83%) of the 206 clinical isolates, which were positive for the production of one or more carbapenemase enzymes by phenotypic methods as the following 24 (14%) by MHT method and Boronic acid screen, 105 (61.5%) by the EDTA test and 42 (24.5%) of the isolates were positive for both EDTA and Boronic acid methods. Details of the carbapenemase activity among different isolates by phenotypic tests are shown in **Table 3**. This suggests that the MBL type is the most prevalent type of carbapenemase hydrolysis enzyme among Gram-negative bacilli in Khartoum state, OXA and KPC types are present at a low level.

Prevalence and distribution of Carbapenemase genes among Gram negative bacilli

Carbapenemase genes were detected in 121 (58.7%) of the 206 study isolates using PCR, one or more carbapenemase genes were detected in the isolates. *bla*NDM was the most commonly detected among the isolates, mainly in *K. pneumonia*, which was the species with the highest number of these genes. *bla*NDM was also detected more often in *A. baumannii*, *P. aeruginosa* and *E. coli*. The most prevalent gene was *blaNDM* 107(88.4%), followed by *blaIMP* 7 (5.7%), *blaOXA-48* 5(4.1%), *blaVIM* 2 (1.6%) and *blaKPC* 0 (0%). ESBL were detected among these isolates, it showed a high prevalence in 183 isolates (88.8%) as the following *bla*CTXM 127(61.6%), *bla*SHV 84(40.7%) and *bla*TEM 80(38.8). The genes were unevenly distributed among the different study isolates. For more details, see **Table 4**.

Co resistance genes carried with NDM gene among Gram-negative bacilli

Many isolates carried more than one gene with *bla*NDM gene. Co-resistance carbapenemase genes were observed in a small number of isolates, *bla*NDM + *bla*OXA-48 were detected in three isolates, while *bla*NDM + *bla*VIM and *bla*NDM + *bla*IMP were detected in two different isolates. On the other hand, ESBL were often observed together with *bla*NDM in 87 (81.3%) of *bla*NDM positive isolates (107). Most of the isolates carried *bla*NDM with one ESBL gene in (43.5%) as the following; *bla*NDM + *bla*CTXM in (24 isolates, 27.6%), *bla*NDM + *bla*TEM (8 isolates, 9.1%), and *bla*NDM + *bla*SHV (6 isolates, 6.8%). Isolates carried *bla*NDM with two ESBL genes in (39.2%) as the following: *bla*NDM + *bla*CTXM + *bla*SHV (10 isolates, 11.5%), *bla*NDM + *bla*CTXM + *bla*TEM (10 isolates, 11.5%), *bla*NDM + *bla*SHV + *bla*TEM (14 isolates, 16.2%). Isolates carried *bla*NDM with three ESBL genes, *bla*NDM + *bla*CTXM + *bla*SHV + *bla*TEM (15 isolates, 17.3%). The distribution of co resistance genes among different Gram negative bacilli is shown in **Table 5**.

The frequency of carbapenemase producer Gram-negative bacilli by type of specimens, hospital units and bacteria species isolates.

Carbapenemase producing isolates were more frequently distributed among clinical specimens including blood (36%) and then followed by wounds (24%), urine (21%), body fluids (7%), catheter tips (6%) and sputum samples were (6%).

With regard to the distribution of Carbapenemase producer among hospital units, the most carbapenemase producing isolates associated with patients were found in the neonatal intensive care unit NICU 32(26%), followed by medicine wards 26(22%) and pediatric wards 22 (18%), Surgery 18(15%), ICU 15(12%) and Renal Unit 8(7%). Carbapenemase genes were predominant in *Klebsiella pneumoniae* isolates: 70(42.6%), followed by *Pseudomonas aeruginosa*: 33(20%); *Acinetobacter baumannii*: 30(18.2%) and *Escherichia coli* 18(10.9%)

Molecular characterization of NDM genes

Out of 107 *bla*NDM genes detected 75 (70%) were *bla*NDM-1. Other subtypes of *bla*NDM were identified by sequencing including *bla*NDM- 5, and *bla*NDM-6. (Fig. 3)

Bioinformatics analysis of blaNDM genes

Fourteen samples were sequenced by and all showed 97–100% similarity with *bla*NDM genes from the NCBI database with accession number MF379688 and MG764089.

Multiple sequence alignment:

The Nucleotide sequences of NDM Deoxyribonucleic acid (DNA) sequences were compared against the DNA databank using BLASTp. Fourteen NDM beta-lactamases genes were compared against the NDM in the database, (http://blast.ncbi.nlm.nih.gov/Blast.cgi), they have produced a significant alignment to NDM-1 beta-lactamase of *Klebsiella pneumoniae* (gb|MK425054|), and the isolates were shown to have 97–100% identity.

When multiple sequence alignment of NDM proteins was undertaken using MEGA7 software version 7.0.9.0 against similar proteins that obtained from BLASTp, NDM-1 from Sudan were similar to |KX100583.1| *Escherichia coli* NDM-1 (blaNDM) gene from India and | MH891562| *Klebsiella pneumoniae* NDM-1 from Bangladesh. NDM-5 from Sudan were similar to | MH991817 |, *Escherichia coli* NDM-5 from India and | MH168510| *Klebsiella pneumoniae* NDM-5 from Bangladesh while NDM-6 from Sudan were similar to | MH683607 | *Escherichia coli* from India, | JN967644 | *Escherichia coli* from the United States and | JQ235754 | *Escherichia coli* from New Zealand.

Nucleotide sequence accession number

The sequence of the 14 NDM genes have been deposited in the GenBank database under accession numbers MK033562, MK033563, MK033564, MK363705, MK363706, MK363707, MK363708, MK363709, MK363710, MK371542, MK371543, MK371544, MK37154 5, and MK371546.

Phylogenetic tree

The phylogenetic analysis of the NDM proteins sequences revealed that the NDM-1 and NDM-5 were related to the same NDM lineage as the Indian and Bangladeshi isolates. The NDM-6 gene was found to be close to NDM-6 from India, New Zealand, and the United States as shown in **Fig. 3.**

Discussion

Carbapenems have become the drugs of choice for the treatment of severe nosocomial infections caused by Gram-negative bacilli; however, carbapenemase producing Gram-negative bacilli have been reported worldwide. Carbapenem resistant *Enterobacteriaceae* (CRE) are a considerable health problem worldwide and associated with increased mortality, therefore rapid detection of carbapenem resistance and adequate treatment of such cases is mandatory. This study was undertaken, to determine the prevalence of different types of carbapenemase producing bacteria among Gram-negative bacilli isolated from various hospitalized patients in Khartoum State. The accurate detection of carbapenemase producing microorganisms is challenging for laboratories, it not only requires phenotypic tests but also genotypic tests to detect of all genes associated with carbapenemase production. In the present study, among 206 isolates 171(83%) were positive by phenotypic analysis including isolates with resistance to carbapenem,

whilst, genotypic analysis detected 121 (58.7%) positive isolates. This finding indicates that the studied carbapenem resistance is not only associated with enzyme encoding resistant genes but also due to other resistance mechanisms such as overproduction of ESBLs, porin loss or mutation (15, 16).

The current situation according to this study, show that the prevalence of carbapenemase production among different Gram negative isolates is increasing up to (83%). This finding is higher than the incidence in a previous study conducted in Khartoum state in 2017 which showed the prevalence was 56% by phenotypic tests (unpublished data) and other done in 2013 by Ali reported the MBL was 37.7% among *Pseudomonas spp.* isolates in Khartoum state (unpublished data). This high frequency of MBL in Khartoum state is a result of the excessive use of meropenem in the treatment of patients associated with ESBL infections. This finding agrees with a study in Egypt, which reported that carbapenem resistance rate was 62.7% among *Enterobacteriaceae* (17). High rates of carbapenem resistance haves also been observed in Uganda in a study conducted by Okoche in 2015. He found 28.6% of isolates were produced carbapenemases (18). In Tanzania the prevalence of carbapenemase producing isolates was 35% (19), in South Africa it was found to be 68% (20). and in Nigeria 11.9% (21). Carbapenem resistance in low and middle income countries in Africa are likely to be increase as long as the use of antibiotics in these countries remains unrestricted and most people consume the antibiotics without a clinical prescription (22).

Carbapenemase genes have been recognized during the past ten years, and these genes are associated with mobile genetic elements that allow their rapid circulation among bacterial isolates, for instance, *bla*NDM have a potential for rapid spread within the country and to other countries (23). In this study, carbapenemase genes were detected by using PCR in 121 (58.7%) of the study isolates. The most prevalent gene among the isolates was *bla*NDM (88.4%) mainly in *K. pneumonia* and other Gram negative bacilli including *A. baumannii*, *P. aeruginosa* and *E. coli*, this agrees with studies in India that reported the *bla*NDM gene was observed between 31% and 55% of Carbapenemase resistant *Enterobacteriaceae* (24, 25), and a study in South Africa published the most carbapenemase gene was *bla*NDM among *K. pneumonia* (20). *Bla*NDM-1 was reported as the most common carbapenemase gene in Saudi Arabia and other Middle Eastern countries (26).

Carbapenemase genes are reported to be more frequent in some regions. For example *bla*KPC genes are dominant in some countries such as Greece, Israel, and USA, while *bla*NDM genes are prevalent in isolates reported from the Far East, India, and Pakistan (16). Carbapenemase production in Turkey mostly occurs in *bla*OXA genes (23). OXA-48 was reported first from Turkey, then followed by reports from Middle Asia and Europe (27). In this current study the genes were unevenly distributed among the different study bacterial isolates. In this study, the *bla*NDM gene was found in high prevalence (88.4%) compared to other genes, such as *blaIMP* (5.7%), *blaOXA-48* (4.1%), *blaVIM* (1.6%) and *blaKPC* (0%). Our finding disagrees with many studies, for instance in the Okoche study, the most common gene was *bla*VIM 1(0.7%), and *bla*NDM-1 (2.6%) was the lowest gene (18), while Mushi reported IMP types were the most predominant at (21.6%) in his study (19). Other studies reported *blaOXA-48* was the most prevalent gene

(28, 29). In this study *bla*KPC wasn't detected among the isolates, which that disagrees with global reports of high prevalence of *bla*KPC genes among international isolates (16, 30).

The *bla*NDM-1 gene was first identified in a clinical isolate of *K. pneumoniae* in New Delhi, India, and has since been disseminated around the world (31). NDM variants have been described, differing by several amino acid changes. A first variant, *bla*NDM-2, has been described in an *A. baumannii* clinical isolate from an Egyptian patient in Germany, *bla*NDM-4, *bla*NDM-5 and *bla*NDM-6 have been detected from *E. coli* in India and *bla*NDM-7 from *E.coli* in France (31). In this study, 107 *bla*NDM producer isolates had been identified using PCR, the most common subtype 75 (70%) was *bla*NDM-1. Other subtypes of *bla*NDM were detected by sequencing including *bla*NDM- 5, and *bla*NDM- 6 among different Gram negative bacilli including *K. pneumoniae, E. coli, A. baumannii, P. aeruginosa* and *Enterobacter spp.*

Carbapenemase genes are becoming highly distributed among *Enterobacteriaceae*, *A. baumannii*, *P. aeruginosa* and other Gram-negative bacilli. The prevalence of carbapenemase production in each species in this study was highest in *A. baumannii* (37.3%) followed by *K. pneumoniae* (27.3%), *P. aeruginosa* (24.8%) and *E. coli* (21.1%). This agrees with many studies that reported *A. baumannii* and *K. pneumonia* were the most predominant carbapenemase producing isolates (32, 33). The prevalence of carbapenemase producing isolates varies from hospital to hospital. This variation could be attributed to differences in collection time of isolates and differences in study populations and designs. A study in Turkey showed the most carbapenemase isolates were *K. pneumoniae* (13.6%), *Pseudomonas spp.* (17.8%), *A. baumannii* (13.8%), *S. maltophilia* 7.5% and *E. coli* 2.8% (34). In Nigeria the highest prevalence of carbapenemase producers was in *P. mirabilis* (16.0%), then *P. aeruginosa*, *K. pneumoniae* (13.3% each) and *E. coli* (11.5%) (21), while in Tanzania, *E. coli* was the most prevalent species with carbapenemase production (14%), followed by *K. pneumoniae* (10.57%), *P. aeruginosa* (10.13%), *K. oxytoca* (1.76%) and *A. baumannii* (1.3%) (19).

Carbapenemase-encoding genes had been commonly associated with bacteria isolated from blood, urine, wounds, and sputum as reported in many studies in Uganda (19), Tanzania (35), Nigeria (21), and India (36). In this study Carbapenem producers were more frequently isolated from blood (39%) followed by wounds (25%) and urine (22%). This is in line with a study in South Africa which reported blood was the most common specimen type (25%), followed by urine (22%) (20).

Many studies considered young patient age as a risk factor for CRE infection which agrees with current finding, that carbapenemase-producing Gram negative bacilli were most frequent in neonate age group isolated from nursery and pediatric wards (26% and 18% respectively). High rates of carbapenem resistant infections were observed among elderly patients from medicine (22%) and ICU (12%), which agrees with another study that found that CRE to be more frequently isolated in the elderly (37).

Carbapenem resistant Gram negative bacilli are usually resistant to other routinely used antimicrobial agents (38–40). The plasmids carrying carbapenemase genes like *bla*NDM-1 are diverse and can harbor a high number of additional resistance genes (e.g., ESBL-alleles) as well as other carbapenemase genes like *bla*Oxa-48, *bla*VIM. These plasmids were considered as the source of multidrug resistance in one

single bacterium (25, 41). Moreover, mechanisms of resistance to β-lactam by producing ESBL, AmpC and carbapenemase were also noticed among the isolates that produce different combinations of the enzymes. In this study co-resistance of *bla*NDM with *bla*OXA-48, *bla*VIM and *bla*IMP were reported in few isolates. In connection to co-resistance with ESBL, *bla*CTXM, *bla*SHV and *bla*TEM was detected in high prevalence 87/107 (81.3%) of *bla*NDM positive isolates. Most of the isolates carried *bla*NDM with one ESBL gene (43.5%), *bla*NDM with two ESBL genes (39.2%) and *bla*NDM with three ESBL genes (17.3%). This agrees with various studies which have reported co-resistance among clinical isolates (42, 43). These co-production genes among some isolates as observed in this study are indicative of the existence of multi-drug resistant pathogens, which are responsible for treatment failure and outbreaks of infections. which impact on treatment outcomes and higher treatment costs (44).

Sudan is a large country which shares its borders with other seven countries. People move freely between these borders with the potential of passaging antibiotic resistance strains. The dynamic movements of the people will make it challenging to monitor AMRs in these countries especially at the boarders. These challenges may also represent an opportunity for a wider continental monitoring collaboration between the countries rather than country- specific. Such approach will aid in universal and intergovernmental initiative to control and limit spread of AMRS.

Conclusion

The increasing numbers of carbapenemase producing bacilli isolated, particularly with the *bla*NDM carbapenemase genes among clinical isolates and belong to Indian lineage. Improved antibiotic stewardship and infection control measures, and close surveillance across all hospitals in Sudan is required. The relative distribution of distribution of Carbapenemase genes among GNB in nosocomial infections in Africa requires to be defined.

Declarations

Ethics approval and consent to participate:

Formal permission was obtained from the managers of Soba University Hospital and the Institutional Research Ethics Committee of the Institute of Endemic Diseases, University of Khartoum, approved this study under reference number IEND_REC 12/2017. Patient consent was waived by the Research Ethics Committee.

Consent for publication:

not applicable

Availability of data and materials

The data for the present study, including the genotypes, isolation location and resistance status of each bacterial isolate, will available under request.

Competing interests

All authors have an academic interest in AMR. All the authors declare no other conflicts of interests.

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Authors' contributions

HE, KE and MA designed the study. HE carried out the microbiological analysis. HE and HA analysed the data. HE and MA wrote the first draft. MA, KE, EM, HA, FN, LE, TM, MO, JT, FV, GI and AZ were major contributors in revising the manuscript critically for important intellectual content. AZ revised the final draft. MA supervised the work. All authors read and approved the final manuscript.

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Tables

Table 1. Primer used for PCR bacterial identification.

Species specific primers	Primer (5' to 3')	Tm (°C)	Band size (bp)	Ref
Universal 16S rRNA	F=AGAGTTTGATCCTGGCTCAG	58		(45)
	R= CTACGGCTACCTTGTTACGA	58		
K. pneumoniae	Pf = ATTTGAAGAGGTTGCAAACGAT	56		(46)
	Pr1=TTCACTCTGAAGTTTTCTTGTGTTC	56	130	
	Pr2 = CCG AAG ATG TTT CAC TTC TGA TT	56	260	
E.coli	ECA75F=GGAAGAAGCTTGCTTCTTTGCTGAC	52	544	(47)
	ECR619R=AGCCCGGGGATTTCACATCTGACTTA	52		
Pseudomonas spp.	PA-SS-F =GGGGGATCTTCGGACCTCA	58	956	(48)
	PA-SS-R =TCCTTAGAGTGCCCACCCG	58		
Acinetobacter	sp4F =CACGCCGTAAGAGTGCATTA	58	294	(49)
	sp4R=AACGGAGCTTGTCAGGGTTA	58		
Acinetobacter baumannii	13TU,sp2F=GTTCCTGATCCGAAATTCTCG	58	490	

Table 2. Primers used for amplification of resistance genes.

Resistance Genes	Primer (5' to 3')	Tm (°C)	Band size (bp)	Ref
BlaIMP	F=GGAATAGAGTGGCTTAATTCTC	54	189	(50)
	R=CCAAACCACTACGTTATC	54		
*Bla*VIM	F=GGTCTCATTGTCCGTGATGGTGATGAG	56	272	(50)
	R=CTCGATGAGAGTCCTTCTAGAG	56		
*Bla*NDM	F=GGTTTGGCGATCTGGTTTTC	56	621	(50)
	R=CGGAATGGCTCATCACGATC	56		
*Bla*NDM-1	F=TCTCGACATGCCGGGTTTCGG	56	580	(51)
	R=ACCGAGATTGCCGAGCGACTT	56		
*Bla*KPC	F=ATGTCACTGTATCGCCGTCT	58	893	(52)
	R= TTTTCAGAGCCTTACTGCCC	58		
*Bla*OXA-48	F=TTGGTGGCATCGATTATCGG	58	745	(52)
	R=GAGCACTTCTTTTGTGATGGC	58		
*Bla*CTXM	F = SCSATGTGCAGYACCAGTAA	58	449	(53)
	R = CCGCRATATGRTTGGTGGTG	58		
*Bla*TEM	F= TCG GGG AAA TGT GCG CG	57	971	(54)
	R = TGC TTA ATC AGT GAG GCA CC	57		
*Bla*SHV	F =GGTTATGCGTTATATTCGCC-3	57	797	(54)
	R=TTAGCGTTGCCAGTGCTC	57		

Table 3. Frequency of carbapenemase producing among Gram-negative bacilli by phenotypic tests.

	Positive isolates for a particular phenotypic test				Total
Bacterial Isolates	EDTA	MHT+BA	EDTA+BA	EDTA+AB+MHT	
	N (%)	N (%)	N (%)	N (%)	N (%)
E.coli (n=28)	17 (16.4)	2 (8.3)	3 (11.1)	4 (26.6)	26 (15.2)
Klebsiella spp. (n=82*)*	41 (39)	10 (41.6)	12 (44.4)	8 (53.3)	71 (41.5)
Pseudomonas spp. (n=31)	19 (18)	9 (37.5)	4 (14.9)	1 (6.6)	33(19.2)
Acin. baumannii (n=36)	19 (18)	3 (12.6)	6 (22.2)	2 (13.3)	30 (17.7)
Burkholderia cepacia (n=3)	2 (1.9)	0 (0)	0 (0)	0 (0)	2 (1.2)
Enterobacter spp. (4)	2 (1.9)	0 (0)	0 (0)	0 (0)	2 (1.2)
Other GNB (n=16)	5 (4.8)	0 (0)	2 (7.4)	0(0)	7 (4)
Total (n=206)	105(50.9)	24 (11.6)	27 (13.1)	15 (7.2)	171

(Other Gram-negative bacilli include *Citrobacter species, Serratia species, Proteus spp., Stenotrophomonas maltophilia, Vibrio vurneficus and Morganella morganii)*

EDTA positive = Metallo-β-lactamase.

-MHT positive = KPC +OXA48 - Boronic acid positive = KPC

-MHT + Boronic acid positive = KPC -MHT positive + boronic acid negative = OXA 48

Bacterial isolate	Carbapenemase genes					ESBL genes		
	NDM	OXA-48	IMP	VIM	KPC	CTXM	SHV	TEM
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
K. pneumoniae (n=82)	59 (55)	1 (20)	3 (42.8)	1 (50)	0 (0)	53 (41.7)	28(33.4)	32 (40)
E.coli (n=29)	9(8.4)	1 (20)	2 (28.5)	0 (0)	0 (0)	14 (11.6)	6 (7.2)	4 (5)
Pseudomonas Spp. (n= 46)	14(13)	2 (40)	2 (28.5)	1 (50)	0 (0)	28 (22.3)	18(21.5)	16 (20)
A. baumannii (n=36)	17 (15.3)	1 (20)	0 (0)	0 (0)	0 (0)	20 (15.7)	24(28.6)	18 (22.5)
Burkholderia (n=2)	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.6)	2 (2.4)	4 (5)
Enterobacter Spp. (n=2)	2 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.6)	1(1.2)	1 (1.25)
Other GNB (n= 11)	7 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)	7 (5.5)	4 (4.7)	5(6.25)
Total (n=206)	107 (51.9)	5(2.4)	7 (3.4)	2 (0.9)	0 (0)	12 7(61.6)	84 (40.7)	80 (38.8)

Table 4. Distributions of carbapenemase and ESBL genes among GNB isolates.

(Other Gram-negative bacilli include *Citrobacter species, Serratia species, Proteus spp., Stenotrophomonas maltophilia, Vibrio vurneficus and Morganella morganii)*

Table 5. Co resistance genes with *bla*NDM among gram-negative bacilli.

Bla genes	*K. pn*	*E.coli*	*P. aer*	*A. bau*	*Enter*	Total N (%)
NDM+CTXM	15	2	5	2	0	24 (27.6%)
NDM+SHV	2	0	2	2	0	6 (6.8%)
NDM+TEM	4	1	1	1	1	8 (9.1%)
NDM+CTXM+ SHV	7	0	1	1	1	10 (11.5%)
NDM+CTXM+ TEM	6	2	0	2	0	10 (11.5%)
NDM+SHV+ TEM	7	0	1	6	0	14 (16.2%)
NDM+ All	9	1	3	2	2	15 (17.3%)
Total	50	6	13	16	2	87 (100%)

(*K. pn = Klebsiella pneumoniae, P. aer = Pseudomonasa aeruginosa,*

A. bau = Acinetobacter baumannii, Enter= Enterobacter spp.)

Figures

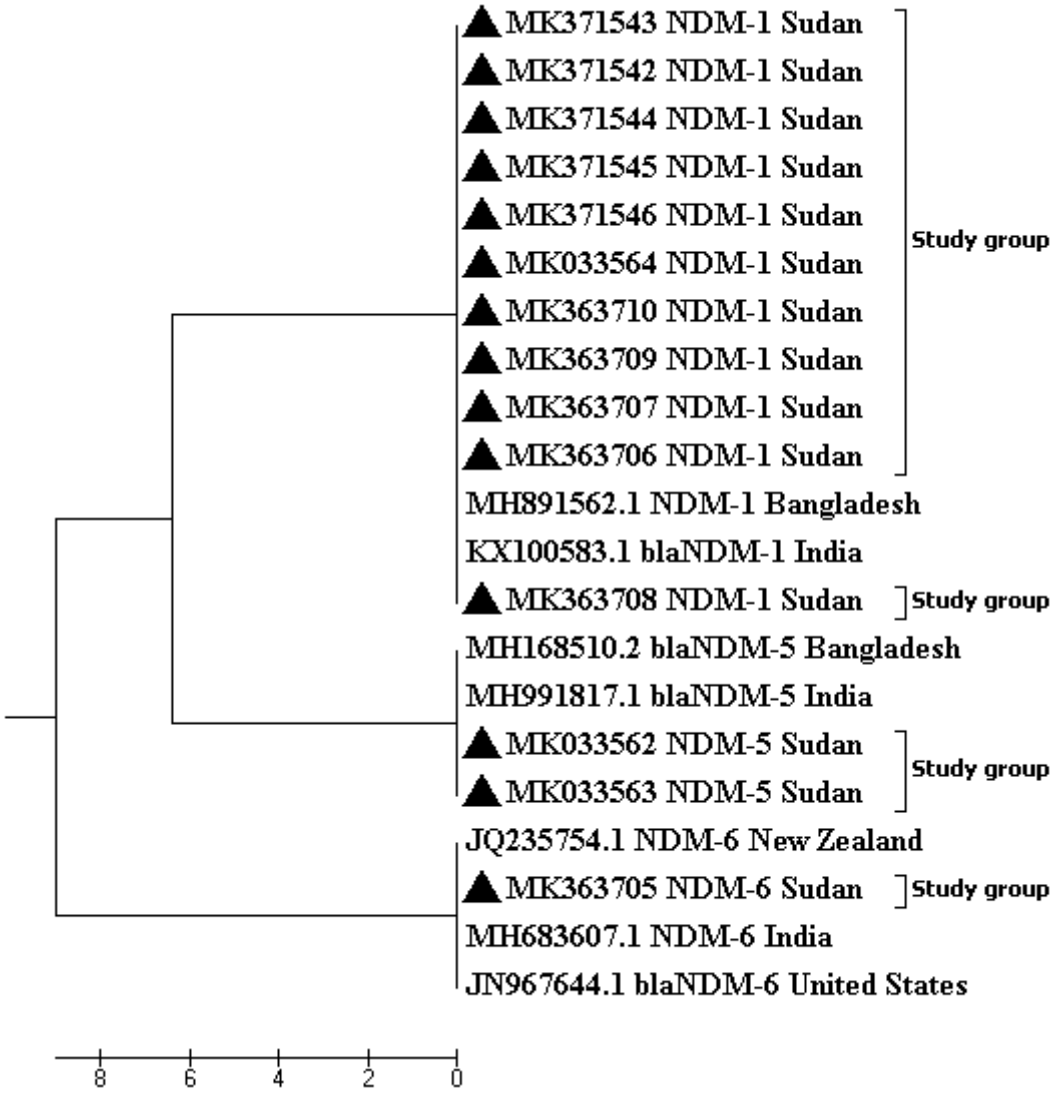

Figure 1

Phylogenetic tree of the 14 NDM isolated from different Gram-negative bacilli. Phylogenetic tree of the 14 NDM genes, Sequences were analysed using MEGA7, the neighbor-joining method, and bootstrap analysis (1,000 replicates) based on the ClustalW algorithm. The scale bar indicates 0.1 nucleotide substitutions per site. Reference sequences shown as: accession number, gene subtype, country. Sequences isolated in this study are designated by grey triangle.

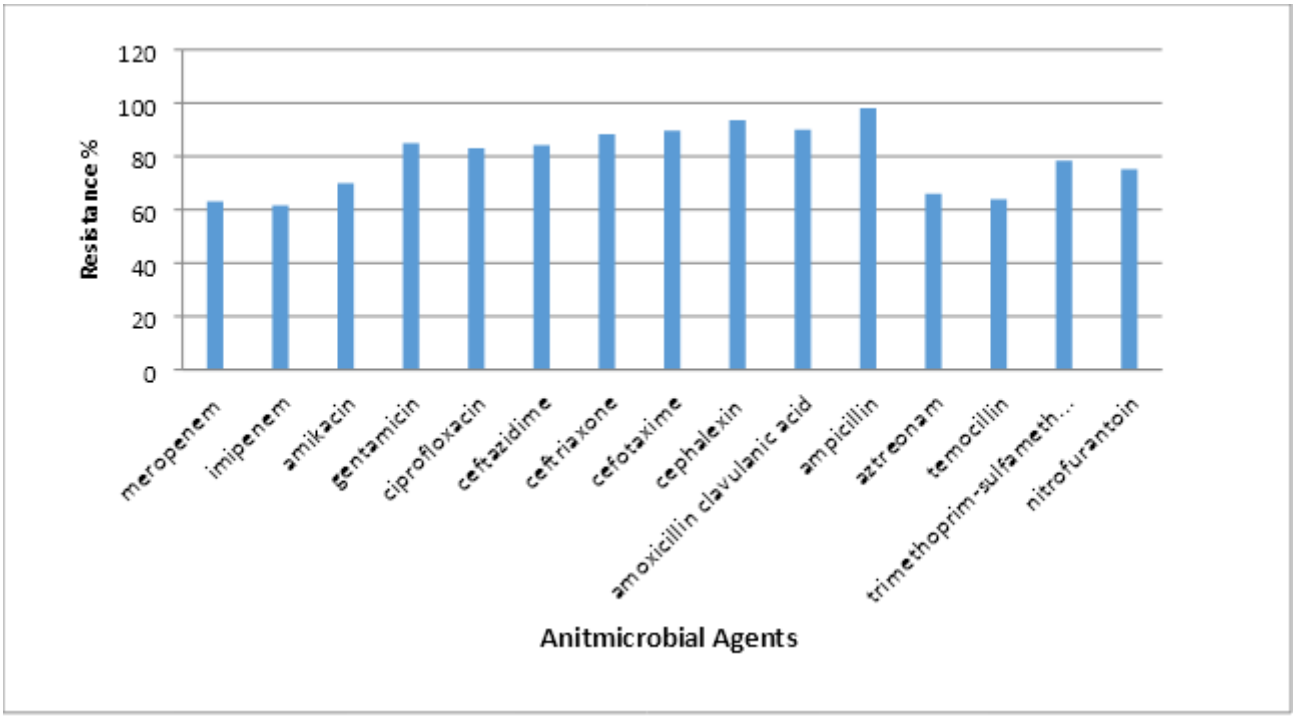

Figure 2

Antimicrobial Resistance pattern among different Gram-negative bacilli isolated from patients treated at Khartoum state hospitals.

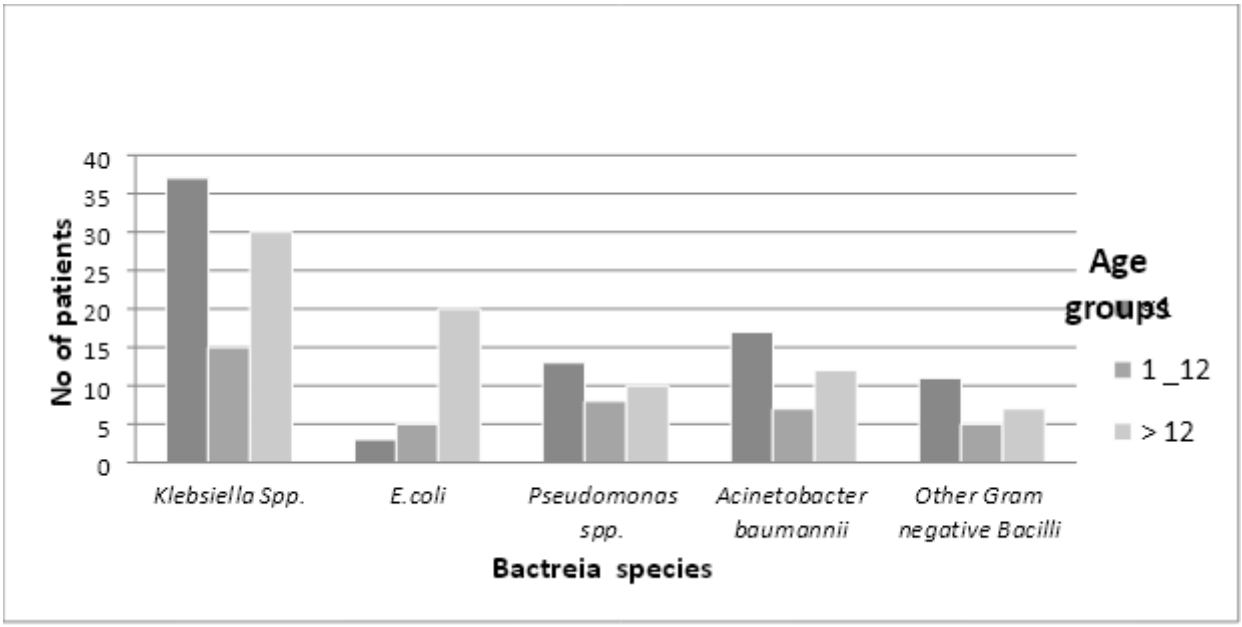

Figure 3

Distribution of bacterial species isolates among different age groups. (Other GNB include Burkholderia cepacia, Citrobacter species, Serratia species, Enterobacter species, Stenotrophomonas maltophilia, Vibrio vurneficus and Morganella morganii)