# Detection and Characterisation of Carbapenem Resistant Gram-negative Bacilli Isolates Recovered From Hospitalised Patients at Soba University Hospital, Sudan

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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). This study aimed to detect and characterize carbapenem resistance Gram negative bacteria isolated from hospitalized patients in Soba University Hospital (SUH) in Khartoum State, Sudan

**Results:** A total of 206 GNB isolates from different clinical specimens were obtained from hospitalised patients between October 2016 to February 2017. Of 206 isolates, 171 (83%) were confirmed resistant phenotypically and 121 (58.7%) isolates were positive for the presence of one or more carbapenemase genes. New Delhi metallo-β-lactamase (NDM) types were the most predominant genes, *bla*NDM 107(52%). Others included *bla*IMP 7 (3.4%), *bla*OXA-48 5(2.4%), *bla*VIM 2 (0.9%) and *bla*KPC 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 < 0.001). NDM1 was the most frequent subtype observed in 75 (70 %) isolates which clusters to the Indian lineage.

**Conclusions:** The frequency of carbapenemase producing bacilli was found to be improperly high in SUH. 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.


# Background

The prevalence and distribution of antimicrobial resistant bacterial infections in the nosocomial settings in Africa is poorly defined [1,2]. Carbapenems have been considered as a robust antibiotic to treat extended spectrum β-lactamase resistant bacteria (ESBL) in the past ten years and are widely prescribed for treatment of multidrug-resistant Gram negative bacilli in systemic infections [3]. The genes encoding ESBL are one of the most commonly distributed among Gram negative bacilli through plasmids and transposons [4]. The novel β-lactamases with direct carbapenem-hydrolysing activity have contributed to an increased prevalence of carbapenem resistant *Enterobacteriaceae* (CRE), which is causing therapeutic failure worldwide [4]. CRE, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii* were rated in the critical and the highest priority pathogen ranks by World Health Organisation (WHO) in 2017 [5].

Carbapenemase enzymes include New Delhi Metallo-beta-lactamase ($bla$NDM), veron integron metallo-beta-lactamases ($bla$VIM), imipenemase ($bla$IMP), *Klebsiella pneumoniae* carbapenemases ($bla$KPC), and oxacillinase-48 ($bla$OXA-48 ) [6]. These enzymes are encoded by what is termed carbapenem resistance determining genes (CRDG), which hydrolyse 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 response to inappropriate of use carbapenem and third generation cephalosporins [9]. These plasmids encoding for carbapenemases may also carry co-resistance genes for resistance to other β-lactam and non β-lactam antibiotics [7]. Detection of carbapenemase producing isolates by clinical microbiology laboratories is essential to provide targeted therapy, antimicrobial stewardship and update local antibiotic guide-lines for clinicians. Furthermore, the use of molecular analysis to detect resistance genes provides confirmation of 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. This study aimed to detect and characterize carbapenem resistance GNB isolated from patients treated at Soba University Hospital in Khartoum state, Sudan.

# Results

### Demographic distribution

The demographic characteristics of the inpatients and the frequency of GNB isolates according to age groups are shown in **Figure 1**. Most of the isolates were from pediatric patients less than one year old (42.5%), followed by age group 13-80 years (38%) and the remainder of paediatric patients age group 1-12 years (19.5%). Males 53.4% (110/206) were predominant among inpatients with females at 46.6% (96/206).

### Antimicrobial susceptibility

The antibiotic resistance pattern is shown in **Figure 2**. Out of 206 isolates tested, the highest percentage resistance was 98% and 93.5%, for ampicillin and cephalexin respectively, followed by amoxicillin clavulanic acid 90%, cefotaxime 89.7%, ceftriaxone 88.4%, ceftazidime 84.2%, aztreonam 66%, temocillin 64%, sulfamethoxazole-trimethoprim 78.4%, nitrofurantoin 75.2%. The resistance rate was also high in ciprofloxacin 83.1%, gentamicin 85% and amikacin 70%. The resistance rate for meropenem and imipenem was 63.1% and 61.6%, respectively.

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

Carbapenemase activity was detected in 171 (83%) of the 206 clinical isolates. These isolates were positive for the production of one or more carbapenemase enzymes by phenotypic methods as the following; 24 (11.7%) by Modified Hodge test (MHT) method and Boronic acid screen, 105 (51%) by the EDTA test and 27 (13.1%) of the isolates were positive for both EDTA and Boronic acid methods while 15 (7.2%) were positive with all methods. Carbapenemase enzymes were predominant in *Klebsiella pneumoniae* isolates: 71(41.5%), followed by *Pseudomonas aeruginosa*: 33(19.3%); *Acinetobacter baumannii*: 30(17.6%) and *Escherichia coli* 26(15.2%). Details of the carbapenemase activity among

different isolates by phenotypic tests are given in **Table 1**. This suggests that the MBL type is the most prevalent type of carbapenemase hydrolysis enzyme among Gram-negative bacilli, 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 *bla*NDM 107(52%), followed by *bla*IMP 7 (3.4%), *bla*OXA-48 5(2.4%), *bla*VIM 2 (0.9%) and *bla*KPC 0 (0%). ESBL were detected among these isolates with high prevalence in 183 isolates (88.8%) as the following; *bla*CTXM 126(61.6%), *bla*SHV 84(40.7%) and *bla*TEM 78(37.8). The genes were unevenly distributed among the different study isolates and more details are given in **Table 2**.

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

Several 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 38(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 in 15 isolates (17.3%). The distribution of co-resistance genes among different Gram negative bacilli is shown in **Table 3**.

### The frequency of carbapenemase producer Gram-negative bacilli by type of specimens and hospital units

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

With regard to the distribution of carbapenemase producers among hospital location, the most carbapenemase producing isolates were found in the neonatal intensive care unit 32(26%), followed by medicine wards 26(22%), pediatric wards 22 (18%), surgery 18(15%), ICU 15(12%) and renal unit 8(7%).

### Molecular characterisation 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 **Figure 3.**

### Bioinformatics analysis of *bla*NDM genes

A subset samples were analysed to confirm the presumed most prevalent (NDM) gene type. Fourteen samples were sequenced 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 sequence of NDM Deoxyribonucleic acid (DNA) sequences were compared against the DNA databank using BLASTp. Fourteen NDM beta-lactamase genes were compared against those NDM genes recorded in the database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The alignment of the NDM genes in the isolates were shown to have identical similarities within range of 97-100% with those available in the database.When multiple sequence alignment of NDM proteins was undertaken using MEGA7 software version 7.0.9.0 against similar proteins obtained from BLASTp, NDM-1 from Sudan was similar to the |KX100583.1| *Escherichia coli* NDM-1 (blaNDM) gene from India and the | MH891562| *Klebsiella pneumoniae* NDM-1 from Bangladesh. NDM-5 from Sudan was similar to | MH991817 |, *Escherichia coli* NDM-5 from India and | MH168510| *Klebsiella pneumoniae* NDM-5 from Bangladesh while NDM-6 from Sudan was 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 the following 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 protein sequences revealed that 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 **Figure 3.**

## Discussion

Carbapenems have become the drug 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 globally and are 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 hospitalised patients at Soba University Hospital, Khartoum State, Sudan. The accurate detection of carbapenemase producing microorganisms is challenging for laboratories and requires phenotypic and genotypic tests to detect all genes associated with carbapenemase production. Of 206 isolates,

171(83%) were positive by phenotypic analysis, including isolates with resistance to carbapenem. Genotypic analysis detected 121 (58.7%) positive isolates. The 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 which were detected among 183 (88.8%) of the study isolates, in addition to porin loss or mutation [10,11].

The current situation according to this study shows that the prevalence of carbapenemase production among different Gram negative isolates is increasing (up to 83%). This finding is higher than the incidence observed in a previous study conducted in Khartoum State in 2017 which showed the prevalence was 56% by phenotypic tests (unpublished data). Other studies completed in reported the MBL as 37.7% among *Pseudomonasspp.* isolated 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. our results are in agreement with a study in Egypt, which reported that carbapenem resistance was 62.7% among *Enterobacteriaceae* [12]. High rates of carbapenem resistance have also been observed in Uganda in a study conducted by Okoche in 2015. Okoche found 28.6% of isolates were carbapenemase producers [13]. In Tanzania, the prevalence of carbapenemase producing isolates was 35% [14]. In South Africa, it was found to be 68% [15] and in Nigeria 11.9% [16]. Carbapenem resistance in low and middle income countries (LMICs) in Africa is likely to increase as result of unrestricted usage of antibiotics in LMICS as the majority of the population consume antibiotics without a clinical prescription [17].

Carbapenemase genes have been recently recognised and these genes are associated with mobile genetic elements that allow their rapid circulation among bacterial isolates. For instance, *bla*NDM have potential for rapid spread within Turkey and to other countries [18]. In this study, carbapenemase genes were detected using PCR in 121 (58.7%) of the sampled 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 is in keeping with studies in India, South Africa, Saudia Arabia and other Middle East countries [15,19,20,21]. A study in India, reported the *bla*NDM gene was observed between 31% and 55% of carbapenemase resistant *Enterobacteriaceae* [19,20]. In South Africa, *bla*NDM was the highest carbapenemase gene among *K. pneumonia* [15]. *Bla*NDM-1 was reported as the most common carbapenemase gene in Saudi Arabia and other Middle Eastern countries [21].

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 [11]. Carbapenemase production in Turkey mostly occurs in *bla*OXA genes [18]. OXA-48 was first reported from Turkey, followed by reports from Middle Asia and Europe [22]. In the current study, the genes were unevenly distributed among the different bacterial isolates. The *bla*NDM gene was found in high prevalence (52%) compared to other genes, such as *bla*IMP (3.4%), *bla*OXA-48 (2.4%), *bla*VIM (0.9%) and *bla*KPC (0%). Our finding, however, differs with several studies. For instance in the Okoche study, the most common gene was *bla*VIM (10.7%), and *bla*NDM-1 (2.6%) was the lowest gene [13], while Mushi reported IMP types were the most predominant at 21.6% in

his study [14]. Other studies reported *bla*OXA-48 was the most prevalent gene [23,24]. In this study, *bla*KPC was not detected among the isolates which is not in keeping with global reports of high prevalence of *bla*KPC genes among international isolates [11, 25].

The *bla*NDM-1 gene was first identified in a clinical isolate of *K. pneumoniae* in New Delhi, India in 2010, and has since been disseminated across the globe [26]. 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 [26]. 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 producers 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 *K. pneumoniae* (41.5%) followed by *P.aeruginosa* (19.3 %), *A. baumannii* (17.6 %) and *E. coli* (15.2%) **Table 1**. This agrees with many studies that reported *K. pneumonia* and *A. baumannii* as the most predominant carbapenemase producing isolates [27,28]. A study in Turkey showed the most carbapenemase producing isolates were *K. pneumoniae* (13.6%), *Pseudomonas spp.* (17.8%), *A. baumannii* (13.8%), *S. maltophilia* 7.5% and *E. coli* 2.8% [29]. 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%) [16], 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%) [14]. The prevalence of carbapenemase producing isolates varies from hospital to hospital. This variation could be attributed to differences in collection time of isolates, study designs and target populations.

Carbapenemase encoding genes have been commonly associated with bacteria isolated from blood, urine, wounds and sputum as reported in many studies in Uganda [13], Tanzania [14], Nigeria [16] and India [30]. In this finding, carbapenem producers were more frequently isolated from blood (39%) followed by wounds (25%) and urine (22%). This is compatible with a study in South Africa which reported blood was the most common specimen type (25%), followed by urine (22%) [15].

Young patient age has long been considered as a risk factor for Carbapenem resistant Enterobacteriaceae (CRE) infection which agrees with current finding that carbapenemase producing Gram negative bacilli were most frequent in children less than one year of age, located in the nursery and pediatric wards with 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 CRE to be more frequently isolated in the elderly (31).

Carbapenem resistant Gram negative bacilli are usually resistant to other routinely used antimicrobial agents [32–34]. 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 [20,35]. Moreover, mechanisms of resistance to β-lactam antibiotics by producing ESBL, AmpC and carbapenemase were also noticed among the isolates that produce different combinations of the enzymes. At the present 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 [36,37]. 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. These multidrug resistant pathogens impact on treatment outcomes and result in higher treatment costs [38].

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

# Conclusions

The frequency of carbapenemase producing bacilli was found to be improperly high in SUH. *bla*NDM was found to be the most prevalent carbapenemase gene among clinical isolates. Improved antibiotic stewardship and infection control measures, and 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 investigated.

# 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 (IEND), University of Khartoum; involving Gram negative clinical bacterial isolates, suspected as carbapenemase producing based on breakpoints zone diameter of carbapenems (CLSI, 2017)[39]. These were isolated from cultures of various clinical specimens; blood, urine, wound swabs, sputum, tips of catheters and other body fluids, between 1$^{st}$ October 2016 and 25$^{th}$ February 2017 from inpatients at Soba University Hospital. Quality control strains [*E. coli* (ATCC #25922) and *P. aeruginosa* (ATCC #27853)] were used in antimicrobial susceptibility testing. Standard biochemical tests were used for primary identification [40] and molecular identification

using PCR [8] was used for all study isolates with universal primer (16SrRNA). For species specific isolates identified on biochemical testing, species specific primers for *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were used for confirmation **Table S1**. All isolates were stored in 20% glycerol at -20 °C until use.

### Subculture and disk diffusion susceptibility testing

Selection of antimicrobial panels and interpretation of disk diffusion for each bacteria was completed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [39].

Isolates were subcultured on blood agar (BA) and then subjected to susceptibility testing with the following antimicrobials (Mast Diagnostic, UK): amoxicillin clavulanate (AMC) (30µg); cefuroxime (CXM) (30µg); cephalexin (CL)(30µg); ceftriaxone (CRO)(30µg); ceftazidime (CAZ) (30µg); cefotaxime (CTX) (30µg); meropenem (MEM)(10µg); imipenem (IPM) (10µg); amikacin (AK)(30 µg); gentamicin (CN)(10 µg); ciprofloxacin (CIP)(5 µg); trimethoprim-sulfamethoxazole (SXT)(25 µg); temocillin (TEM)(30 µg); 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 [39, 40].

### Phenotypic screening and confirmatory test for carbapenemase

Bacterial isolates were screened for carbapenemase production according to CLSI guidelines (CLSI, 2017). In this method, meropenem and imipenem discs (10 µg, each) (Mast Diagnostic, UK) were used. Isolates that showed intermediate or resistant to imipenem or meropenem were considered as suspected carbapenemase producers. Phenotypic confirmatory tests 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 *Enterobactercea* to detect KPC and OXA-48 producers in addition to temocillin sensitivity [41].

### 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 were used **Table S2**. The reaction was carried out in a total reaction volume of 25 µl (5µl master mix, Maxime RT premix kit) [8]. The purity and integrity of each PCR product was evaluated and the amplified product was confirmed with reference to 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

Firstly we ensured the ambiguous sites were clarified and determined the overall quality of the sequences by reviewing nucleotide chromatogram by using Finch TV software version 1.4.0 (http://www.geospiza.com/Products/finchtv.shtml). Thereafter, nucleotide sequences of the NDM genes identified were searched for sequence similarity using nucleotide BLAST [42] (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment for highly similar sequences, was retrieved from NCBI using the MEGA version 7 software [43]. Phylogenetic tree of *bla* NDM genes and their evolutionary relationship with those obtained from the NCBI database were completed using MEGA version 7 [43].

### Statistical analysis

Data were analysed using SPSS software version 20.0. Cross tabulation was used to present the relationships between data of antimicrobial sensitivity, phenotypic tests and resistant gene detection among the study isolates, qualitative data were performed through $\chi^2$test and significance was set at $p \leq 0.05$.

## Abbreviations

*A. baumannii:*AK: Amikacin;AMC: Amoxicillin clavulanate; Amp C: Class C β-lactamases;AMR: Antimicrobial resistance; ATCC: American Type Culture Collection; AZT: Azteroname;BLAST: Basic local alignment search tool; bp: Base pair;CAZ: Ceftazidime; CIP: Ciprofloxacin; CL: Cephalexin; CLSI: Clinical and Laboratory Standards Institute; CN: Gentamicin; CPR: Carbapenemase producer *Enterobacteriaceae;* CRDG: Carbapenem resistance determining genes; CRE: Carbapenem resistance *Enterobacteriaceae;* CRO:Ceftriaxone; CTX: Cefotaxime; CTX-M: CTX for cefotaximase and M for Munich; CXM:Cefuroxime; DNA: Deoxyribonucleic acid; *E. coli: Escherichia coli;* EDTA: Ethylenediaminetetraacetic acid; ESBL: Extended-Spectrum β-Lactamase; GNB: Gram-negative bacilli; ICU: Intensive care unit IEND: Institute of Endemic Diseases; IMP: Imipenemase Metallo-β-lactamase; IPM:Imipenem; *K. pneumonia: Klebsiella pneumonia;* KPC: *Klebsiella pneumoniae* carbapenemase-producer; LMICs: Low and middle income countries; MBL: Metallo-β-lactamas;MDR: Multi drug resistance; MEM: Meropenem; MHT: Modified Hodge test; NDM: New Delhi Metallo-β-lactamase; OXA: oxacillinases; *P. aeruginosa: Pseudomonas aeruginosa;* PCR: Polymerase Chain Reaction; *S. maltophilia: Stenotrophomonas maltophilia;* SHV: Sulphydryl Reagent Variable; SPSS: Statistical Package for the Social Sciences; SUH: Soba University Hospital; SXT: Trimethoprim-sulfamethoxazole; TEM: Named after patient Temoniera; TEM: Temocillin; VIM: Verona integron-encoded Metallo-β-lactamase; WHO: World Health Organisation; °C: Celsius degree; 16SrRNA: 16 small ribosomal RNA; µg: Microgram

## Declarations

### Ethics approval and consent to participate

This study was approved by Institutional Research Ethics Committee of the Institute of Endemic Diseases (IEND), University of Khartoum and Soba University Hospital (Ref: 12/2017). Written informed consent was obtained from all participants.

## 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 be available upon reasonable request.

## Competing interests

The authors declare that they have no competing interests.


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## Authors' contributions

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


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# Tables

**Table 1.** 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=45) | 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 (17.6) |
| *Burkholderia cepacia* (n=2) | 2 (1.9) | 0 (0) | 0 (0) | 0 (0) | 2 (1.2) |
| *Enterobacter spp.* (2) | 2 (1.9) | 0 (0) | 0 (0) | 0 (0) | 2 (1.2) |
| *Other GNB* (n=11) | 5 (4.8) | 0 (0) | 2 (7.4) | 0(0) | 7 (4) |
| Total (n=206) | 105(50.9) | 24 (11.7) | 27 (13.1) | 15 (7.2) | 171(83) |

(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

Note: MHT applied only for *Enterobacterceai*.

* These isolates just positive by BA and not MHT.

| 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) | 58 (54.3) | 1 (20) | 3 (43) | 1 (50) | 0 (0) | 53 (41.7) | 28(33.4) | 32 (40) |
| *E.coli* (n=28) | 9(8.4) | 1 (20) | 2 (28.5) | 0 (0) | 0 (0) | 14 (11.6) | 6 (7.2) | 4 (5) |
| *Pseudomonas Spp*. (n= 45) | 14(13.1) | 2 (40) | 2 (28.5) | 1 (50) | 0 (0) | 28 (22.3) | 18(21.5) | 16 (20) |
| *A. baumannii* (n=36) | 17 (15.8) | 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 (5.7) | 5(6.25) |
| Total (n=206) | 107 (52) | 5(2.4) | 7 (3.4) | 2 (0.9) | 0 (0) | 126 (61.1) | 84 (40.7) | 78 (37.8) |

**Table 2.** 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 3.** 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

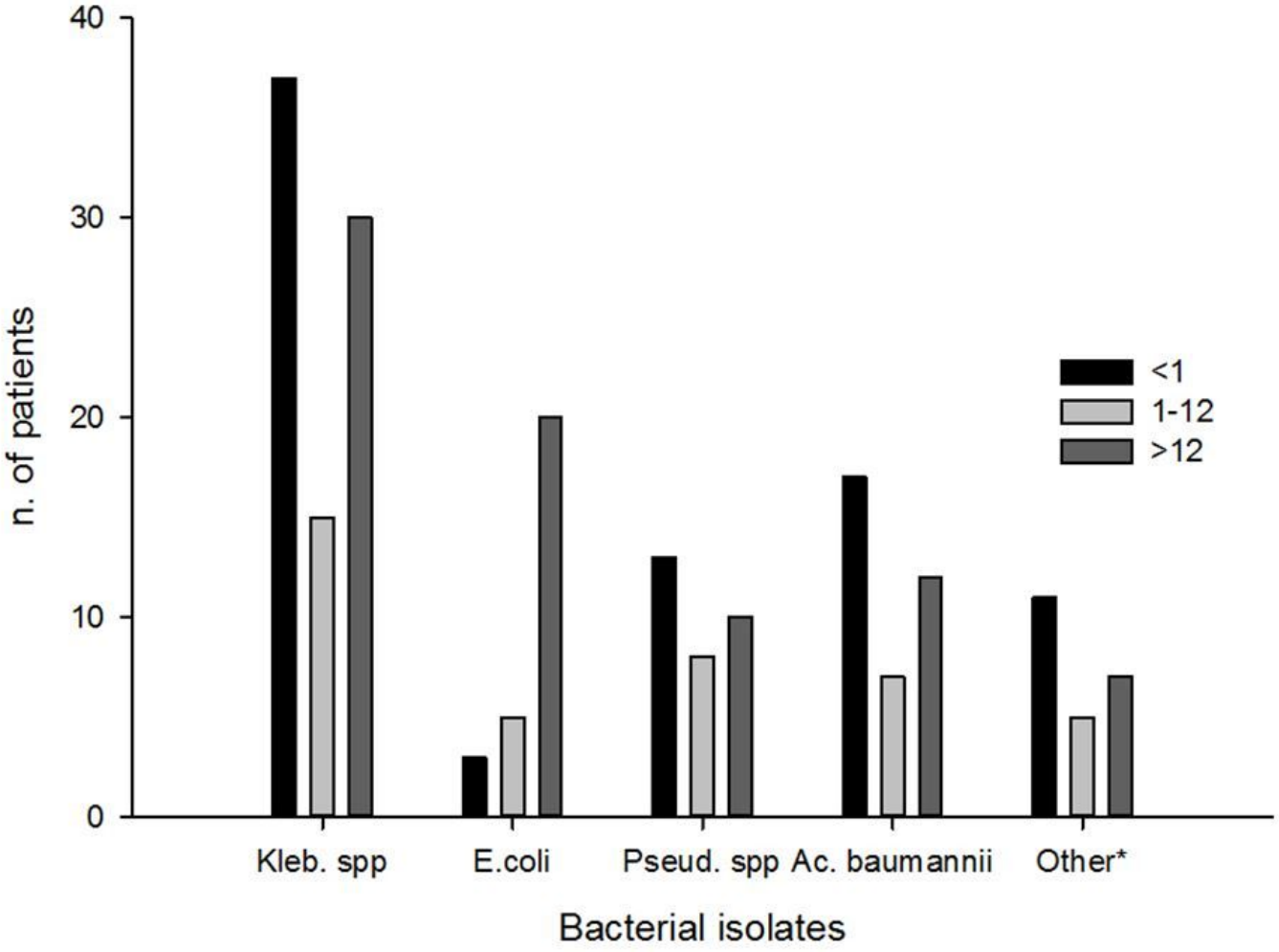
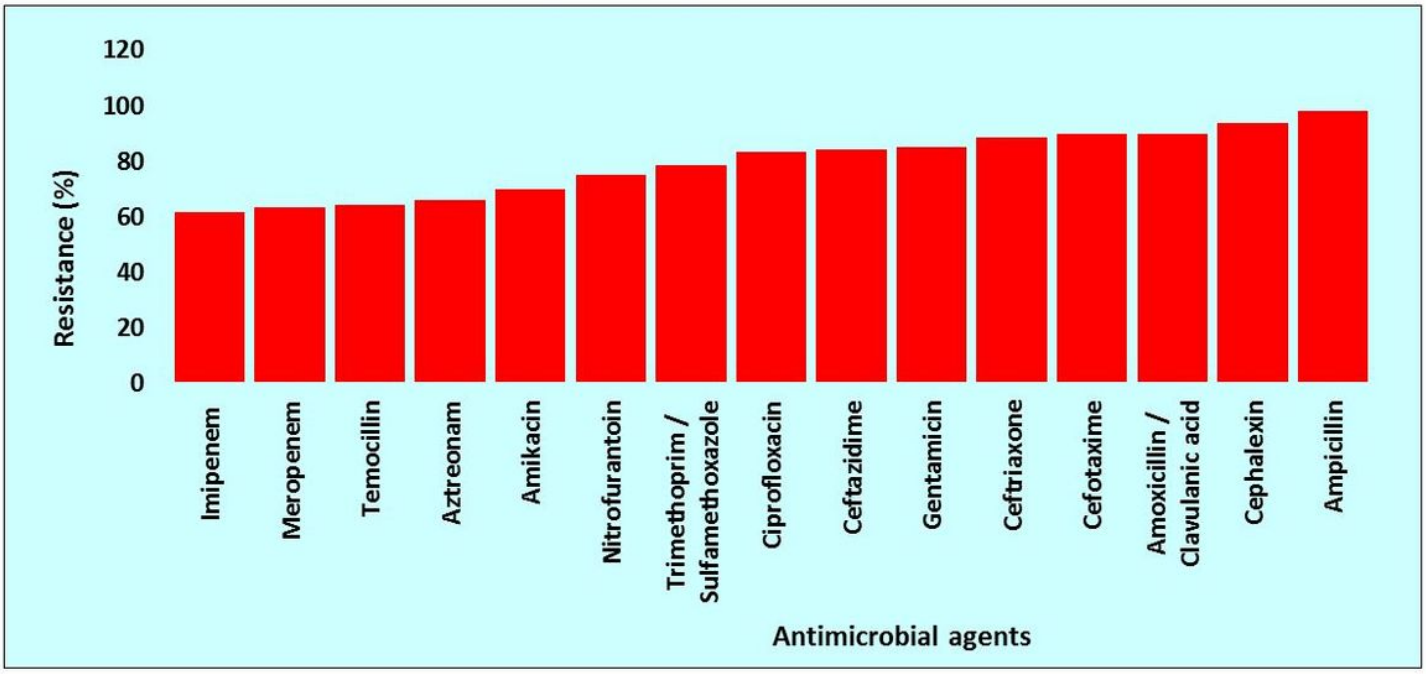


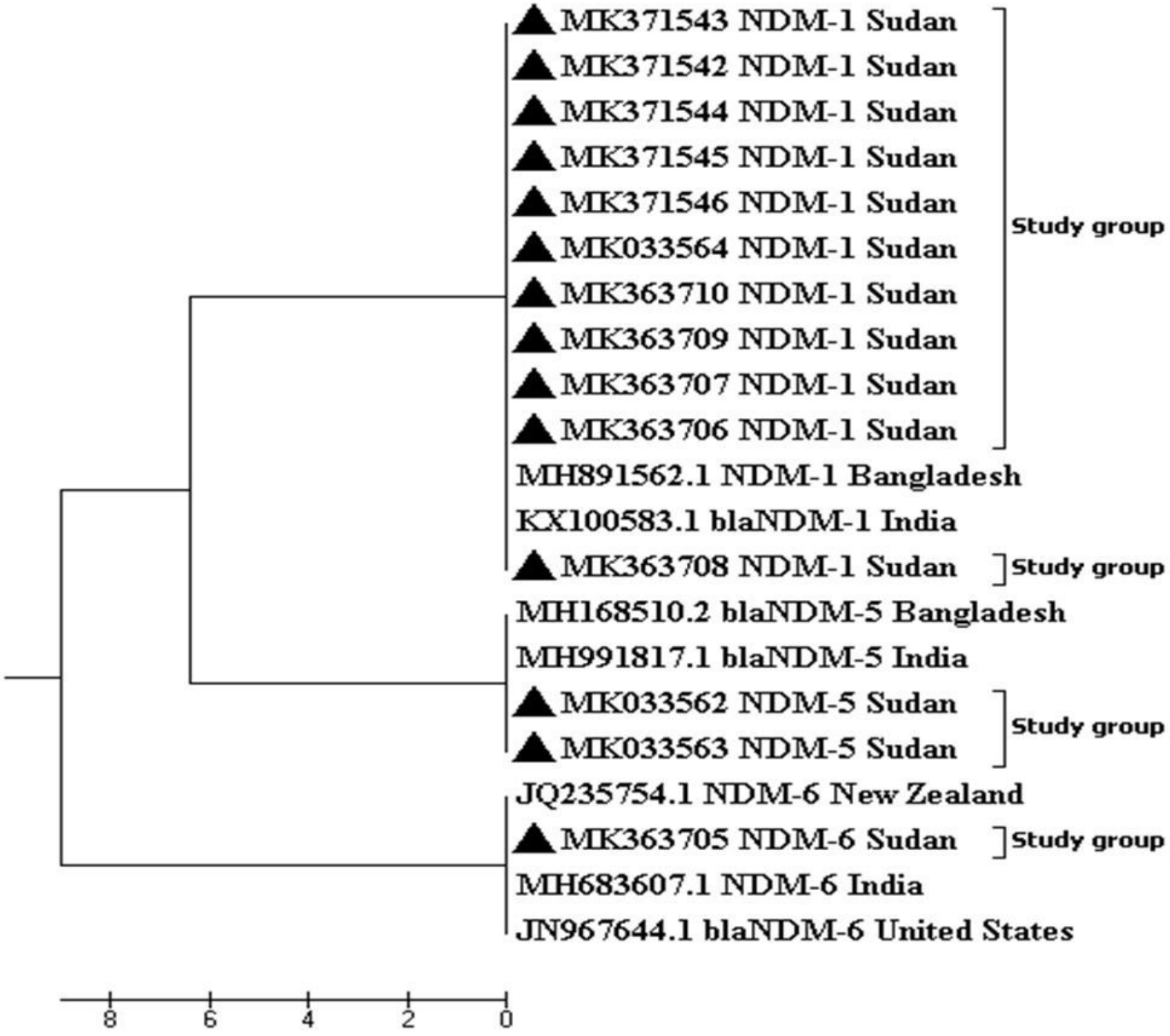

**Figure 1**

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)




## Figure 2

Antimicrobial resistance pattern among different Gram-negative bacilli isolated from patients treated in Soba University Hospital, Khartoum State.




## Figure 3

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.