

## Detection of Carbapenem resistance genes among selected Gram Negative bacteria isolated from patients in-Khartoum State, Sudan

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### Abstract

**Background:** Carbapenem-resistant Gram-negative rods (CR-GNR) are gaining increasing importance in healthcare settings, especially in high-dependency units and among critically ill patients. These bacteria are frequently resistant to all antibiotics except colistin, some aminoglycosides and variably tigecycline, posing a serious challenge for treatment. CR-GNR cause infections associated with significant morbidity and mortality. Data on the prevalence of carbapenem resistant genes in Sudan is limited. This study, aimed to determine the prevalence of (CR-GNR) isolated from clinical specimens in Khartoum, Sudan during January 2015 to August 2015.

**Methods:** A total of 83 Carbapenem resistant clinical isolates (*Klebsiella pneumoniae* n=21 *Escherichia coli* n=7, *Pseudomonas aeruginosa* n=15, *Citrobacter* n=2, *Proteus* n=1 and *Acinetobacter baumannii* n= 37) were screened for the presence of carbapenemases (blaTEM, blaVIM, blaIMP, blaSHV, blaCTX and blaKPC genes) by using Multiplex PCR.

**Results:** Out of 83 isolates 68 were Tem gene positive while, 50 isolates were Vim gene positive, Imp gene was present in 42 isolates, Kpc gene in 41 isolates, Ctx gene present in 40 isolates and Shv gene in 15 isolates. TEM gene was the predominant gene among the positive (antibiotic resistant) species.

**Conclusion:** Detection of the genes related with carbapenemase production indicated widespread prevalence and multiplicity of these genes in carbapenem resistant clinical isolates. The results also showed that the multiplex PCR as a reliable, fast method for the detection of these genes.

**Keywords:** Tem, Vim, Kpc, Imp, Shv, Ctx, Carbapenemases, Multiplex PCR.

### Background

Multidrug resistance in Gram-negative rod (GNR) is an increasing problem and might lead to dangerous limitations of treatment options. Carbapenems are considered as the most potent agents used for chemotherapy of infectious diseases caused by (GNR) due to their high affinity for PBP 2, stability to most  $\beta$ -lactamases including extended-spectrum  $\beta$ -lactamases, and excellent permeation across bacterial outer membranes [14]. Carbapenemases are versatile  $\beta$ -lactamases that have the ability to hydrolyse penicillin, cephalosporin, and monobactams. Carbapenems exhibit a broader spectrum of antibacterial activity towards Gram-positive and Gram-negative bacteria than other beta-lactams [16]. Resistance to carbapenems, which is caused mainly by carbapenemase production or by porin loss, combined with the expression of beta-lactamases like an extended spectrum beta-lactamase (ESBL) or AmpC [1]. The most prevalent carbapenemases in (GNR) are KPC, VIM, IMP, TEM, and CTX [2]. So far, SHV are rarely found in other members of Gram-negative bacteria [3]. These resistant genes are located on transferable plasmids and can be freely transferred between bacteria, from one region to another and from one country to the other and then spread all over the world [17].

Carbapenemase-producing (GNR) have been associated with increasing mortality and with serious hospital outbreaks that present major therapeutic and infection control challenges [4]. CR-GNR has been associated with the use of medical devices such as: intravenous catheters, ventilators, urinary catheters, and through wounds caused by injury or surgery [15]. Detection of Carbapenem resistance genes producing bacteria may be difficult based on routine antibiotic susceptibility testing. Therefore it is essential to implement efficient infection control actions to limit the spread of these pathogens [18]. Tests based on molecular techniques are considered the standard tests for the identification of carbapenemase genes [5]. Considering the fact that information on the subject is limited in our country, utilization of a suitable method for determination of the carbapenemase production is important in the microbiology laboratory [19]. Therefore the aim of the present study was to test the feasibility of nucleic acid extraction and a multiplex PCR amplification assay for identification of blaTEM, blaSHV, blaIMP, blaKPC, blaVIM and blaCTX genes in a series of clinical isolates of (CR-GNR). Such data serve an important role in understanding the spread of carbapenem-resistant gram-negative pathogens.

## Methods

### Bacterial Isolates:

A total number of 83 CR-GNR isolates including (*Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Citobacter freundii* and *Proteus mirabilis*) and 12 negative controls isolated from three private hospitals, during January till August 2015, Khartoum, Sudan were investigated using multiplex PCR. The isolates were identified using microbiological and biochemical methods.

### Antibiotic Resistance Investigation:

Measurement of antibiotic resistance was conducted by disk diffusion method for the following antibiotics: Ceftazidime (CAZ), Ceftriaxone (CRO), Cefotaxime (CTX), Cephalexin (CN30), Cefixime (CFM), Penicillin (P), Cefuroxime (CXM), Meropenem (MRP) and Imipenem (IMP) according to the standards of Clinical and Laboratory Standards Institute (CLSI).

### Investigation of carbapenem resistance genes by PCR:

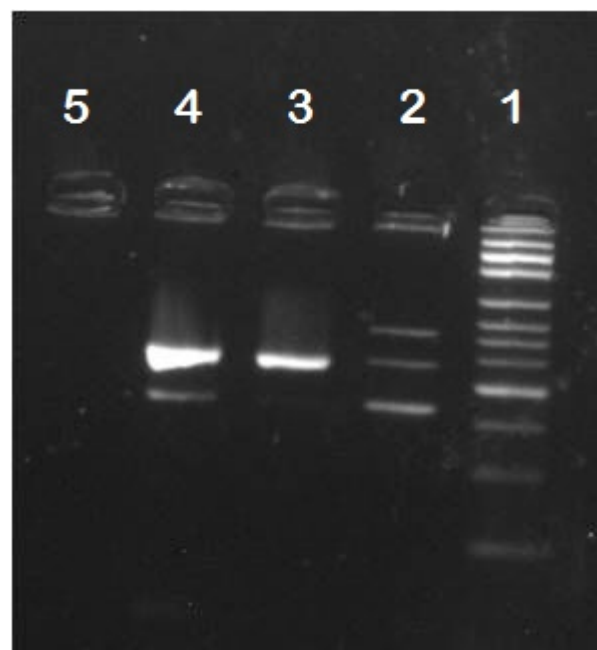
Carbapenem resistant isolates were used to investigate bla-VIM, bla-IMP, blaTEM, blaSHV, blaKPC and blaCTX genes by PCR. For DNA extraction the boiling method was applied [13]. Firstly, three to five colonies were picked from fresh culture medium and then a suspension was prepared using 200 ml of distilled water boiled at 100 °C for 30 minutes. The suspension was then centrifuged at 12000 rpm for 30 minutes, and the supernatant containing DNA was transferred to new Eppendorf tubes for PCR in order to amplify the genes, (bla-VIM, bla-IMP, blaKPC, blaTEM and bla-CTX). The primers used to amplify the genes are shown in the table below:

Gene	Sequence (5'- 3')	Product size	Reference
KPC FP	TCGAACAGGACTTTGGCG	201	6
KPC RP	GGAACCAGCGCATTTTTGC		
IMP FP	GAAGGCGTTTATGTTTCATAC	587	6
IMP RP	GTAAGTTTCAAGAGTGATGC		
VIM FP	GTTTGGTCGCATATCGCAAC	382	6
VIM RP	AATGCGCAGCACCAGGATAG		
TEM FP	TCGCCGCATACACTATTCTCAGAATGA	445	12
TEM RP	ACGCTCACCGGCTCCAGATTTAT		
SHV FP	ATGCGTTATATCGCCTTG TG	747	7
SHV RP	TGCTTTGTTATTCGGGCCAA		
CTX FP	ATGTGCAGYACCAGTAARGTKATGGC	593	8
CTX RP	GGGTRAARTARGTSACCAGAAYCAGCGG		

**Table 1:** Primer sequences of 6 target genes (blaKPC, blaIMP, blaVIM, blaTEM, blaSHV and blaCTX genes) for Multiplex PCR.

To perform PCR, 2 µl from the primers, 5 µl of the extracted DNA, 13 µl of distilled water was added to the PCR Master Mix (Maxime

PCR Permixon Kit, Korea) with a final volume of 20 µl. Thermal cycling (Aeris Machine Pelter technology Thermo Assis) for 30 cycles was done at 94 °C for 1 min, 60 °C for 1 min and 72 °C for one and half min. the final extension step was performed for 5 min at 72 °C. The PCR products were applied and electrophoresed in 2% agarose gel along with ladder DNA and then stained using ethidium bromide. The result was observed by transilluminator system (Biometer an analytical Jena company).



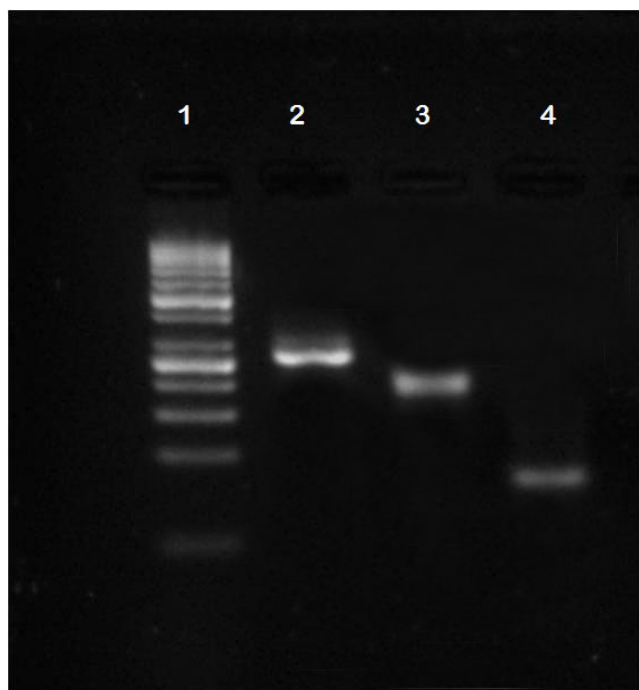
**Figure 1:** Agarose gel result of Shv, Tem and Ctx, (lane 1: 100bp ladder; lane 2: positive control; lane 3: Ctx positive sample; lane 4: Tem and Ctx positive sample; lane 5: negative control).

## Results

The results of the Multiplex PCR for the six target genes are shown in Table 2.

Total of 99 clinical isolates which successfully grew, 83 were identified to be resistant to Meropenem, while 12 isolates were susceptible to Meropenem.

Out of 83 CR-GNB 37 (44.5%) were *A. baumannii* as the most predominant species followed by *K. pneumoniae* 22 (26.5%), *P. aeruginosa* 14 (16.8%), *E. coli* 7 (8.4%), *C. freundii* 2 (2.4%) and *P. mirabilis* 1 (1.2%). The prevalence of the resistance genes in the 83 resistant isolates were as follows TEM (81.9%) was the most predominant gene, VIM (56.6%), IMP (50.6%), KPC (45.7%), CTX (48.1%) and SHV (18.1%). *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* isolates harbored all six resistance genes (TEM, KPC, CTX, IMP, VIM, SHV), *C. freundii* contained five of the genes but lacked (SHV) gene, While *P. mirabilis* on the other hand contained only one gene (CTX).



**Figure 2:** Agarose gel result of IMP, VIM and KPC (Lane 1:100bp ladder; Lane 2: IMP Positive sample; Lane 3: VIM positive sample; Lane 4: KPC positive sample).

Resistance gene	TEM	VIM	IMP	KPC	CTX	SHV
A.baumannii	31	25	17	19	16	6
K.pneumoniae	19	15	16	11	11	6
P.aeruginosa	10	2	5	4	7	1
E.coli	6	6	3	5	4	2
P.mirabilis	-	-	-	-	1	-
C.frendii	2	2	1	2	1	-
<b>Total</b>	<b>68</b>	<b>50</b>	<b>42</b>	<b>41</b>	<b>40</b>	<b>15</b>
<b>%</b>	<b>0.819</b>	<b>0.566</b>	<b>0.42</b>	<b>0.457</b>	<b>0.481</b>	<b>0.181</b>

**Table 2:** Results of Genotypic test (Multiplex PCR).

## Discussion

Different antibiotics including beta lactamases, aminoglycosides and quinolones are applied to treat the infections caused by *Acintobacter baumannii*, *Klebsiella pneumonia*, *Pseudomona aeruginosa*, *E.coli*, *Proteus mirabilis* and *Citrobacter freundii*. However, it is becoming a great challenge to treat infections caused by these bacteria due to its resistance against drugs and the rapid changes in the pattern of resistance. Additionally, the resistance of the bacteria against antibiotics fluctuates, especially regarding Imipenem, Carbapenems and Beta lactam antibiotics are being used increasingly to treat infections due to CR-GNR since they are resistant against most of the beta lactamases and have great membrane permeability [10].

Carbapenems and Beta lactam antibiotics are used considerably to treat infections due to multidrug-resistant Gram Negative bacteria. Resistance of Carbapenem agents is due to carbapenemase and presence of other resistance mechanisms, such as ESBLs, and porin mutations [9]. In the current study, these genes were mostly detected in a high percentage of antibiotic resistant isolates of *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *C. freundii* and *P. mirabilis*. findings where TEM is reported as the commonest MBL to be found (81.9%), followed by VIM (56.6%), IMP (50.6%), KPC (45.7%), CTX (48.1%), while SHV (18.1%) had the lowest prevalence in the tested resistant isolates. Of 83 bacterial isolates detected of having carbapenemase genes, 70 had multiple genes coding for carbapenem resistance especially in *A. baumannii* and *K. pneumoniae*. The presence of multiple resistance genes in one strain provides selection advantage of these strains; such phenomenon has not been commonly detected in a large number of studies probably due to the limited number of genes studied since most of the studies research on one or two genes. Since various reports indicate the increased resistance of these bacteria against antibiotics (especially imipenem), proper use of these antibiotics and time-consuming identification of isolates generating MBL should be considered. This can lead to successful treatment and prevents propagation of resistant genes, which can be seriously harmful for societies [11] Testing of carbapenem resistant genes among MDR-GNB isolates should routinely be used to determine these species in clinical laboratories. In addition, increased effort at discovery of more effective antimicrobial compounds with new effecting mechanisms should be noted. Finally, as the most of MDR-GNB infection are hospital acquired every effort should be exerted to prevent spread of these bacteria in our health facilities.

## Conclusion

Identification of carbapenem resistance and their resistance pattern of genes is necessary for the surveillance of their transmission in hospitals and to overcome the problems associated with G-ve carbapenem resistance. The multiplex PCR described here is a reliable and rapid method for detection of the most prevalent carbapenemase genes.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

SBS carried out the molecular genetic studies and drafted the manuscript. AIE conceived of the study and contributed to the conception and design of the study. AME, IME and MAA revised the manuscript. KAE contributed to the conception and design of the study and acquisition of funding. All authors read and approved the final manuscript.

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