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Antimicrobial Susceptibility and Molecular Detection of Carbapenem Resistant Genes NDM-1 and IMP-1 of *Pseudomonas aeruginosa* Isolated from Clinical Samples in Khartoum State-Sudan

Aisha Mohammed Abdallah[1,4], Mutaz Fathelrhman Saad[1]*, Hind Haidar Ahmed[1], Tagwa Salah Ahmed[2,3], Samar Mohammed Saeed[1] and Wafaa Mohammed Abdalla[1]

[1]Department of Microbiology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan.
[2]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Saudi Arabia.
[3]Department of Parasitology, Medical Entomology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum – Sudan.
[4]Department of Microbiology, College of Medical Laboratory Science, National Ribat University, Khartoum, Sudan.
*Corresponding Author E-mail: mutazsaad74@gmail.com

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Carbapenems are the most important ß-lactam antibiotics against bacteria especially multidrug resistant isolates like Pseudomonas aeruginosa. This study was conducted to detect carbapenem resistance genes of P. aeruginosa from clinical samples in Khartoum State, Sudan. Fifty (n=50) isolates of p. aeruginosa were included in this descriptive cross-sectional laboratory-based study. P aeruginosa were isolated from urine, blood, wound swab, sputum and Body fluids samples, which were attained from different hospitals in Khartoum State. The samples were collected from both males and ladies with different age using non-self-constructing information list. The isolated organism was stored in 20% Glycerol peptone media and was inoculated on CLED agar. Antibiotic susceptibility tests were carried out using Kirby Bauer technique. DNA was extracted using boiling procedure. The isolates were tested for the presence of carbapenem resistance genes (NDM, IMP) using Multiplex PCR. Out of 50 P. aeruginosa isolates, 19 (38%) were positive for one or both carbapenem resistance genes; 6 (12%) were positive for NDM gene and 13(26%) were positive for IMP gene. The study concluded that, there was a high percentage and multifariousness of carbapenem resistance genes among P. aeruginosa isolates in Khartoum State, Sudan.

Keywords: Carbapenem; IMP; Multiplex PCR; NDM genes; P. aeruginosa; Sudan.

P. aeruginosa, one of the most common opportunistic pathogen associated with nosocomial infections, including pneumonia, urinary tract infections, and wound infections. Although carbapenems are frequently used as a therapeutic agent for treating infections caused by *P. aeruginosa*, the high emergence of carbapenem resistance significantly decreases their utility.[1] Resistance

of *Pseudomonas aeruginosa* to different classes of antibiotics such as penicillin, cephalosporin, quinolone, aminoglycoside and carbapenem. Metallo beta lactamases (MBL) are enzymes that catalyze the hydrolysis of broad-spectrum beta lactam antibiotics including carbapenems. Resistant to carbapenems especially in Gram negative pathogens is global public health issue because of the spread of transferable carbapenemase encoding genes. The antibiotic resistant elements could be transferred to other Gram-negative bacterial strains and contribute towards spread of antimicrobial resistance rate making the treatment of infected cases complex. [2] Carbapenems are the antibiotics which are utilized for treating multidrug-resistant *P.aeruginosa* (MDRP) isolates. Carbapenem antibiotics used to be effective agents against MDRP when first presented. However, the growing frequence of carbapenem resistant *P. aeruginosa* (CRPA) has turned into a severe health problem recently. These strains lead to high mortality rates in cases infected by *P. aeruginosa* and there are also few effective drugs against them. Resistance to carbapenems can be related to the production of carbapenemase enzymes such as serine carbapenemases (containing KPC and GES enzymes), metallo-â-lactamases (MBLs) such as IMP, VIM, and NDM enzymes, and oxacillinases (such as OXA enzymes). blaIMP and blaVIM are the most constantly acquired MBLs. However, the recently emerged NDM type (New Delhi metallo-â lactamases) is getting the most important carbapenemase. [3]

Carbapenems have become the drug of choice for the treatment of severe nosocomial infections caused by Gram-negative bacilli. Carbapenem resistant Enterobacteriales (CRE) is a considerable health problem globally and are associated with increased mortality also There is a significant challenge in controlling the spread of carbapenemases, Surveys of the molecular epidemiology of carbapenemases revealed that the dissemination of carbapenemases, including NDM, VIM, IMP, OXA-48 and KPC producers, are rapid and widespread among healthcare facilities. Molecular techniques remain the gold standard for the precise identification of carbapenemase genes. [4] Most of these techniques are based on PCR and may be followed by a sequencing step if a precise identification of the carbapenemase gene is needed (e.g. VIM type, KPC type, NDM type or OXA-48 type). [4] They are either single or multiplex PCR techniques.[4]

In Africa, data on the frequence and distribution of carbapenem resistance among the multidrug resistant Gram-negative bacteria is still limited.[5] In addition, detection discovery of carbapenem resistance genes producing bacteria may be difficult based on routine antibiotic susceptibility testing.[6] Considering the fact that there were no comprehensive studies about prevalence of carbapenem resistance genes in Sudan so detection of those genes in *P. aeruginosa* from clinical samples will be of a great value. Such data serve an important part in understanding the spread of this pathogen and help in good treatment for it. So this study aimed to detect carbapenem resistance genes of *Pseudomonas aeruginosa* from clinical samples in Khartoum State, using conventional and PCR based method.

MATERIALS AND METHODS

Study sample and data collection

A total of fifty non-repetitive *P.aeruginosa* (n=50), isolated from different clinical specimens

Data included sample type, gender, and age of patients were collected from hospitals' medical records checklist.

Isolation and identification of bacteria

One colony from each isolate was sub-cultured on Cysteine Lactose Electrolyte Deficient media (CLED) and Nutrient agar and incubated aerobically at 37°C overnight, for further Re-identification and preservation. *P.aeruginosa* showed smooth blue non-lactose fermenting colonies on CLED, were identified by Gram stain and conventional biochemical tests.

Antimicrobial Susceptibility test

All confirmed isolates were subculture from 15% glycerol on nutrient agar getting overnight and pure growth. Modified Kirby Bauer was performed and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2022).

DNA Extraction

The boiling method was used for isolation of bacterial genomic DNA which extracted from fresh overnight incubated isolate that suspended in 200ul of d H2O in 1.5 eppendrof tube, bolied for

10 min at 100°C in thermal block incubator, vortex, then cooling at -20°C following by centrifuged for 10 min at 12000xg. Supernatant were carefully collected and store at -20°C until analyzed. The purity of the extracted DNA was determined by running the DNA sample on 1.5% agarose gel.

Polymerase Chain Reaction Amplification

PCR was done by multiplex PCR, amplification which done using Maxime PCR Premix kit and specific primer which sensitized by Specific primers were used to amplification indicated in table (1). The PCR assay was carried out in a total volume of 25 ìL of mixture containing 2 ìLMaxime PCR Premix containing 1X PCR buffer, 1.5 mM MgCl2, 200M ì of each dNTP, and 1 U Taq DNA polymerase, 0.5 ìL of each of the virulence gene-specific primers (2 ìL), 5 ìL of template DNA and 13 ìL of water for injection. The amplification conditions included three steps: heating at 95°C for 5 min; 34 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1min for (NDM -1 and IMP -1 genes) and extension at 72°C for 1min; and the final extension at 72°C for 5 min. [7]

Gel electrophoresis

The gel casting tray was put into the electrophoresis, tank flooded with 1x TBE buffer just to cover the gel surface, 2ìl of PCR products from each 50samples was added to wells of electrophoreses, 5 ìl of100-bp DNA ladder, was added to the well in each run. The electrophoresis was carried out at 120Volts for 2 Hours. Then the gel was visualized for DNA bands by U.V transilluminator and photographed.

Statistical analysis

All outcome data were analyzed by chi-square using Statistical Package for Social Sciences (SPSS; Version16).

RESULTS

Out of the 50 isolates, 18 were isolated from urine samples, 11 from wound swabs, 6 from blood, 2 from high vaginal swabs, 8 from sputum, 3 from ear swabs, and 2 from body fluids, after culturing most strains were show pigmentation 32 (74%), the most pigmented strain was found in urine (26%) and wound samples (20%) table (2). Demographic data show out of 50 the male 23 were (46%),where female were 27 (54%) with ratio1 : 0.85. Ages ranged from 4 to 76 years, with an average of 49. Majority of the samples (37%) were found in the age group (40 – 60) year table (3).

Table 1. Primers used for amplification of carbapenem resistance genes of *P.aeruginosa* isolates

Target gene	Sequence (5'- 3')	Amplicon size (bp)
IMP-F	GGAATAGAGTGGCTTAAYTC	Bla IMP -1 232
IMP-R	GGTTTAAYAAAACAACCACC	
NDM-F	GGTTTGGCGATCTGGTTTTC	Bla NDM -1621
NDM-R	CGGAATGGCTCATCACGATC	

Table 2. Frequency of pigmented strains among different clinical samples

		Pigment appearance	
		Green Pigment	Non pigmented
Sample	Urine sample	13 (26%)	5 (10%)
	Sputum	7 (14%)	1 (2%)
	Wound	10 (20%)	1 (2%)
	Blood	4 (8%)	2 (4%)
	Ear swab	1 (2%)	2 (4%)
	High vaginal swab	2 (4%)	0 (0%)
	Body fluids	1 (2%)	1 2%)

Approximately one-third 18 (36%) of isolates were resistant to Aztreonam, 10 (20%) were resistant to Ciprofloxacine and Colistin, 11 (22%) resistant to Gentamicin, Amikacin, and Pipracillin/tazobactam combinations, only one isolate was resistant to Imipenem, from 18 urine samples 3 (19%) isolates were resistant to Norfloxacin table (4).

Out of 50 *P. aeruginosa* isolates; only 14 (28%) were MDR according to the preliminarily described definition , most isolates were sensitive to all antibiotics 10 (36%), 12 (24%) isolates resist

Table 3. Distribution of positive samples among different gender and age groups

		Gender		
		Male	Female	Total
Age group	<20	1 (2%)	0 (0%)	1(2%)
	20-40	3 (6%)	10 (20%)	13 (26%)
	40-60	10 (20%)	10 (20%)	20 (40%)
	>60	9 (18%)	7 (14%)	16 (32%)

Table 4. Antimicrobial susceptibility pattern of *P . aeruginosa* isolates

Sample / Antibiotic		Urine (N=18)	Wound swab (n=11)	Blood (N=6)	HVS (N=2)	Sputum (N=8)	Ear swab (N=3)	Body fluid (N=2)	Total (N=50)
CIP	Resistant	3	3	2	0	1	2	0	11(22%)
	Sensitive	15	8	4	2	7	1	2	39(78%)
CN	Resistant	4	5	2	0	2	1	1	15(30%)
	Sensitive	14	6	4	2	6	2	1	35(70%)
TPZ	Resistant	5	3	0	0	1	2	0	11(22%)
	Sensitive	13	8	6	2	7	1	2	39(78%)
CAZ	Resistant	6	4	3	0	2	1	1	17(34%)
	Sensitive	12	7	3	2	6	2	1	33(66%)
ATM	Resistant	12	4	3	1	7	1	1	30(60%)
	Sensitive	6	7	3	1	1	2	1	20(40%)
AK	Resistant	2	5	1	0	3	1	1	13(26%)
	Sensitive	16	6	5	2	5	2	1	37(74%)
IMP	Resistant	2	0	0	0	0	0	0	2 (4%)
	Sensitive	16	11	6	2	8	3	2	48(96%)
CT	Resistant	5	1	0	1	2	1	0	10(20%)
	Sensitive	13	10	6	1	6	2	2	40(80%)

Table 5. Association between sex and Antimicrobial susceptibility results

Antibiotic	Sex group			
	male		Female	
	Sensitive	Resistant	Sensitive	Resistant
CAZ	15	8	18	9
AZT	11	12	10	17
TPZ	17	6	22	5
CT	21	2	19	8
CN	13	10	22	5

Table 6. Association between *P . aeruginosa* Resistant genes and infection sites

Resistant genes	Sample sites					
	Urine N=18	Wound N=11	Ear swab N=3	Blood N=6	Body fluid N=2	Sputum N=8
NDM-1	4	2	0	0	0	0
IMP-1	6	4	0	1	1	1

Table 7. Association between the presence of IMP-1gene and NDM -1 gene WITH antibiotics susceptibility

Antibiotic		IMP -1		NDM -1	
		(+ve)	(-ve)	(+ve)	(-ve)
Ciprofloxacin	R	5	12	2	15
	S	8	25	4	29
Colistin	R	3	10	3	10
	S	10	27	3	34
Gentamicin	R	4	18	2	20
	S	9	19	4	24
Amikacin	R	1	5	0	6
	S	12	32	6	38
Pipracillin	R	7	19	1	25
	S	6	18	5	19
Impenem	R	5	18	2	21
	S	8	19	4	23
Aztreonam	R	8	24	3	29
	S	5	13	3	15
Ceftazidime	R	10	26	3	33
	S	3	11	3	11

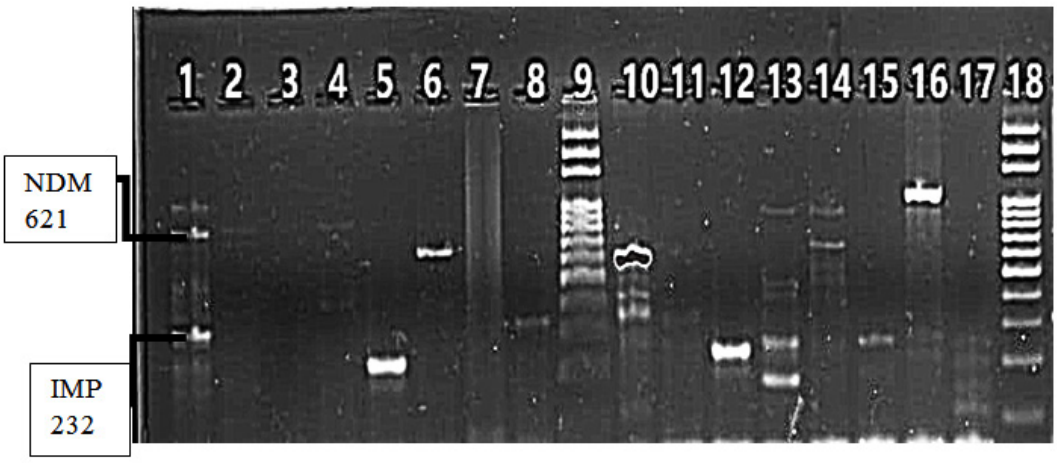

Fig. 1. Gel electrophoresis of NDM -1 gene. The gene was detected in 6 isolates out of the 50 isolates. Lane: 9 and 18 ladder 100 bp. Lane: 1/5/12/13/15/and 17 positives for IMP -1 product (232bp). Lane:7/8 and 11 show negative results. Lane: 1/2/4/13 /14 and16 show positive for NDM -1product (621bp).

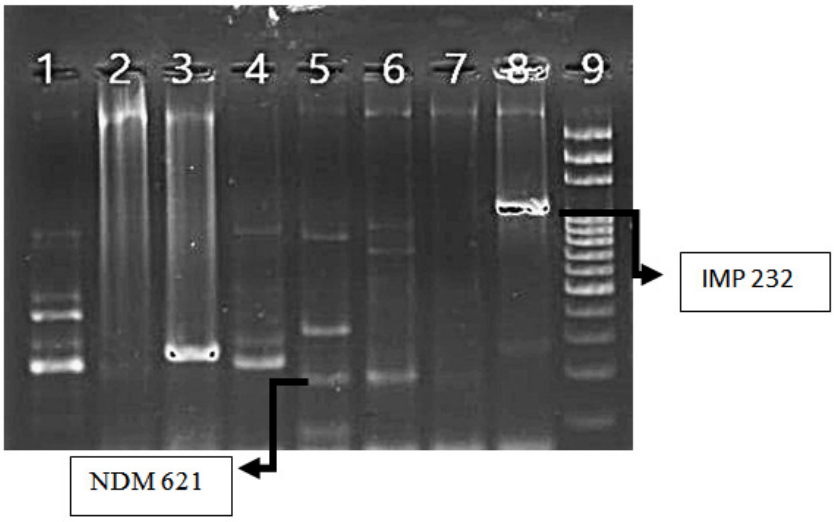

Fig. 2. Gel electrophoresis of IMP -1 gene, the gene was harbored in13samples Lane: 9 ladder 100 bp. Lane: 1/4/5and 6 positive for NDM -1 product (621bp). Lane: 2/7 show negative results. Lane: 1/3 and 8 positive for IMP -1 product (232bp)

to one antibiotic, 6 (12%) isolates resist to two antibiotics. In MDR isolates we found two strains were resistant to >7 antibiotics, whereas just three isolates were found to be resistant to 6 antibiotics. There was insignificant Association between sex and antimicrobial susceptibility results table (5). The NDM-1gene was detected in 6 isolates (12%) and negative in 44 isolate (88%). They were recovered from urine (8%), wound (4%), ear swab (0%), sputum (0%), blood (0%) high vaginal swabs (0%) and body fluid (0%) table (6). While the IMP-1 gene was detected in 13(26%), and negative in 37(74%) isolate table (6). There was insignificant difference in the percentage of resistant genes among the different infection site. The association between the presence of NDM-1 and IMP-1genes and antibiotics was significant with Pipracillin, Impenem, Aztreonam, Ceftazidime table (7).

DISCUSION

In the current study, most of isolates harboring carbapenem resistance genes were phenotypically susceptible to imipenem and most of them were positive for IMP gene. This confirm what [8] said that this gene is not strong and relies upon other synergistic mechanisms to mediate resistance against carbapenems. In addition to imipenem other antibiotics were analyzed in this study. Majority of the isolates showed varying degrees of resistance to the other antibiotics (ciprofloxacin, gentamicin and ceftazidime). Resistance to these antibiotics may also be due to the presence of ESBLs and other mechanisms like efflux pumps and porin mutations [9], which were not covered in this study.

In the current study, the most prevalent gene among the 50*P. aeruginosa* isolates was IMP gene (26 %). NDM gene was also present but on a smaller scale (12%, n =6). This is consistent with previous findings by in Turkey[10], which detected OXA-48 gene (58%) and NDM gene (2%) [11], detected OXA-48 gene (64.7%) and NDM gene (5.8%) and [12] detected OXA-48 gene (78%) and NDM gene (20%) both from Saudi Arabia, IMP gene were not detected in these studies

These variations in results may be because, they only detect resistant genes in carbapenem-resistant isolates or due to differences in method of detection as the first one used real-time PCR or differences in the sample size of tested organism and that was seen with the other two studies .

Of 50 *P. aeruginosa* isolates detected of having carbapenem resistance genes, two had multiple genes. This finding agree with [13] and [6] which showed multiplicity of genes in their

isolates. The presence of multiple resistance genes in one isolate have not been generally 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. [6] Regarding NDM and IMP gene, 6 (12%) and 13(26%) were positive respectively. Ceftazidime resistant Isolates showed significant association with both NDM and IMP gene as detected 3, 10 isolates respectively. One of the limitations of this study; it was conducted on a limited number of samples so future studies should be carried with large sample size and different clinical isolates to and sequencing technique to document the prevalence of these resistant genes and to help on therapeutic strategies.

CONCLUSION

In conclusion, the findings of the present study disclosed a high percentage andmultiplicity of carbapenem resistance genes among *P. aeruginosa* isolates in Khartoum State. This study showed that the NDM-1 and IMP-1 genes were generally circulated among the *P. aeruginosa.*

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Conflect of interest

The author(s) do not have any conflict of interest

Data availability

This statement does not apply to this article.

Ethics statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval

Informed consent statement

This study did not involve human participants, and therefore, informed consent was not required

Clincal trial registeration

This research does not involve any clinical trials.

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Not Applicable.

Authors contributions

Aisha Mohammed Abdallah methodology, and conducted the study; Mutaz Fathelrhman Saad supervised the study, data analysis conceptualized, visualized, designed, interpretation of the results, and wrote the drafted paper; Hind Haidar Ahmed, Samar Mohammed Saeed, Tagwa Salah Ahmed, Wafaa Mohammed Abdalla data analysis and check the methodology, editing and approved the manuscript critically for remarkable intellectual contents. All authors read and approved the final manuscript

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