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Molecular Detection of Some High Resistance Genes Metallo B-Lactams of *Klebsiella Pneumoniae* using Multiplex PCR

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ABSTRACT

In the last decade, organisms resistant to multiple drugs have become a threatening problem worldwide. Considering the lack of infection control practices to contain outbreaks and the increase in bacterial resistance in many hospitals in Khartoum, this study aimed to investigate the pattern of antibacterial susceptibility and prevalence of resistance genes (blaNDM, blaAIMblaGIM, blaIMP) of *Klebsiella pneumoniae* using multiplex PCR from clinical samples with multidrug resistance. The cross-sectional study was conducted in the period from October 2020 to January 2021 in multidrug-resistant bacteria. The bacteria were collected from a different hospital in Khartoum, Sudan. String test highly virulent *Klebsiella pneumoniae* was identified by a positive string test. The disc diffusion method on Mueller-Hinton agar (Merck Company Germany) was used to determine antibiotic susceptibility. Genomic DNA was extracted from colonies of *Klebsiella pneumoniae* and analyzed by multiplex PCR for the genes (blaNDM, blaAIM, blaGIM and blimp. A total of 51 isolates of *Klebsiella pneumoniae* were recorded from samples of patients of whom 29 (56,9%) were male and 22 (43.1%) were female, the mean age of the patients was 40 years (median 41 years) and ranging from 1 to 71 years. Samples types in various cases included urine 23(45.1%)**,** sputum 18(35.3%), blood 2(3.9%), wound swabs 7(13.7%) and pus aspirate 1(2.0%)**.** Antimicrobial susceptibility testing of the resistant isolates by disc disk diffusion method showed that the lowest and highest resistance, the rate of the antibiotic resistance; meropenem (56.9%), trimethoprim/sulfamethoxazole (74.5%), ceftazidime (84.3%), cefotaxime (96.1%) ampicillin (98.0%), ceftriaxone (96.1%), tetracycline (54.9%), aztreonam (84.3%), colistin (25.5%) and ampicillin/clpxacilin (100%). A multiplex PCR amplification test was performed to detect the prevalence of genes NDM 27 (52.9%), AIM 7 (13.7%) IMP 3 (5.9%) GIM 3 (5.9%), and no genes 11 (21.6%) in the samples. The study concluded that these isolates became a source of warriors because they can develop resistance in a short period. Controlling and preventing resistant isolates necessitates close monitoring and rapid intervention.

Keywords: Molecular detection, High resistance genes, Klebsiella pneumoniae, multiplex PCR.

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INTRODUCTION

The metallo-β-lactamase (MBL) has become a series of problems globally due to the increased prevalence of carbapenem-resistant in gram-negative bacteria [1]. It has numerous virulence factors that play the main role in the severity of infection that is concerned about the increase in the antimicrobials resistance spread through a different mechanism from the endemic *K pneumoniae* carbapenemase KPC [2, 3]. It is both a public hospital-acquired pathogen and a prospective community-acquired pathogen that can cause infection of the urinary tract (UTI), pneumonia acquired in a hospital, intra-abdominal infection, and more infections in patients admitted to the ICU [3, 4]. Reducing the risk of public health problems and best management of the development and the spread of *Klebsiella pneumoniae* is important. This can be done by developing more prevention strategies for controlling [5]. *Klebsiella pneumoniae* has developed enzymes that degraded β-lactams the result became drug resistance [6]. Ambler classifies this enzyme into four categories (A, B, C, and D). carbapenemases genes were coded for transferable plasmids and be able to be easily transmitted among bacteria [7]. Carbapenems are the ultimate antimicrobials for the treatment of prolonged spectrum β-lactamases (ESBLS) producing Enterobacteriaceae [8]. *Klebsiella pneumoniae* is a fast-growing multidrug-resistant gene [9]. Moreover, known genes of resistance produced by bacteria might be challenging based on repetitive antibiotic vulnerability testing [7]. The knowledge about bacterial resistance plays a meaningful role control of the spread of pathogens [10]. An energetic longstanding investigation should be done on both drug resistance features and virulence influences [11]. In Sudan, multi-drug resistance *Klebsiella pneumoniae* is spread among hospitals in Khartoum, and incidence of carbapenems multi-resistant genes in very severe type of *Klebsiella pneumoniae* detection from patients in the hospitals [12, 13]. The study was designed to investigate antibacterial susceptibility patterns and prevalence of resistance by using multiplex PCR from Klebsiella Pneumoniae isolated from clinical specimens in the Khartoum capital of Sudan.

MATERIALS AND METHODS

Study design

The cross-sectional study was conducted in the period from October 2020 to January 2021. Conducted in multidrug-resistant bacteria. The bacteria were collected from a different hospital in Khartoum, Sudan. Sample size in this study enrolled according to this equation: n =z2*p(1_p)/c2 n = sample size Z = power (1.96), P = prevalence of disease, (0.5) C= stander deviation. Clinical Isolate of Klebsiella pneumoniae collected from the clinical samples including urine (n=23), blood (n=2), sputum (n=18), pus aspiration (n=1) and wound swab (n=7). Personal data were obtained by a directly interviewed questioner in the hospitals in Khartoum. The patient agrees to participate in the research, verbal, and publication of this data. Thus, obtaining permission from the Administration of Health in Khartoum. The reason for just obtaining oral consent without the need for written consent is that collection is part of routine clinical laboratory work for the diagnosis of these infections.

Method

String test: The high virulent of Klebsiella pneumoniae was identified by a positive string test [14]. Gram stain and Biochemical test: were done according to medical laboratory manual for tropical countries volume 2 [15]. Sensitivity Test: By using the disc diffusion method on Mueller-Hinton Agar (Merck Co., Germany). A plate of Mueller Hinton agar was inoculated with the suspension by the use of a sterile cotton swab. The swab flippantly over the surface of the medium become streaked and meropenem (MEM) 10 µg Trimethoprim/Sulfametaxazol (SXT;1.25/23.75µg), Ceftazidime (CAZ;30µg), Cefotaxime (CTX;30µg), Ampicillin (AM; 10µg), Ceftriaxone (CRO; 30µg), Tetracycline (TE;30Mg), Aztreonam (ATM;30 Mg), Colistin (CT) and, Ampicillin/Clpxacilin (AX;10 µg) antimicrobial discs had been placed. As a result, in this observe carbapenem resistance becomes described as testing non -susceptible to meropenem. However, susceptibility turned into described as checking out susceptible to meropenem and/ or other tested antibiotics.

Multiplex-PCR Genomic DNA has been extracted from colonies of Klebsiella pneumoniae that are suspended in 200µl sterile distilled water, boiled at 95c for 10-15 min, cooled on ice for 10 min and

then boiled to 2 min and cooled to 2 min and centrifuged for 10min at 10,000×g. The concentration and quality of the extracted cellular DNA were measured using gel electrophoresis and screening for genes β-lactamase genes (blaNDM, blaAIM, blaGIM, blaIMP) using multiplex-PCR by a specific primer for gene amplification NDM-Forward GGTTTGGCGATCTGGTTTT, 621bp and NDM Reveres [16]. AIM-Forword CTGAAGGTGTACGGAAACAC 322 bp, AIM-Reveres GTTCGGCCACCTCG AATTG, GIM-Forward TCGACACACCTTGGTCTGAA 477 bp and GIM-Reveres AACTTCC AACTTTGCCATGC [17]. In the reaction was used 5µl genomic DNA, mixed with 6µl of master mix and 0.5µl of each primer and 7µl of sterile distilled water. The reaction condition was 36 cycles, with worming stage initial denaturation at 95°C for 10 min; denaturation at 95°C for the 30s, annealing at 52°C for 40s, and extension at 72°C for 50 min; and a final extension at 72°C for 5 min: and reading the result by gel electrophoresis using the ladder 100 pb.

Data analysis

The data were analyzed by using statistical program (SPSS) software. The Chi-square was used to determine the association between categorical variables and significant parameters at the level at p-value 0.05.

RESULTS AND DISCUSSION

A total of 51 isolates of *Klebsiella pneumoniae* were obtained from different clinical specimens of patients, out of these males were 29 (56,9%) and females were 22 (43,1%), the average age of patients was 40.7 years (median, 40 years) ranging from 1 to 71 years, this average indicates that most patients over 40 years have susceptible to infection in the urinary tract. Samples types in various cases included urine23(45.1%) sputum , 18(35.3%) blood ,2(3.9%), wound swabs 7(13.7%), and pus aspirate 1(2.0%), the Frequency of study population according to samples **Table 1**. The results of antimicrobial sensitivity tests indicated 51 isolates **Table 2**. Antimicrobial susceptibility testing of the resistant isolates by disc disk diffusion method showed that the lowest and highest resistance, the rate of the antibiotic ;resistance meropenem (56.9%), trimethoprim/sulfamethoxazole (74.5%), ceftazidime (84.3%), cefotaxime (96,1%), Ampicillin (98.0%), ceftriaxone (96.1%), tetracycline (54.9%), Aztreonam (84.3%), colistin (25.5%), Ampicillin/Clpxacilin (100%). The variation in sensitives between tested available antibiotics uses in sub-therapeutic doses, non-laboratory oriented therapy, and poor storage. Overall, this resistance refers to the misuse of antibiotics by health care providers, unskilled practitioners, and drug consumers. A multiplex PCR amplification assay was performed to detected genes (NDM (27: 52.9%), AIM (7: 13.7%), IMP (3: 5.9%), GIM (3: 5.9%), and no detected genes (11: 21.6%) samples, the percentage of Gene among study population: **Figure 1**, the Correlation between gene and sample **Table 3**.

Table 1. Frequency of study population according to samples

Samples	Frequency	Percent
Urine	23	45.1
Sputum	18	35.3
Blood	2	3.9
Wound	7	13.7
pus aspirate	1	2.0
Total	51	100.0

Table 2. Frequency of study population according to sensitivities

Antibiotic	Sensitive	Resistance
Meropenem (MEM)	22(43.1%)	29(56.9%)
Ampicilin/Clpxacilin (AX).	0	51(100%)
Teatracycline (TE)	23(45.1%)	28(54.9%)
Ampicillin (AM)	1(2.0%)	50(98.0)
Colistin (CT)	38(470.5%)	13(25.5%)
Ceftriaxone (CRO)	2(3.9%)	49(96.1%)
Aztreonam (ATM)	8(15.7%)	43(84.3%)
Cefotaxime (CTX)	13(25.5%)	38(74.5%)
Ceftazidime (CAZ)	8(15.7%)	43(84.3%)
Trimethoprim/Sulfametaxazol (SXT)	2(3.9%)	49(96,1%)

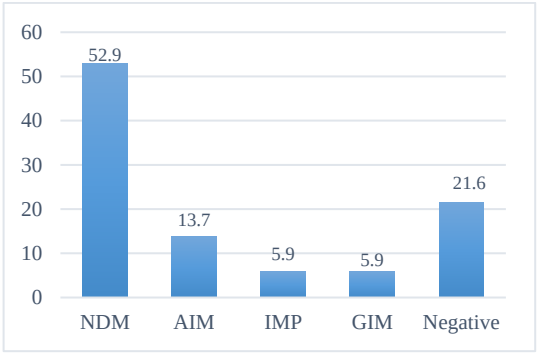

Figure 1. Percentage of Gene among study population

Table 3. Correlation between gene and sample

		Sample						
		Urine	Sputum	Blood	Wound	pus aspirate	Total	P-value
Gene	NDM	11	9	1	6	-	27	
	AIM	4	3	-	-	-	7	
	IMP	3	-	-	-	-	3	
	GIM	1	1	-	1	-	3	
	Negative	4	5	1	-	1	11	0.641
Total		23	18	2	7	1	51	

Level of significant (0.05).
P-value (0.641) is not significant between genes and samples.

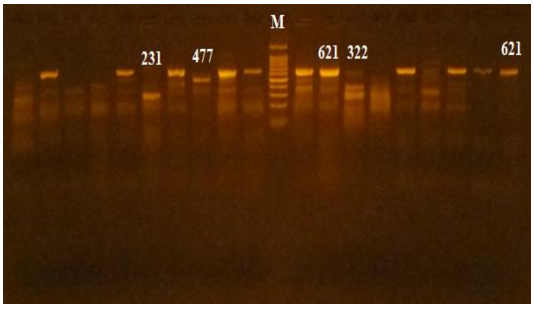

Figure 2. Multiplex PCR of samples of *Klebsiella pneumoniae* isolates. Legend M= Molecular marker, 100-1000 bp; 621bp =NDM; 477bp=GIM; 322bp=AIM; 232bp= IMP.

Antibiotic-resistant microorganisms have been described as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world [18]. In the current study, they found that *Klebsiella pneumoniae* was the most bacteria-made problem in ICU in Khartoum hospitals, especially in patients with an average age of 40 infected with the urinary tract. Due to lacking infection-control practices for containment of outbreaks and increased bacterial resistance in many hospitals in Khartoum. The results of this lacking reflect in the situation of resistance *Klebsiella pneumoniae* and became a major problem for medication with tested antibiotics, the study refers to this resistance in tested antibiotics to use in sub-therapeutic doses, non-laboratory oriented therapy, and poor storage. Overall, this resistance refers to the misuse of antibiotics by health care providers, unskilled practitioners, and drug consumers. The principal factor responsible for the development and spread of bacterial resistance is the injudicious use of antimicrobial agents, resulting in most bacteria continuously developing resistance to the antimicrobials in regular use at different periods [19]. Change in the efficiency of antimicrobial agents to bacterial pathogens is steady. The adequacy of another medication may change as it is utilized in clinical settings, and resistant bacterial clones result from the encounter of drug and organism. A large portion of the notable antibiotics is insufficient to a few microbial pathogen. Ampicillin, metronidazole, amoxicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, nalidixic acid, gentamicin, and ceftazidime are basic antibiotic antibiotics agents that have whose have been resisted recently raised [20]. The global incidence and extent of genes of bacterial resistance such as Metallo Beta-lactamase and carbapenemase genes in *Klebsiella pneumoniae* current is an important risk to public health [21]. Shoja *et al*. mentioned that, tigecycline turned into the best antimicrobial agent with 96.5% susceptibility. Similarly, 6.5% of the isolates were carbapenem-resistant. Bla NDM-1 become identified in four isolates (isolate A-D) and all of them were multidrug-resistant. mlst discovered that Bla NDM-1 -fine isolates have been clonally associated and belonged to two distinct clonal complexes [1]. In the current study, *Klebsiella pneumoniae* isolates were positive genes which in arrangement with results obtained by Ali and Omer, [22], who reported that the percentage in Sudan 17% while in South Africa was 71% reported Singh-Moodley and Perovic, [23]. In Saudi Arabia was recorded *Klebsiella pneumoniae* Ceftriaxone (CRO)(49%, 33%) [24]. The bacteria acquired resistant genes due to the lack of practices and application of antibiotics in hospitals and pharmacies most of Klebsiella pneumoniae isolation found resistance majority antibiotics with varying degrees [25]. Many studies done by other researchers in Sudan they're finding the NDM gene (52.9%), IMP (5.9 %), GIM (5.9%), and AIM (13.7 %) were different from our finding this variation refers to methods applied and samples size [22]. Regarding, (bla_{AIM}, bla_{GIM}, and bla_{IMP}) qualities, were positive separately, qualities showed importance with both examined tests orientation and age. This might be expected to become microorganisms more powerful to contaminated patients who have immunodeficiency [4, 26]. *Klebsiella pneumoniae* examined in Saudi Araba was tracked down a high variety in antibiotics resistance genes [27]. Walsh [28], proposed that

this gene is very weak and they have other factors that help it to produce antibiotic resistance. Adam and Elhag [29] were the first to report the prevalence of MBL encoding genes among *Klebsiella pneumoniae* in Khartoum State, Sudan. Our result agrees with a study done in Khartoum state, where there were genes detected in clinical samples were isolated from *Klebsiella pneumoniae*.

CONCLUSION

This study revealed a high prevalence of MBL genes in *Klebsiella pneumoniae* isolates from Khartoum state Hospital, all the four-gene assayed (bla_{AIM}, bla_{IMP}, bla_{AIM}, and bla_{GIM}) were detected in study samples. Detecting of MBL gene among isolate which reported as sensitive to genes were acting reservoir. Those outcomes imply co-carriage of several β-lactamase genes and antibiotic resistance integrons at the identical plasmids harboring multi-drug resistance genes. It seems that those houses assist to decrease treatment headaches due to resistant bacterial infections by speedy detection, contamination-manipulate packages, and prevention of transmission. Furthermore, these isolates became a source of warriors because they can develop resistance in a short period. Controlling and preventing resistant isolates necessitates close monitoring and rapid intervention.

Limitation of study

This study used only a few tested antibiotics because the research was conducted within short time and they used available antibiotics in Khartoum. The number of isolation is very low, specially that isolated from blood culture.

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CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: This work was approved by the Ethics Committee of, National University. The data obtained from this work were analyzed according to guidelines of ethical standards of the Declaration of Helsinki. Permission to carry out the study will take it to run this study. All subjects examined will be informed of the purpose of the study before the collection of the specimens.

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