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Original Article

Identification and phylogenetic analysis of carbapenemase genes from clinical strains of *Klebsiella pneumoniae*

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Abstract

Introduction: *Klebsiella pneumoniae* is an encapsulated Gram-negative bacterium that is responsible for numerous infections in healthcare facilities worldwide and is frequently isolated. The World Health Organization has listed *K. pneumoniae*as as a critical antibiotic resistant bacterial pathogen for which new antibiotics are urgently needed. This study aimed to use molecular tools to identify and examine antibiotic resistance in clinical strains of *K. pneumoniae*.

Methodology: A total of 15 unduplicated *K. pneumoniae* strains isolated from patient samples with multidrug-resistant (MDR) profiles were subjected to polymerase chain reaction (PCR) to amplify the most common carbapenem resistance genes. (GTG)5 PCR and phylogenetic analysis were performed to identify the genetic relationship between the strains.

Results: All strains yielded a (GTG)5-PCR profile, and this allowed us to group these strains into 8 groups according to the size and number of characteristic bands. Phylogenetic analysis was done using the free software UPGMA and a single bacterial clone with a correlation coefficient of over 97% was identified. New Delhi metallo-beta-lactamase NDM-like (*bla*NDM) carbapenem resistance genes were detected in three strains of *K. pneumoniae*, which represented a resistance rate of 20%. However, carbapenemases type A [*Klebsiella pneumoniae* carbapenemase (KPC) and imipenem-hydrolysing beta-lactamase (IMI), type D [oxacillinase-48 (OXA-48)], and other metallo-β-lactamase [Verona integron-encoded metallo-beta-lactamase (VIM), and enzyme active on imipenem (IMP)] were not detected.

Conclusions: We identified and grouped the *bla*NDM resistance genes of *Klebsiella pneumonia* strains.

Key words: *Klebsiella pneumoniae*; carbapenemases; (GTG)5-PCR.

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative pathogen that is the causative agent of a variety of diseases, including urinary tract and soft tissue infections, bacteremia, and pneumonia. *K. pneumoniae* was traditionally considered to be an opportunistic agent responsible for nosocomial infections; however, it is increasingly involved in community infections [1]. This opportunistic, encapsulated, non-motile bacterium has the ability to survive and persist in the hospital environment and can be transmitted via personal contact, contaminated environments, and medical equipment [2]. *K. pneumoniae* is considered to be one of the most frequent agents of infectious diseases and is a significant menace to public health due to its multidrug resistance (MDR). Carbapenem was used as the drug of choice for the treatment of infections caused by MDR *K. pneumoniae*. However, the increased prevalence of carbapenem-resistant *K. pneumoniae* greatly compromised the efficacy of carbapenem antibiotics. It is often resistant to all currently available antimicrobial agents; or remains susceptible only to older, potentially more toxic agents, such as the polymyxins, leaving limited and sub-optimal options for treatment [3]. The treatment options for MDR bacteria such as *K. pneumoniae* are questionable due to antibiotic resistance enzyme production; therefore, they are included in the World Health Organization (WHO) critical list [4]. *K. pneumoniae* is one of the species where most antibiotic resistance enzymes have been described, including carbapenem resistance enzymes *Klebsiella pneumoniae* carbapenemase (KPC),

imipenem-hydrolyzing beta-lactamase (IMI), enzyme active on imipenem (IMP), Verona integron-encoded metallo-beta-lactamase (VIM), and New Delhi metallo-beta-lactamase (NDM) [5–8]. Other β-lactamases are also produced by *Klebsiella pneumoniae* including oxacillinase (OXA), temoneira (TEM), sulfhydryl variale (SHV), and cefotaximase Munich (CTX-M). As a result, treatment of *K. pneumoniae* infections are complicated, and, in the many cases, ineffective [9].

In view of the MDR of the *K. pneumoniae* species, polymerase chain reaction (PCR) using (GTG)5 PCR was carried out to group the strains of this study. Subsequently, a search for the most frequent carbapenemase genes (*blaKPC*, *blaIMI*, *blaVIM*, *blaIMP*, *blaNDM*, and *blaOXA-4*8) was carried out to monitor the spread of antibiotic resistance and verify whether the presence of a resistance gene is associated with a specific group.

Methodology

Sample collection

This was a retrospective study of clinical strains collected in the laboratory for routine analysis. The clinical strains included in this study were derived from urine and pus cultured specimens. Strains of MDR enterobacteria, including *K. pneumoniae*, were collected from the analysis of inpatient and outpatient samples at the biomedical laboratory from February to September 2020. Fifteen MDR *K. pneumoniae* strains were included in this study. The bacteria were identified by standard bacteriological methods using Gram stain, and biochemical method using API 20E kits (bioMérieux, Marcy-l'Étoile, France). Pure colonies were kept at -20 °C in LB medium supplemented with 20% glycerol for subsequent tests and analyses.

Screening of MDR K. pneumoniae

Antimicrobial drug susceptibility was determined by the disk-diffusion method on Mueller-Hinton (MH) agar plates (HiMedia Laboratories, Thane, India). The following antibiotics (Liofilchim, Roseto degli Abruzzi, Italy) were tested on the strains in routine examination: Fosfomycin (200 µg), norfloxacin (10 µg), gentamicin (10 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ceftriaxone (30 µg), cefixime (5 µg), chloramphenicol (30 µg), cefuroxime (30 µg), imipenem (10 µg), amikancin (10 µg), ticarcillin-clavulanate (75/10 µg), ceftazidime (30 µg), meropenem (10 µg), cefepime (30 µg), ticarcillin (75 µg), aztreonam (30 µg), netilmicin (10 µg), nitrofuran (100 µg), amoxicillin-clavulanate(20/10 µg), according to the guidelines of the Antibiogram Committee—French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) guidelines [10], which are used for the detection of the effect of MDR [11]. All strains were resistant to at least one antibiotic from three different families.

Molecular characterization

DNA extraction

A pure identical colony of *K. pneumoniae* was resuspended in 100 µL of sterile distilled water in a 1.5 mL Eppendorf tube. This was boiled at 100 °C for 10 min and immediately frozen at -20 °C for 10 min, centrifuged at 13,000 rpm for 5 min, and the supernatant containing the DNA was collected for PCR analysis [12].

(GTG)5 PCR conditions

Repetitive element sequence-based polymerase chain reaction (Rep-PCR) was performed with the universal (GTG)5 primer (5'-GTGGTGGTGGTGGTG-3'). Amplification was carried out in 25 µL of reaction mixture containing 4 µL of Master Mix (FIREPol®) Inqaba Biotec, Ibadan, Nigeria), 1 µL of (GTG)5, 17 µL of nuclease-free water, and 3 µL of extracted DNA. Amplification was done for

Table 1. Primers used for detection of carbapenemases genes in *K. pneumoniae.*

Target	Sequence (5′ → 3')	Annealing temperature	Amplicon size (bp)	Reference
NDM	**F**: TGGCAGCACACTTCCTATC **R**: AGATTGCCGAGCGACTTG	58	488	[13]
VIM	**F**: AGTGGTGAGTATCCGACAG **R**: TCAATCTCCGCGAGAAG	52	212	[13]
IMP	**F**: GTTTATGTTCATACWTCG **R**: GGTTTAAYAAAACAACCAC	45	432	[14]
IMI	**F:** CTACGCTTTAGACACTGGC **R:** AGGTTTCCTTTTCACGCTCA	57	481	[15]
KPC	**F**: CTGTCTTGTCTCTCATGGCC **R**: CCTCGCTGTRCTTGTCATCC	60	796	[16]
OXA-48	**F**: TTGGTGGCATCGATTATCGG **R**: GAGCACTTCTTTTGTGATGGC	58	744	[17]

IMI: imipenem-hydrolyzing beta-lactamase; IMP: imipenemase; KPC: *Klebsiella pneumoniae* carbapenemase; NDM: New Delhi metallo-beta-lactamase; OXA-48: oxacillinase-48; VIM: verona integron-encoded metallo-beta-lactamase.

Table 2. Characteristic bands of rep-PCR fingerprint profiles.

Groups	Bacterial strains	NCB	Sizes of different bands (pb)
I	714, 743, 912, 942, 1707	1	100
II	P58	1	400
III	1075, 1686	2	200, 700
IV	1098, 1148	3	600, 700, 1000
V	1258, 1848	3	200, 500, 800
VI	1113	4	200, 300, 400, 800
VII	114	4	200, 400, 500, 700
VIII	1299	4	200, 300, 400, 500
	Kp	5	100, 200, 400, 700, 800

Kp: *Klebsiella pneumoniae* ATCC 700603; NCB: number of characteristic bands; pb: basic pair; rep-PCR: (GTG)5 repetitive element sequence-based polymerase chain reaction.

30 PCR cycles in a thermocycler (Mastercycler nexus gradient, Inqaba Biotec West Africa, Pretoria, South Africa). The PCR program was as follows: initial denaturation at 94 °C for 4 min; followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 1 min, and elongation at 65 °C for 8 min; a final extension at 65 °C for 16 min, and cooling the amplified product at 4 °C [12]. The *K. pneumoniae* ATCC 700603 strain was used for standardization of rep-PCR reactions to assess reproducibility.

PCR conditions for detection of carbapenemases genes

Carbapenemase genes were amplified by PCR on a mastercycler nexus gradient (Eppendorf, Flexlid Inqaba Biotec, Pretoria, South Africa) using gene specific primers for the detection of *blaNDM*, *blaVIM*, *blaIMP*, *blaOXA*, *blaKPC*, and *blaIMI*. The reaction mixture consisted of 4 µL of master mix (Inqaba Biotec West Africa, Ibadan, Nigeria), 2 µL of primers (forward and reverse), 17 µL of nuclease-free water, and 2 µL of DNA extract; in a total volume of 25 µL. The PCR reagents were provided by Inqaba BiotecWest Africa (Ibadan, Nigeria). The sequences of the primers used for detection of carbapenemases genes are listed in Table 1. Amplification reactions were performed in a thermal cycler (Mastercycler nexus gradient Inqaba Biotec, Pretoria, South Africa) according to the following program: initial denaturation at 95°C for 5 minutes; 36 cycles of denaturation at 95°C for 45 seconds, annealing at specific primer temperature (58 °C for *NDM*, 52 °C for *VIM*, 45 °C for *IMP*, 58 °C for *OXA*, 60 °C *KPC*, and 57 °C for *IMI*), extension at 72 °C for 1 minute; and final extension at 72 °C for 10 minutes.

Electrophoresis of PCR products

Ten microliters of each (GTG)5 PCR product were visualized in a 1.5% agarose gel, by adding to 4 µL of ethidium bromide (1mg/mL) in 1X TAE buffer. The band sizes were compared using a 100 bp molecular weight DNA ladder. The agarose gel electrophoresis was carried out for two hours at 60 V and 40 mA. The

Table 3. Antibiotic profiles of *Klebsiella pneumoniae* strains.

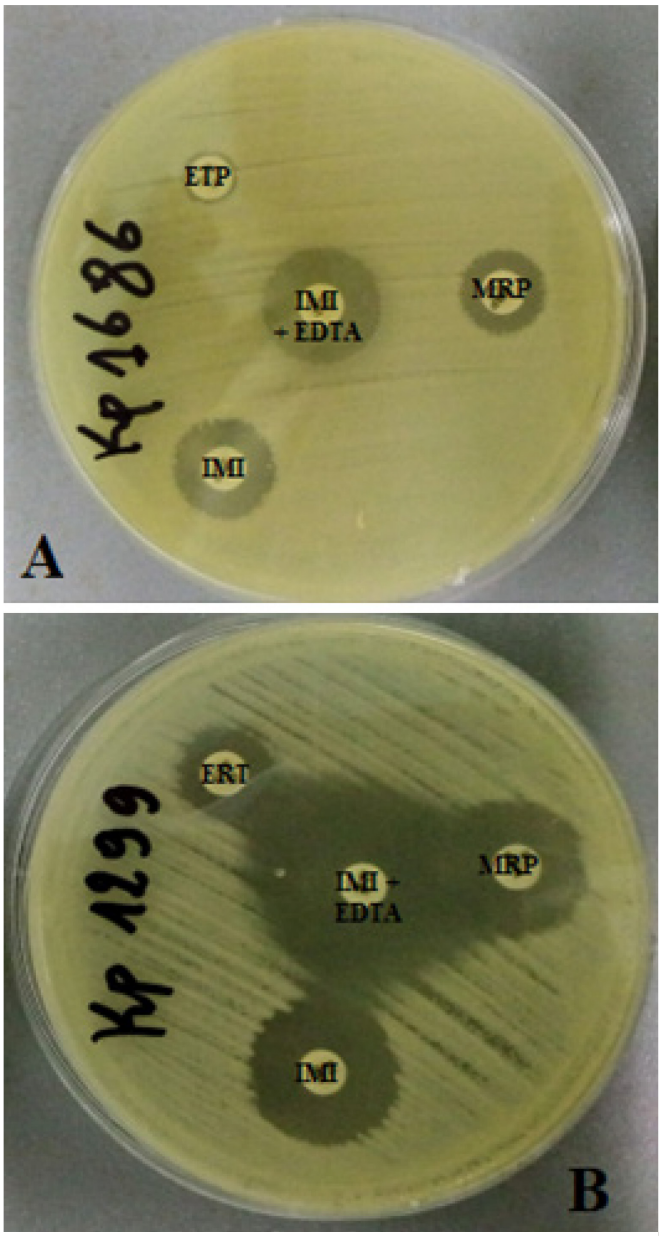
Strains	Patient's gender / age (years)	Susceptible	Intermediate	Resistant
K. pneumoniae 714	M/74	IMI^{27}, FOS^{24}		SXT^{6}, CN^{6}, NOR^{6}, CIP^{12}, CRO^{10}, TCC^{15}, AMC^{15}
K. pneumoniae 743	M/60	IMI^{27}, FOS^{25}, CN^{21}, AMC^{20}		CIP^{6}, NOR^{6}, SXT^{6}, CRO^{6}, CFM^{14}
K. pneumoniae 912	M/62	AMC^{20}, C^{23}		NOR^{6}, CN^{6}, SXT^{6}, CIP^{6}, CRO^{12}, CFM^{10}, CXM^{15}, IMI^{23}
K. pneumoniae 942	M/77	FOS^{25}		CFM^{10}, AMC^{10}, CRO^{9}, TCC^{19}, FEP^{14}, SXT^{6}, CIP^{6}, NOR^{6}, CN^{10}, LEV^{10}, IMI^{22}
K. pneumoniae 114	M/17	AK^{20}, C^{30}, TCC^{23}	IMI^{25}	SXT^{6}, CRO^{10}, CN^{10}, NET^{12}, CIP^{17}, FEP^{15}, NOR^{21}
K. pneumoniae P58	F/76	CN^{19}, CN^{28} AMC^{18}		SXT^{6}, CRO^{8}, CIP^{18}, CFM^{10}, IMI^{22}
K. pneumoniae 1075	F/75	IMI^{26}, C^{30}		AMC^{17}, CRO^{6}, AK^{17}, TCC^{20}, CIP6, NOR^{6}, CN^{6}
K. pneumoniae 1098	F/25	AK^{20}, CN^{20}	TCC^{20}	AMC^{16}, CRO^{14}, CFM^{14}, C^{6}, CIP^{25}, SXT^{20}, IMI^{25}, NOR^{20},
K. pneumoniae 1113	F/39	AK^{19}		AMC^{15}, CRO^{9}, TCC^{10}, CXM^{6}, CN^{9}, NOR^{15}, CIP^{18}, SXT^{6}, IMI^{22}
K. pneumoniae 1148	M/40	CN^{20}, CIP^{28}, CFM^{18}		CRO^{17}, AMC^{10}, IMI^{18}, CXM^{15}, C^{10}, SXT^{20}
K. pneumoniae 1258	M/75	C^{21}, AK^{18}		CN^{6}, SXT^{6}, NOR^{6}, CRO^{6}, CIP^{6}, AMC^{10}, CFM^{10}, FOS^{17}
K. pneumoniae 1299	M/69			IMI, MEM, FOS, TZP, C, NOR, AK, CXM, AMC, SXT, TCC, ATM, CFM, CIP, CRO
K. pneumoniae 1686	M/73	AK^{19}	FOS	TCC^{6}, SXT^{6}, CFM^{6}, AMC^{6}, CN^{6}, CIP^{6}, CXM^{6}, CRO^{6}, NOR^{6}
K. pneumoniae 1707	M/80	FOS^{24}, TCC^{28}, AK^{22} CN^{20}		AMC^{6}, CFM^{6}, CRO^{17}, C^{6}, CIP^{20}
K. pneumoniae 1848	F/20	SXT^{25}, AK^{21}, AMC^{18}		CRO^{6}, CN^{6}, FEP^{6} CIP^{22}, IMI^{25}, NOR^{22}

The subscript number indicates the inhibition diameter of the antibiotic in millimeters according to CA-SFM / EUCAST guidelines. AK: amikancin (10 µg); AMC: amoxicillin-clavulanate (20/10 µg); ATM: aztreonam (30 µg); C: chloramphenicol (30 µg); CFM: cefuroxime (30 µg); CIP: ciprofloxacin (5 µg); CN: gentamicin (10 µg); CRO: ceftriaxone (30 µg); CXM: cefixime (5 µg); FEP: cefepime (30 µg); FOS: fosfomycin (200 µg); IMI: imipenem (10 µg); LEV: levofloxacin (5 µg); MEM: meropenem (10 µg); NET; netilmicin (10 µg); NOR: norfloxacin (10 µg); SXT: co-trimoxazole (25 µg); TCC: ticarcillin-clavulanate (75/10 µg); TZP: piperacilline/tazobactam.

gel was visualized and photographed by the UVP PhotoDoc-lt Imaging System (Inqaba Biotec, Pretoria, South Africa). The photographs were visually analyzed and strains with identical profiles (characteristic bands) were assigned to the same group (Table 2).

In order to detect the carbapenemase genes, 10 µL of the PCR amplified products were separated by 1.5% agarose gel for 120 min at 80 V in 1X TAE buffer.

Figure 1. Carbapenem disc diffusion test. A, Klebsiella pneumoniae (kp) 1686; B, kp 1299.



IMI: imipenem (10 µg); MRP: meropenem (10 µg); ERT/ETP: ertapenem (10 µg); IMI + EDTA: imipenem + ethylenediamine tetraacetic acid.

Phylogenetic affiliation of strains

IBM SPSS version 25 for Windows (IBM Corp. Armonk, NY, USA) was used for data analysis. The genetic profiles of the strains obtained through rep-PCR were used for phylogenetic analysis. The band profiles for each isolate were converted to a binary matrix, indicating the presence or absence of a characteristic band. The similarity matrix was calculated using the Jaccard coefficient index. The unweighted pair group method with arithmetic (UPGMA) algorithm was applied to the similarity matrix with at and above mean Jaccard coefficient (standard deviation) value of 97%. A phylogenetic tree was developed by hierarchical classification analysis using the dendrogram construction utility DendroUPGMA.

Results

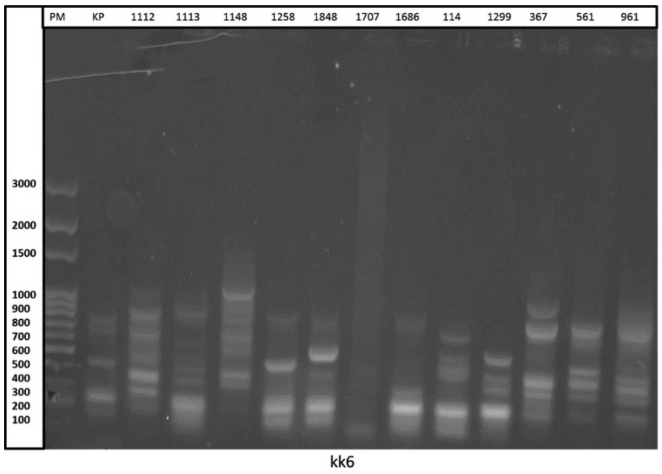
Bacterial multidrug resistance

Most of the strains of *K. pneumoniae* included in this study were isolated from urine samples. Only two strains (*K. pneumoniae* 114 and *K. pneumoniae* P58) were isolated from pus samples. The antibiograms of the different strains of MDR *K. Pneumoniae* are presented in Table 3.

Carbapenem diffusion disc

The phenotypic resistance profiles of *K. pneumoniae* strains were studied. The three strains harboring the *NDM* genes showed resistance to carbapenems (imipenem, meropenem and ertapenem) (Figure 1). The presence of EDTA along with imipenem showed a larger inhibition diameter than the imipenem disc alone, indicating the chelating action of

Figure 2. Representative gel profiles obtained after (GTG)5 repetitive element sequence-based polymerase chain reaction (rep-PCR). The different lanes represent rep-PCR amplified products from the different K. pneumoniae strains.



EDTA on the enzyme. This technique allowed the detection of metallo-beta-lactamase as had been described by several authors [18,19].

DNA fingerprinting with(GTG)$_5$ rep-PCR

Rep-PCR profiles of the 15 clinical *K. pneumoniae* isolates were obtained using the (GTG)5 primer (5'-GTGGTGGTGGTGGTG-3'). Figure 2 shows the fingerprint profiles of the different strains [20]. The strains were divided into 8 groups according to the number of characteristic bands as summarized in Table 2. At least one characteristic band was observed within each isolate of *K. pneumoniae*, which allowed them to be classified into 8 groups according to the size and number of characteristic bands (Table 2). The (GTG)5-PCR fingerprint approach is a novel genotyping method with the poly-trinucleotide (GTG) primer targeting the conserved and repetitive poly GTG sequences present in the bacterial genome. The assay's effectiveness has been deemed acceptable in comparison with other molecular typing analyses [9]. Using this technique, we wanted to understand if the MDR of *K. pneumoniae* strains was related to mutations within this repetitive sequence. Indeed, the dendrogram obtained showed that the strains belonged to the same phylogenetic clade with a cophenetic correlation coefficient (CP) of 97% (Figure 3).

PCR detection of carbapenemase genes

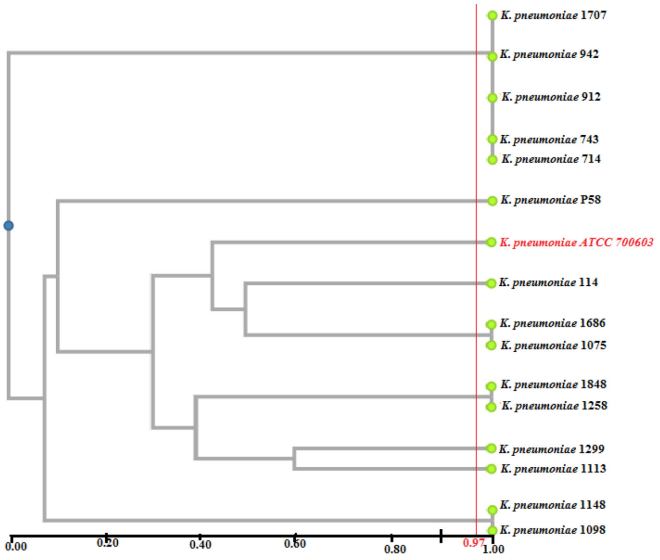
The 15 *K. pneumoniae* strains were tested by PCR for the most frequent carbapenemase genes including *IMI*, *KPC*, *IMP*, *VIM*, *NDM*, and *OXA-48*-like. Three strains (3/15, 20%) of the strains, were found to carry the *NDM* gene, including *K. pneumoniae* 114, *K. pneumoniae* 1299, and *K. pneumoniae* 1686. This proportion seems to be high and it is probably because the total number of strains tested (15) was low. The other types of carbapenemase genes were not detected.

Discussion

K. pneumoniae is one of the most important pathogenic bacterial species that infects immunocompromised people, and is the most frequent cause of nosocomial infections. It is resistant to common antibiotics and results in a high mortality rate [21,22]. In our study, we included 13 non-duplicated *K. pneumoniae* strains isolated from urinary tract infections in patients of average age 59.15 years (range 25–80 years). The patients included 4 females. We did not know the state of health of our patients, but upon bacteriological analysis of urine samples, most elderly male patients were found to be suffering from renal insufficiency and cancer of the prostate. Indeed, Cristea *et al*. had reported that the maximum age of patients with chronic renal failure with *K. pneumoniae* urinary tract infection was 82 years [23]. All the strains in their study were MDR. MDR has been previously defined as those strains that were resistant to at least two of the antibiotic classes, in addition to the β-lactams [24].

All antibiotics that were tested in this study on the *K. pneumoniae* 1299 strain showed no inhibition diameter, indicating that the strain was resistant to all antibiotics tested. Regardless of the number of strains detected harboring the *NDM* gene, this constitutes a public health emergency, and actions should be taken to prevent the spread of these resistance genes. NDM carbapenemase is the only carbapenemase that we have detected in *K. pneumoniae* strains. It is one of the most important carbapenemases and the most frequently detected among the strains of Enterobacteriaceae. Mitra *et al.* had also detected only this metallo-beta-lactamase in a study carried out on strains of Enterobacteriaceae [25]. Navon-Venezia *et al.* described antibiotic resistance in *K. pneumoniae*, including more than 52 fully sequenced antibiotic resistance plasmids carrying carbapenemases and extended spectrum beta-lactamases (ESBL) genes [26]. Mathlouthi *et al.* studied 87 strains of *Escherichia coli* and *K. pneumoniae* and reported that about two-thirds of the strains produced ESBL [27]. Zongo *et al.* reported 92.85% (n = 26/28) resistance by ESBL production in MDR *K. pneumoniae* in Burkina Faso [28]. Other resistance genes relating to

Figure 3. Dendrogram based on (GTG)5-PCR fingerprints of Klebsiella pneumoniae clinical strains based on UPGMA algorithm.



Cophenetic correlation coefficient (CP) = 0.970058206967055.

other families of antibiotics such as quinolone and colistin have also been described in *K. pneumoniae* [29–31]. The multitude of these resistance genes within *K. pneumoniae* makes the species MDR and difficult to treat in the event of infection. Indeed, phylogenetic analysis does not show any particularity of the strains harboring the *NDM* gene compared to the other strains of *K. pneumoniae* since they all belong to the same clone. However, previous studies have demonstrated the presence of strains of *K. pneumoniae* belonging to several clones [31,32].

Conclusions

We detected carbapenems resistance genes phenotypically, and confirmed our results using molecular methods. The genes present in the isolates that we studied were of the *NDM* type. We did not detect other types of carbapenemase resistance genes. Phylogenetic analysis of the strains indicated that they belonged to the same clone. Therefore, continuous surveillance and monitoring of resistance phenotypes and the mechanism of resistance is important for improving antimicrobial stewardship and antimicrobial prescription.

In future studies, we will consider extending our analysis to a larger number of bacterial strains, and then sequencing to determine the type of circulating *NDM*.

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Authors' contributions

KB, HSO, AGO: investigation, analysis, and writing-original draft; HC, KJZ, OZ, BZ: paper revision; IS, AS: results validation and review of paper. All authors have read and approved the final manuscript.

References

1. Hennequin C, Robin F (2016) Correlation between antimicrobial resistance and virulence in *Klebsiella pneumoniae*. Eur J Clin Microbiol Infect Dis 35: 333–341. doi: 10.1007/s10096-015-2559-7.
2. Lagha R, Ben F, Alkhammash AAH, Amor N, Hassan MM, Mabrouk I, Alhomrani M, Gaber A (2021) Molecular characterization of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from King Abdulaziz Specialist Hospital at Taif City, Saudi Arabia. J Infect Public Health 14: 143–151. doi: 10.1016/j.jiph.2020.12.001.
3. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler J F, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18: 268–281. doi: 10.1111/j.1469-0691.2011.03570.x.
4. Gnanasekaran C, Alobaidi AS, Govindan R, Muhammad S, Alagarsamy S, Alharbi NS, Kadaikunnan S, Govindan R, Manoharan N (2021) Piperacillin/tazobactum and cefotaxime decrease the effect of beta lactamase production in multi-drug resistant *Klebsiella pneumoniae*. J Infect Public Health 14: 1777–1782. doi: 10.1016/j.jiph.2021.10.017.
5. Li B, Xu X, Zhao Z, Wang M, Cao Y (2014) High prevalence of metallo-beta-lactamase among carbapenem-resistant *Klebsiella pneumoniae* in a teaching hospital in China. Can J Microbiol 60: 691–695. doi: 10.1139/cjm-2014-0291.
6. Mathlouthi N, Al-bayssari C, Bakour S, Rolain J M, Chouchani C (2017) Prevalence and emergence of carbapenemases-producing Gram-negative bacteria in Mediterranean basin. Cri Rev Microbiol. 43: 43–61. doi: 10.3109/1040841X.2016.1160867.
7. Hoang CQ, Nguyen HD, Vu HQ, Nguyen AT, Pham BT, TranTL, Nguyen HTH, Dao YM, Nguyen TSM, Nguyen DA, Tran HTT, Phan LT (2019) Emergence of New Delhi metallo-beta-lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC) production by *Escherichia coli* and Klebsiella *pneumoniae* in southern Vietnam and appropriate methods of detection: a cross-sectional study. BioMed Res Intl 2019: 9757625. doi: 10.1155/2019/9757625.
8. Yu H, Hern A, Est G, Casares MH, Han X, Baldoqu W, Quiñones D (2022) A retrospective study of risk factors, mortality, and treatment outcomes for infections with carbapenemase-producing Enterobacterales in a tertiary hospital in Havana, Cuba. Antibiotics 11: 942. doi: 10.3390/antibiotics11070942.
9. Ranjbar R, Afshar D (2019) Evaluation of (GTG)5-PCR for genotyping of *Klebsiella pneumonia* strains isolated from patients with urinary tract infections. Iran J Public Health 48: 1879–1884. doi: 10.18502/ijph.v48i10.3496.
10. Jehl F, Bru JP, Caron F, Cattoen C, Cattoir V, Dubreuil L, Lina G, Merens A, Plesiat P, Ploy MC, Soussy CJ, Varon E (2020) Antibiogram Committee of the French Society of Microbiology. French Society Microbiol 1: 1–181. [Article in French].
11. Rajivgandhi G, Maruthupandy M, Manoharan N (2018) Detection of *TEM* and *CTX-M* genes from ciprofloxacin resistant *Proteus mirabilis* and *Escherichia coli* isolated on urinary tract infections (UTIs). Microb Pathog 121: 123–130. doi: 10.1016/j.micpath.2018.05.024.
12. Khalid S, Ahmad N, Ali SM, Khan AU (2020) Outbreak of efficiently transferred carbapenem-resistant *blaNDM*-producing Gram-negative bacilli isolated from neonatal intensive care unit of an Indian hospital. Microb Drug Resist 26: 284–289. doi: 10.1089/mdr.2019.0092.
13. Kirtikliene T, Naugzemys D, Steponkiene A, Bogdevic R, Vizuje G, Zvingila D, Kuisiene N (2018) Evaluation of the inter- and intra-hospital spread of multidrug resistant Gram-negative bacteria in Lithuanian hospitals. Microb Drug Resist 25: 326–335. doi: 10.1089/mdr.2018.0160.
14. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, Ecker DJ, Massire C, Eshoo MW, Sampath R, Thomson JM, Rather PN, Craft DW, Fishbain JT, Ewell AJ, Jacobs MR, Paterson DL, Bonomo RA (2006) Analysis of

antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 50: 4114–4123. doi: 10.1128/AAC.00778-06.
15. Mlynarcik P, Roderova M, Kolar M (2016) Primer evaluation for PCR and its application for detection of carbapenemases in Enterobacteriaceae. Jundishapur J Microbiol 9: 1–6. doi: 10.5812/jjm.29314.
16. Lixandru BE, Cotar AI, Straut M, Usein CR, Cristea D, Ciontea S, Tatu-Chitoiu D, Codita I, Rafila A, Nica M, Buzea M, Baicus A, Ghita MC, Nistor I, Tuchiluș C, Indreas M, Antohe F, Glasner C, Grundmann H, Jasir A, Damian M (2015) Carbapenemase-producing *Klebsiella pneumoniae* in Romania: a six-month survey. PLoS One 10: e0143214. doi: 10.1371/journal.pone.0143214.
17. Poirel L, Héritier C, Tolün V, Nordmann P. (2004) Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48: 15–22. doi: 10.1128/AAC.48.1.15-22.2004.
18. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (2009) Characterization of a new metallo-β-lactamase gene, *blaNDM-1*, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 53: 5046–5054. doi: 10.1128/AAC.00774-09.
19. Ali A, Ahmad K, Rahat S, Ahmad I (2021) Genetic diversity and molecular analysis of metallo beta lactamases among imipenem resistant clinical isolates of *Pseudomonas aeruginosa* from Peshawar, Pakistan. Pak J Med Sci 37: 1865–1870. doi: 10.12669/pjms.37.7.4303.
20. Kaboré B, Ouédraogo GA, Cissé H, Ouédraogo HS, Sampo E, Zongo KJ, Zeba B, Traoré Y, Gnankiné O, Sanou I, Savadogo, A (2022) (GTG)5-PCR fingerprinting of multi-drug resistant *Escherichia coli* bacteria isolates from hospital in Ouagadougou, Burkina Faso. BMC Microbiol 22: 1–8. doi: 10.1186/s12866-022-02537-7.
21. Kim TJ, Lee KH, Choe YH., Lee KS (2021) Pulmonary Infection (pneumonia). In Kim TG, Lee KH, Choe YH, Lee KS, editors. Emergency Chest Radiology in Springer. Singapore: 119–141. doi: 10.1007/978-981-33-4396-2_8.
22. Choby JE, Howard-Anderson J, Weiss DS (2020) Hypervirulent *Klebsiella pneumoniae*-clinical and molecular perspectives. J Intern Med 287: 283–300. doi: 10.1111/joim.13007.
23. Cristea OM, Avrămescu CS, Bălășoiu M, Popescu FD, Popescu F, Amzoiu MO (2017) Urinary tract infection with *Klebsiella pneumoniae* in patients with chronic kidney disease. Curr Health Sci J 43: 137.
24. Barguigua A, El Otmani F, Talmi M, Reguig A, Jamali L, Zerouali K, Timinouni M (2013) Prevalence and genotypic analysis of plasmid- mediated beta-lactamases among urinary *Klebsiella pneumoniae* isolates in Moroccan community. J Antibiotics 66: 11–16. doi: 10.1038/ja.2012.91.
25. Mitra S, Mukherjee S, Naha S, Chattopadhyay P, Dutta S, Basu S (2019) Evaluation of co-transfer of plasmid-mediated fluoroquinolone resistance genes and *blaNDM* gene in Enterobacteriaceae causing neonatal septicaemia. Antimicrob Resist Infect Control 8: 1–15. doi: 10.1186/s13756-019-0477-7.
26. Navon-Venezia S, Kondratyeva K, Carattoli A (2017) *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 41: 252–275. doi: 10.1093/femsre/fux013.
27. Mathlouthi N, Al-Bayssari C, El Salabi A, Bakour S, Gwierif SB, Zorgani AA, Jridi Y., Slama KB, Rolain JM, Chouchani C (2016) Carbapenemases and extended-spectrum β -lactamases producing Enterobacteriaceae isolated from Tunisian and Libyan hospitals. J Infect Dev Ctries 10: 718–727. doi: 10.3855/jidc.7426.
28. Zongo KJ, Dabire AM, Compaore LG, Sanou I, Sangare L, Simpore J, Zeba B (2015) First detection of *blaTEM*, *SHV*, and *CTX-M* among Gram negative bacilli exhibiting extended spectrum -lactamase phenotype isolated at University Hospital Center, Yalgado Ouedraogo, Ouagadougou, Burkina Faso. Afr J Biotechnol 14: 1174–1180. doi: 10.5897/AJB2014.13908.
29. Wang CX, Cai PQ, Guo Y, Huang ZM, Mi ZH (2006) Emerging plasmid-mediated quinolone resistance associated with the *qnrA* gene in Enterobacter cloacae clinical isolates in China. J Hosp Infect 63: 349–350. doi: 10.1016/j.jhin.2006.02.013.
30. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, Zhang S, Shen J, Shen Z, Wang Y (2018) Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. Emerg Microbes Infect 7: 1–9. doi: 10.1038/s41426-018-0206-y.
31. Arabzadeh B, Ahmadi Z, Ranjbar R (2022) Molecular characterization of antibiotic resistance and genetic diversity of *Klebsiella pneumoniae* strains. Can J Infect Dis Med Microbiol 2022: 2156726. doi: 10.1155/2022/2156726.
32. Sharma S, Banerjee T, Kumar A, Yadav G, Basu S (2022) Extensive outbreak of colistin resistant, carbapenemase (blaOXA-48, blaNDM) producing *Klebsiella pneumoniae* in a large tertiary care hospital, India. Antimicrob Resist Infect Control 11: 1–9. doi: 10.1186/s13756-021-01048-w.

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