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ORIGINAL RESEARCH

First Emergence of NDM-5 and OqxAB Efflux Pumps Among Multidrug-Resistant *Klebsiella pneumoniae* Isolated from Pediatric Patients in Assiut, Egypt

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Introduction: New Delhi metallo-β-lactamase (NDM)-producing *K. pneumoniae* poses a high risk, especially among Egyptian pediatric patients who consume carbapenems antibiotics very widely and without adequate diagnostic sources. In addition, presence of efflux pump genes such as *OqxAB* increases resistance against many groups of antimicrobials which exacerbates the problem faced for human health. This study aimed to determine NDM variants among *K. pneumoniae* strains isolated from pediatric patients in Egypt, analyze the presence of *OqxAB* genes, and molecular characterization of $bla_{\text{NDM-5}}$-positive *K. pneumoniae*.

Methods: Fifty-six *K. pneumoniae* isolates were recovered from pediatric patients, and tested for carbapenemase by modified carbapenem inactivation methods (mCIM) test. Minimum inhibitory concentrations of meropenem and colistin were determined by meropenem E-test strips and broth microdilution, respectively. PCR was used for the detection of the resistant genes (ESBL gene ($bla_{\text{CTX-M}}$), carbapenemase genes (bla_{NDM}, bla_{KPC}) colistin resistant (*mcr1, mcr2*)) and genes for efflux pump (*oqxA* and *oqxB*). Bla_{NDM} was sequenced. The effect of efflux pump in NDM-5-producing isolates was assessed by measuring MIC of ciprofloxacin and meropenem before and after exposure to the carbonyl cyanide 3-chlorophenylhydrazone (CCCP). The horizontal gene transfer ability of $bla_{\text{NDM-5}}$ was determined using liquid mating assay and PCR-based replicon typing (PBRT) was done to determine the major plasmid incompatibility group.

Results: Twenty-nine isolates were positive for $bla_{\text{NDM-1}}$, nine isolates were positive for $bla_{\text{NDM-5}}$, and 15 isolates were positive for bla_{KPC}. There is a significant increase of meropenem MIC of NDM-5-positive isolates compared with NDM-1-positive isolates. In addition, 38 isolates were positive for CTX-M, and 15 isolates were positive for *mcr1*. Both *OqxA* and *OqxB* were detected in 26 isolates and 13 isolates were positive for OqxA while 11 isolates were positive for *OqxB* only. All NDM-5-producing isolates except one isolate could transfer their plasmids by conjugation to their corresponding transconjugants (*E. coli* J53). Plasmid replicon typing showed that FII was predominant in NDM-5-producing *K. pneumoniae*. Similar strains were found between the three isolates and similarity was also detected between the two isolates.

Conclusion: The highly resistant *K. pneumoniae* producing $bla_{\text{NDM-5}}$ type was firstly isolated from pediatric patients. The association of efflux pump genes such as *OqxAB* is involved in resistance to ciprofloxacin. This highlighted the severity risk of $bla_{\text{NDM-5}}$-positive *K. pneumonia* as it could transfer $bla_{\text{NDM-5}}$ to other bacteria and has more resistance against carbapenems. This underlines the importance of continuous monitoring of infection control guidelines, and the urgent need for a national antimicrobial stewardship plan in Egyptian hospitals.

Keywords: $bla_{\text{NDM-5}}$, *OqxAB*, carbapenem resistant *K. pneumoniae*, efflux pumps

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Introduction

Currently, there is increased awareness of the impact of Gram-negative bacteria that are resistant to carbapenem as it is the last choice treatment for bacterial infections.[1,2] *Enterobacteriaceae* exhibit resistance to carbapenems by three possible mechanisms: production of carbapenemase enzymes, efflux pump activity, and porin loss.[3,4] Carbapenemase production constitutes the basic mechanism of carbapenem resistance and study of this mechanism is essential for the best choice of recently effective antibiotics.[5,6]

New Delhi metallo-β-lactamase (NDM) is considered the latest threat for public health and numerous variants of NDM-type carbapenemases have been identified among Gram-negative bacteria worldwide.[7] Among them, NDM-5 was a variant with increased carbapenemase activity in comparison with NDM-1.[8] The $bla_{\text{NDM-5}}$ gene has been identified in *E. coli* and *Klebsiella pneumoniae* from many countries.[9] In addition, $bla_{\text{NDM-5}}$ was reported to be carried on different incompatibility typing plasmids that are responsible for transferring as IncF, IncN and IncX3.[10] These plasmids can facilitate the dissemination of $bla_{\text{NDM-5}}$ among the members of *Enterobacteriaceae* through horizontal gene transfer[11] that cause the bacterial cells to become a severely hazardous strain.[12]

Efflux pumps are found in nearly all bacteria and are responsible for mediating resistance to antimicrobials by reducing intracellular concentration of antibiotics and promoting site mutation accumulation. Presence of *OqxAB* efflux pump confers resistance to many drugs such as quinolones, quinoxalines, tigecycline, chloramphenicol and nitrofurantoin.[13,14] Efflux pumps are considered as one of the key mechanisms of antibiotic resistance in *K. pneumoniae* isolates. OqxAB, encoded by the *oqxA* and *oqxB* genes, is a plasmid-encoded multi-drug efflux pump first identified in *E. coli* (located on a conjugative plasmid). Their presence was found to develop multi-drug resistance against different groups of antimicrobials, detergents, and disinfectants and on the chromosome of *K. pneumoniae*.[15] This study aimed to determine NDM variants among *K. pneumoniae* strains isolated from pediatric patients in Assiut governorate, Egypt, analyze the presence of *OqxAB* genes and carry out the molecular characterization of $bla_{\text{NDM-5}}$-producing *K. pneumoniae*.

To the best of our knowledge, this study is the first to isolate *K. pneumoniae* carrying $bla_{\text{NDM-5}}$ and *OqxAB* from pediatric patients in Egypt.

Materials and Methods

K. pneumoniae Isolates and Identification

Fifty-six *K. pneumoniae* isolates were isolated from pediatric patients admitted to different departments at Assiut University Children's Hospital over a period of 6 months, from September 2022 to February 2023. This study was approved by scientific research ethics committee at the faculty of pharmacy, Al-Azhar University, Egypt (reference number of ZA-AS/PH/12/C/2022). *K. pneumoniae* were isolated from blood samples, sputum, endotracheal aspirate samples, urine samples, stool, wound or throat swabs. Isolates were identified by API20E kit (BioMerieux, Marcy L Etoile. France). Demographic and clinical data of patients were obtained from the hospital information system.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the isolated *K. pneumoniae* was tested by the Kirby-Bauer disc diffusion method according to recommendations of the clinical laboratory standards institute.[16] The following 14 commercial antimicrobial discs were used: ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), piperacillin (100 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefoperazone (75 μg), meropenem (10 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), aztreonam (30 μg), amikacin (30 μg), nitrofurantoin (300 μg), levofloxacin (5 μg), tetracycline (30 μg), and cefepime (30 μg). The *E. coli* ATCC 25922 isolate was used for quality control. The test was repeated twice for each isolate.

Detection of Carbapenemase-Producing Isolates

Isolates were considered carbapenemase producing by modified carbapenem inactivation methods (mCIM) test.[17] In addition, the minimum inhibitory concentrations (MIC) of meropenem against the tested *K. pneumoniae* were tested using Meropenem E-test strips (bioMérieux, Solna, Sweden).

Phenotypic Evaluation of Colistin Resistant *K. pneumoniae*

Minimum inhibitory concentrations (MICs) were determined by broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for colistin against *Enterobacteriaceae*.[18]

PCR for Detection of Resistant Genes

DNA was extracted from overnight grown culture using Wizard Genomic DNA Purification Kit (Promega, WI, USA). The extracted DNA was stored at −20°C. *K. pneumoniae* isolates were tested by PCR for the detection of resistant genes. *K. pneumoniae* isolates were analyzed for the presence of the ESBL gene ($bla_{\text{CTX-M}}$), carbapenemase genes (bla_{NDM}, bla_{KPC})[19] and for colistin resistant genes (*mcr1, mcr2*).[20,21] Amplified DNA fragments of bla_{NDM} were purified using QIAquick PCR Purification Kit (QIAGEN, Crawley, UK) and sequenced in both directions. Nucleotide and deduced amino acid sequences were analyzed and compared by BLAST, as implemented by the National Center for Biotechnology Information website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Detection of *oqxA* and *oqxB* Genes Efflux Pump

The *oqxA* and *oqxB* genes were screened using a PCR-based technique. The presence of *oqxA* and *oqxB* was detected using primers *oqxA*F (5′-CTCGGCGCGATGATGCT-3′) and *oqxA*R (5′-CCACTCTTCACGGGAGACGA-3′), with products of 392 bp, and *oqxB*s (5′-TTCTCCCCCGGCGGGAAGTAC-3′) and *oqxB*a2 (5′-CTCGGCCATTTTGGCGCGTA-3′), with products of 512 bp, as described previously.[15]

Statistical Analysis

R-packages (version 4.2.3) were used in data analysis and visualization. Wilcoxon rank sum test and Mann–Whitney *U*-test were used to compare meropenem MIC between NDM-5 and NDM-1-positive isolates and meropenem MIC before and after addition of CCCP, respectively. *P*-value of less than 0.05 was regarded as significant. Clustering of samples was elucidated based on the Euclidean distance matrix.

Phenotypic Evaluation of the Efflux Pump in NDM-5-Producing *K. pneumoniae*

Phenotypic evaluation of the efflux pump in NDM-5-producing *K. pneumoniae* was assessed by measuring the minimum inhibitory concentrations (MICs) for ciprofloxacin and meropenem before and after exposure to the efflux pump inhibitor (EPI), carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (Sigma-Aldrich, Dorset, UK) at a concentration of 20 mg/L. If the MIC values decreased 4-fold or greater in the presence of EPI, this was defined as a significant inhibition effect.[22]

Conjugation Experiment and Plasmid Replicon Typing

The horizontal gene transfer ability of $bla_{\text{NDM-5}}$ was determined using liquid mating assay for nine *K. pneumoniae* isolates that harbored $bla_{\text{NDM-5}}$. *E. coli* J53 was used as recipient isolate, and transconjugants selection was performed on MacConkey agar plates containing meropenem (2 μg/mL) and sodium azide (100 μg/mL). Transconjugants were tested for $bla_{\text{NDM-5}}$ by PCR.[23] PCR-based replicon typing (PBRT) was done to determine the major plasmid incompatibility group by using 14 pairs of primers in which multiplex PCR for FII, FIA and FIB was performed while 11 simplex PCRs were performed for IncL, IncM, IncT, FIC, FIIK, IncN, IncX3, IncH12, IncW, IncY and IncA/C.[24]

Genotyping by ERIC

PCR amplification was performed using the primers ERIC1 (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′) for *K. pneumoniae* isolates.[25] The gel bands were presented by PyElph version 1.4.[26] The dendrogram was computed by the unweighted pair group method with arithmetic averages (UPGMA).

Results

Bacterial Isolates and Patient Details

Fifty-six *K. pneumoniae* isolates recovered from 56 (male 31, female 25) pediatric patients were analyzed; 21 patients were from chest unit and eight from gastroenterology, six from the intensive care unit, five from neurology, and five from hematology wards. Most of the isolates were recovered from endotracheal aspirate (25%, 14/56), followed by sputum (23.21%, 13/56) and blood (21.42%, 12/56). The clinical details of all patients are given in Table 1.

Table 1 Clinical Details and MIC of Colistin and Meropenem for All Isolates

Isolate ID	Wards	Specimen	Colistin MIC by Broth Microdilution	Meropenem MIC by E-Test
St-1	Neurology	Urine	2	32
St-2	Chest	Endotracheal aspirate	0.5	8
St-3	Gastroenterology	Sputum	4	8
St-4	Hematology	Blood	2	0.19
St-5	General surgery	Wound swap	4	0.19
St-6	Clinical Nutrition	Stool	4	2
St-7	General surgery	Swap	2	2
St-8	Cardiology	Blood	32	0.25
St_9	General surgery	Endotracheal aspirate	16	0.19
St-10	Orthopedic	Blood	2	1
St-11	Chest	Sputum	4	4
St-12	Neurology	Blood	2	1
St_13	Intensive care	Endotracheal aspirate	32	0.75
St-14	Chest	Sputum	2	16
St-15	Gastroenterology	Stool	4	0.19
St-16	General surgery	Swap	2	0.75
St-17	Orthopedic	Swap	1	4
St-18	Intensive care	Endotracheal aspirate	0.5	12
St-19	Chest	Sputum	16	1
St-20	Hematology	Blood	0.5	8
St-21	General surgery	Blood	1	8
St-22	Chest	Sputum	1	8
St-23	Chest	Blood	4	16
St-24	Chest	Blood	32	12
St-25	Gastroenterology	Urine	16	8
St-26	Gastroenterology	Urine	1	12

(*Continued*)

Table 1 (Continued).

Isolate ID	Wards	Specimen	Colistin MIC by Broth Microdilution	Meropenem MIC by E-Test
St-27	Hematology	Endotracheal aspirate	16	2
St-28	Intensive care	Endotracheal aspirate	2	4
St-29	Neurology	Endotracheal aspirate	2	8
St-30	Chest	Swap	0.5	4
St-31	Chest	Endotracheal aspirate	2	8
St-32	Intensive care	Endotracheal aspirate	2	8
St-33	Chest	Endotracheal aspirate	4	4
St-34	Chest	Swap	2	8
St-35	Gastroenterology	Stool	2	4
St-36	Chest	Sputum	2	8
St-37	Hematology	Blood	16	8
St-38	Gastroenterology	Sputum	0.5	12
St-39	Chest	Sputum	0.5	4
St-40	Chest	Urine	1	8
St-41	Gastroenterology	urine	8	4
St-42	Chest	Endotracheal aspirate	16	8
St-43	Intensive care	Endotracheal aspirate	8	2
St-44	Chest	Endotracheal aspirate	8	2
St-45	Clinical Nutrition	Blood	16	2
St-46	Chest	Sputum	1	4
St-47	Chest	Sputum	16	2
St-48	Chest	Blood	0.5	3
St-49	Hematology	Blood	0.5	12
St-50	Neurology	Sputum	4	4
St-51	Chest	Sputum	0.5	8
St-52	Gastroenterology	Stool	0.5	16
St-53	Intensive care	Endotracheal aspirate	0.5	4
St-54	Clinical Nutrition	Stool	4	4
St-55	Neurology	Urine	0.5	8
St-56	Chest	Sputum	1	4

Abbreviation: MIC, minimum inhibitory concentrations.

Antimicrobial Susceptibility Testing

The antibacterial susceptibility profiles to all isolates showed that all tested isolates (n = 56) were resistant to ampicillin, amoxicillin-clavulanate, piperacillin, ceftriaxone, ceftazidime, cefoperazone and aztreonam (Figure 1).

Isolates Producing Carbapenemase

mCIM test showed that 46 isolates (82.1%) of the tested *K. pneumoniae* were carbapenemase enzyme producers.

The MIC Values for Colistin and Meropenem

It was found that 67.85% (38/56) of *K. pneumoniae* isolates were meropenem resistant [MIC ≥4 µg/mL], 17.85% (10/56) isolates were meropenem sensitive [MIC ≤1 µg/mL] and 14.28% (8/56) isolates were meropenem intermediate [MIC >1<4 µg/mL] as illustrated in Table 1.

MIC values for all 56 isolates for colistin by broth microdilution ranged from 0.5–32 µg/mL and meropenem MIC by E-test ranged from 0.19–32 µg/mL (Table 1). There is a significant increase of meropenem MIC of NDM-5-positive isolates (*P*-value = 1.704e-14) than MIC of NDM-1-positive isolates.

Detection of Resistant Genes and *OqxAB* by PCR

Out of 56 isolates, 29 isolates were positive for $bla_{\text{NDM-1}}$, 9 isolates were positive for $bla_{\text{NDM-5}}$, and 15 isolates were positive for bla_{KPC} carbapenemase genes. In addition, 38 isolates were positive for $bla_{\text{CTX-M}}$ ESBL gene, and 15 isolates were positive for *mcr1*. Both *OqxA* and *OqxB* were detected in 26 isolates. Thirteen isolates were positive for *OqxA* only and 11 isolates were positive for *OqxB* only (Figure 2).

Effect of the Efflux Pump in NDM-5-Producing *K. pneumoniae*

MIC of ciprofloxacin in NDM-5-producing *K. pneumoniae* ranged from 4–10 µg/mL due to the inhibition of efflux pump by CCCP. Eight of the nine (8/9) NDM-5-producing *K. pneumoniae* had 4-fold or more decrease in MIC after CCCP exposure. Only isolates St-26 and St-42 had an MIC fold change of 2 after CCCP addition (*P*-value = 0.00042). Conversely, no change in MIC of meropenem after CCCP addition was observed (Table 2).

Plasmid Replicon Typing and Conjugation Experiment

All NDM-5-producing isolates except one isolate (St-29) could transfer their plasmids by conjugation to their corresponding transconjugants *(E. coli* J53). The $bla_{\text{NDM-5}}$ gene was located on six different types of plasmids (FII, FIIK, FIB, FIC, L and Inc M) and the FII type was predominant in NDM-5-producing *K. pneumoniae* (Table 2).

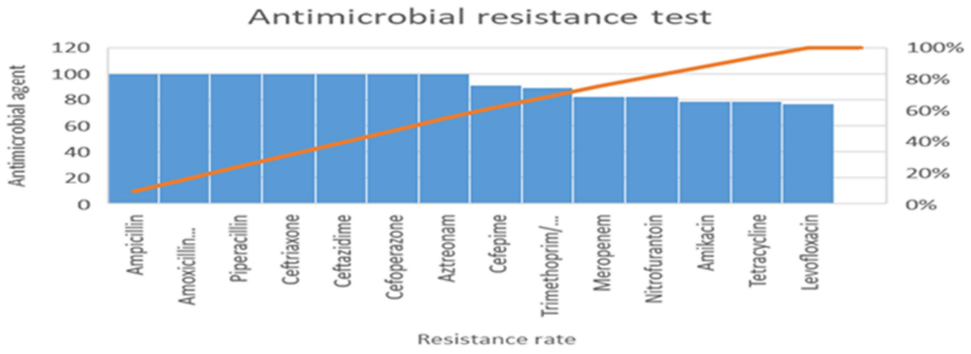

Figure 1 Antibacterial resistance pattern of *K. pneumoniae* isolates.

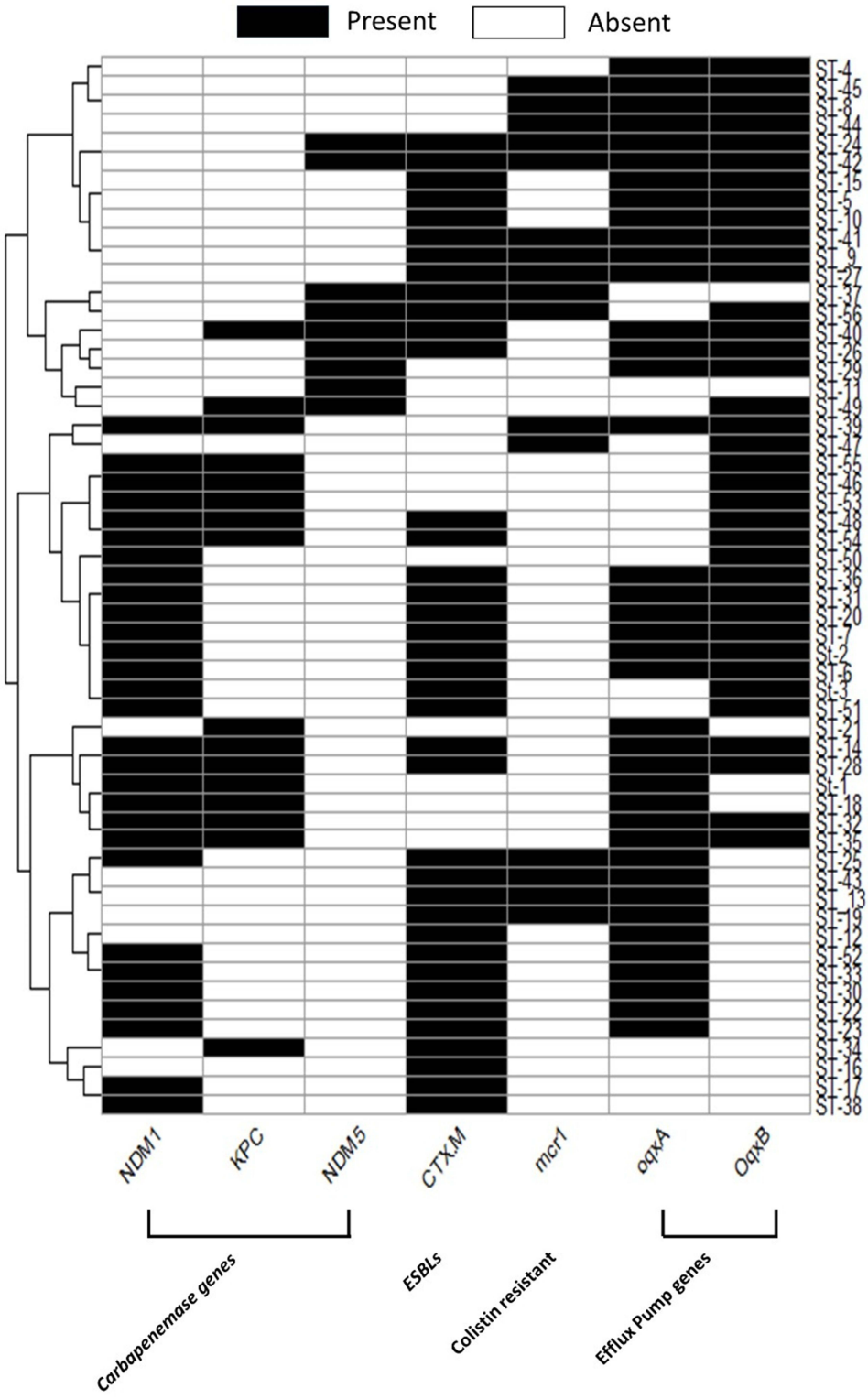

Figure 2 Dendrogram based on the Euclidean distance matrix between resistance factors of *K. pneumoniae* isolates in all samples. The presence and absence of the resistance factors are represented by black or white blocks, respectively.

Table 2 Characterization of NDM-5-Producing *K. pneumoniae*

Isolates ID	PBRT	MIC of CIP	MIC of CIP+CCCP	MIC of MEM+ CCCP
St-11	FII, L	4	0.5	4
St-24	FIIK, M	6	1	12
St-26	FII, FIB	4	2	12
St-29	FIC	8	1	8
St-37	FIB	4	0.5	8
St-40	FII, FIB	5	0.5	8
St-42	FIIK	6	3	8
St-49	FII	10	2	12
St-56	FIIK	9	1	4

Abbreviations: PBRT, PCR-based replicon typing; CIP, ciprofloxacin; MEM, meropenem; MIC, minimum inhibitory concentrations; CCCP, carbonyl cyanide 3-chlorophenylhydrazone.

Genotyping by ERIC

Dendrogram of NDM-5-producing *K. pneumoniae* presented by PyElph showed that similar strains were found between these isolates (St-37, St-29, St-40) from hematology, neurology and chest and similarity was also detected between two isolates (St-42, St26) from the chest and gastroenterology departments (Figure 3).

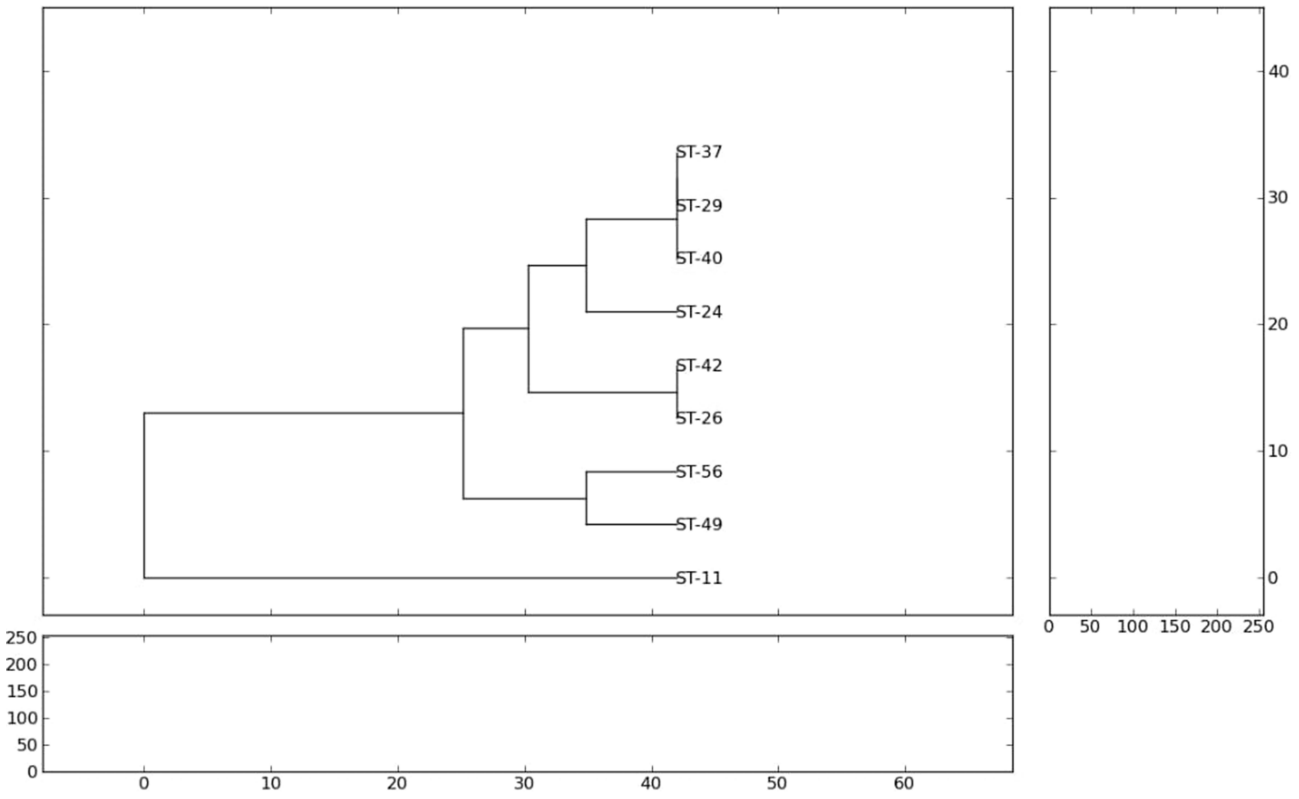

Figure 3 Dendrogram of NDM-5-producing *K. pneumoniae* presented by PyElph.

Discussion

Carbapenem antibiotics are used in the treatment of infections caused by multi-drug resistant *K. pneumoniae*. However, the emergence and spread of NDM-producing *K. pneumoniae* has been a serious challenge to manage in the clinic because of the rapid worldwide spread of multi-drug resistance.[27] As one main type of carbapenemases, New Delhi metallo-β-lactamase (NDM) can resist almost all β-lactams, including carbapenems. NDM-5, compared with NDM-1, has two amino-acid substitutions (Val88→Leu) and (Met154→Leu), which confer enhanced hydrolytic activity against carbapenems.[11] Most studies focus on the dissemination of carbapenemase-producing Gram negative strains among adult patients more than from children. There was a spread of carbapenem-resistant *K. pneumoniae* and endemicity of NDM was reported in many hospitals of our region.[28,29]

In this study 56 *K. pneumoniae* isolates were recovered from hospitalized pediatric patients at Assiut University Children's Hospital. Mostly patients were from the chest unit and most of the specimens were recovered from endotracheal aspirate. We observed previously that many MDR *K. pneumoniae* isolates can maintain and spread easily in the ward of PICU of this hospital.[30] So, it is essential to estimate molecular characterization of this pathogen from other wards in the same hospital and to show the responsibility of this pathogen to cause a great challenge for infection monitoring among pediatric patients. The antibacterial susceptibility profiles of all isolates showed that all tested isolates were multi-drug resistant as each isolate were resistant to at least three different classes of antimicrobial agents.[31]

Among the 56 *K. pneumoniae* isolates, 46 isolates were confirmed phenotypically to produce carbapenemase by mCIM. However, only 40 isolates were found to produce carbapenemase genes by PCR and six other isolates exhibited other unknown mechanisms of carbapenem-resistance. The high level of production of carbapenemase genes among the isolates is that mostly of pediatric patients treated with carbapenem empirically. Sequencing of NDM-producing isolates revealed that the majority of bla_{NDM} genes were $bla_{\text{NDM-1}}$ type as with many studies[32–34] and we found nine $bla_{\text{NDM-5}}$ type. It was the first to isolate NDM-5 type from *Klebsiella pneumoniae* isolates in Egypt but recovered previously from *Escherichia coli* isolates with associated OXA-181.[35] There is a significantly higher meropenem MIC of NDM-5-positive isolates than of NDM-1-positive isolates. NDM-5-producing strains (containing the V88L substitution) have been reported repeatedly to exhibit higher MIC against carbapenem than those against NDM-1-producing strains which suggests that this substitution may have a significant impact on carbapenemase activity even though not being located at the active site, and the mechanism of enhanced activity remains uncertain.[8] NDM-5-positive isolates are continuing to be a critical challenge for treatment and the continuous monitoring of infection control policies is critically important.

Out of 56 isolates, 15 colistin-resistant isolates were positive for *mcr-1* gene (26.7%) but *mcr-2* gene was not detected at all among the isolated *K. pneumoniae* as colistin is not used in this hospital to treat infections. Moreover, extended spectrum b-lactamase was shown by the tested isolates as 38 isolates were positive for $bla_{\text{CTX-M}}$ gene. The present study revealed massive co-existence of different resistance genes among the tested isolates which may greatly contribute to the observed raised variability in resistance genotypes among *K. pneumoniae* in Egypt.[36,37]

It was known that efflux pump is one of the mechanisms that is involved in antibiotic resistance in *K. pneumoniae*,[38] and the presence of efflux pump genes (*OqxAB*) in many Gram-negative pathogens causes an increase in the rates of resistance to many different antibiotic families including beta-lactams, carbapenems, macrolides, fluoroquinolone, tetracyclines that results from their inherent ability to develop resistance.[39,40] So, we need to know if efflux pump plays a role in carbapenem and fluoroquinolone resistance or not by testing the effect of CCCP on meropenem and ciprofloxacin resistance. There is a significant decrease in MIC after CCCP is added to ciprofloxacin which indicates that the drug efflux system is involved in resistance to ciprofloxacin. Our study showed no effect of CCCP on meropenem resistance among isolates.

PCR was used to screen for *oqxA* and *oqxB* genes. Both *OqxA* and *OqxB* were detected in 26 isolates, 13 OqxA only and 11 OqxB only were also detected. Presence of these efflux pump genes seems to contribute to the high resistance of clinical isolates of *K. pneumoniae* to other antibiotics. Previous research found that OqxAB was detected in all of *K. pneumoniae* chromosome isolates and suggested the genome of *K. pneumoniae* is a possible reservoir of oqxAB.[41]

In the current study we could not observe any effect of CCCP on susceptibility to meropenem as no change in MIC of meropenem after CCCP addition was detected (Table 2). However, the addition of the CCCP decreased resistance to

ciprofloxacin (4-fold or more in MIC) in NDM-5- producing *K. pneumoniae* isolates. Meanwhile only St-26 and St-42 isolates had an MIC fold change of 2 upon CCCP addition. We observed that eight of the nine NDM-5-producing *K. pneumoniae* had 4-fold decrease in MIC after CCCP exposure. Similarly, other studies reported greater reductions in MIC of ciprofloxacin with the use of efflux pumps inhibitors compared with their initial MIC.[42–44] In the present study, the resistance to meropenem could be greatly affected by the presence of beta lactamases, which are not targeted by efflux pump inhibitors[45] and the use of CCCP may not improve in vitro susceptibility of *K. pneumoniae* to meropenem.[46]

All NDM-5-producing isolates except one isolate could transfer their plasmids carrying $bla_{\text{NDM-5}}$ by conjugation to their corresponding transconjugants (*E. coli* J53). Our results supported what we had previously reported about the vital role of the plasmid in the transfer of resistant genes.[47] This plasmid may promote the rapid dissemination of $bla_{\text{NDM-5}}$ among Gram-negative bacterial pathogens. This may indicate the possible transition of this type ($bla_{\text{NDM-5}}$) between different species by conjugative plasmid and further highlights the importance of carbapenemase genes which could facilitate their dissemination on mobile genetic elements.[48]

Plasmid replicon typing showed that the FII type was predominant in NDM-5-producing *K. pneumonia*. It has been reported that $bla_{\text{NDM-5}}$ closely associated with different types of plasmids; among these, the IncF plasmid was found mainly in *Enterobacteriaceae* and has the ability to transfer its antimicrobial resistance determinants.[49] On the other hand, genotyping similarity between strains was found between these isolates (St-37, St-29, St-40) and between two isolates (St-42, St26). This indicated the capability of these NDM-5-producing strains to disseminate between different wards of this pediatric hospital.

Conclusions

The highly resistant *K. pneumoniae* producing $bla_{\text{NDM-5}}$ type was firstly isolated from pediatric patients. The association of efflux pump genes such as *OqxAB* is involved in resistance to ciprofloxacin but not to meropenem. This highlights the severity risk of $bla_{\text{NDM-5}}$-positive *K. pneumonia* as it could disseminate $bla_{\text{NDM-5}}$ to other bacterial cells and has more resistance against carbapenems and other antibiotics. This emphasizes the importance of continuous monitoring of infection control policies, and the urgent need for a national antimicrobial stewardship plan in Egypt.

Institutional Review Board Statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the faculty of pharmacy, Al-Azhar University, Egypt (9/2022, reference number of ZA-AS/PH/12/C/2022).

Informed Consent Statement

Written informed consent has been obtained from the parents or legal guardians of the pediatric patients to participate in this study.

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Disclosure

The authors declare no conflicts of interest in this work.

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