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Original article

Genotyping and antimicrobial resistance patterns of *Klebsiella pneumoniae* clinical isolates in Suez Canal University Hospitals

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ABSTRACT

Background: Health crisis of multi-drug resistant *Klebsiella pneumoniae (K. pneumoniae)* seems overwhelming. Genotyping for such an important pathogen is a demand to control aggressive *Klebsiella* infections. Therefore, this study was conducted to identify the different phylogenetic groups of *K. pneumoniae* complex and to assess their prevalence and antimicrobial resistance patterns. **Methods:** Seventy-six *K. pneumoniae* isolates were collected and identified using conventional microbiological methods. Isolates were confirmed to be *K. pneumoniae* by amplification of *gyrA* gene by PCR. The antimicrobial susceptibility testing was performed by disk diffusion method. Restriction Fragment Length Polymorphism-PCR (RFLP-PCR) was performed for the identification of the phylogenetic groups and the relationships of these phylogroups to antimicrobial susceptibility were investigated. **Results:** RFLP-PCR showed that 62 isolates (81.6%) were identified as *KP I*, 9 isolates (11.8%) as *KP II* and 5 isolates (6.6 %) as *KP III*. Among the 62 *KP I* isolates, 39 isolates (63%) showed MDR and 12 (19.4%) showed XDR. While among the 9 *KP II* isolates, 7 isolates (77.7%) showed MDR and only one (11.1%) showed XDR. Among the 5 *KP III* isolates, 2 isolates (40%) showed MDR, while only one isolate (20%) showed XDR. **Conclusion:** Reliable identification of the three *K. pneumoniae* phylogenetic groups can be obtained by *gyrA* PCR-RFLP. The level of resistance for most of the antibiotics was highest among phylogroup I, which may start future outbreaks that could be difficult to manage by the available sets of antibiotics. This ensures the value of genomic surveillance in research, clinical and public health settings.

Introduction

The Gram-negative bacteria *Klebsiella* has posed a serious threat to public health during the past few decades, contributing significantly to global morbidity and mortality rates. It is known to be an opportunistic pathogen that has gained global medical significance due to its multiple virulence factors, resistance to many antibiotics, severity of disease and difficulty of treatment [1]. This microorganism poses a significant risk to hospitalized patients since it can lead to a range of healthcare associated infections (HCAIs) including sepsis, endocarditis, meningitis, pneumonia, surgical site infections (SSIs) and urinary tract infections (UTIs) [2].

The occurrence of multidrug-resistant (MDR) *Klebsiella pneumoniae (K. pneumoniae)* in healthcare facilities has increased globally. This increase could be attributed to the bacterium's acquisition of new resistance genes, the improper

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use of antibiotics to treat infections, immunosuppressed conditions, the use of invasive medical equipment, and inadequate diagnostic and surveillance systems [3].

With the recent discovery of multiple new species and subspecies, *Klebsiella* taxonomy is rapidly evolving. *K. pneumoniae* and the associated species complex which are collectively known as the *Klebsiella pneumoniae complex* (*KPC*) is classically and taxonomically classified into seven phylogenetic groups as follows: *K. pneumoniae* (Kp1), *K.quasipneumoniae* subsp. *qua sipneumoniae* (Kp2), *K.variicola* subsp. *variicola* (Kp3), *K.quasipneumoniae* subsp. *similipneumoniae* (Kp4), *K. variicola* subsp. *tropicalensis* (Kp5), *K. quasivariicola* (Kp6) and *K. africanensis* (Kp7). Among them, *K. pneumoniae*, *K. variicola* and *K. quasipneumoniae* are the most frequently reported in human clinical samples. Accurate phenotypic identification of these species is difficult and can be achieved by molecular techniques [4].

Klebsiella pneumoniae complex is responsible for around one-third of all Gram-negative infections and is one of the most common causes of outbreaks in healthcare settings [5]. KPC strains reduce the treatment options for several antibiotics by acquiring multiple antibiotic resistance genes [6]. Unfortunately, KPC that produce different kinds of ESBLs and carbapenemases are found everywhere in the world [4].

Molecular typing of *K. pneumoniae* is a powerful tool used to trace the sources of infection in hospital settings for better control of HCAIs. Furthermore, identifying the dominant genotype in isolates is essential for determining the infection's origin and implementing preventative measures [7]. To date, a number of techniques have been used to identify the phylogenetic type of *Enterobacteriaceae*, including restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR), phylogenetic analysis of the *ori* region, and phylogenetic analysis by sequencing the *dnaJ* and *gyrA* genes (6]. Sequence analysis of *gyrA* gene has demonstrated that it is a reliable phylogenetic marker because it is not prone to repeated horizontal translocation across clusters [8].

Among the benefits of RFLP-PCR over other molecular typing techniques are its capacity to distinguish between closely related bacterial strains and its simplicity, speed, affordability, and dependability as a high throughput genotyping method [4]. Therefore, this study aims to investigate *gyrA* PCR- RFLP as a molecular typing method of *K. pneumoniae* clinical isolates at Suez Canal University hospitals (SCUHs) and to find out the relationship between different phylogenetic groups and antimicrobial resistance patterns.

Materials and Methods

This was a descriptive cross-sectional study carried out during the period from May 2022 to April 2024, on 143 patients admitted to different wards at SCUHs including ICU, urology, surgery, burn, internal medicine and pediatric wards and clinically diagnosed to have UTIs, ventilator-associated pneumonia (VAP), blood stream infection (BSI), SSI, wound infections, pneumonia and intra-abdominal infections (IAI).

Patients were from all age groups and of both sexes. A thorough medical history was obtained, covering the patient's name, age, occupation, date of admission, underlying chronic conditions (such as diabetes mellitus, hypertension, chronic renal failure, cancer, chronic heart disease, or liver disease), antibiotic therapy, previous hospital stays, and recent surgical procedures. Informed consent was taken from all the study participants and/or their guardians. Approval was obtained from the Research Ethics Committee at Faculty of Medicine, Suez Canal University (Research 4905#). The procedures used in this study adhere to the ethical standards of the Declaration of Helsinki.

Collection and processing of specimens

Various clinical specimens were collected, one from each patient, under aseptic conditions and transported to be processed at the Microbiology department laboratory, Faculty of Medicine, Suez Canal University.

Identification of K. pneumoniae species

Specimens were inoculated onto blood agar and MacConkey's agar plates (OXOID, UK) incubated aerobically at 35±2˚C for 24-48 hrs. Gram-stained smears were prepared from the colonies and examined microscopically and conventional biochemical reactions were performed. *K. pneumoniae* isolates were identified as being Gram negative bacilli with mucoid lactose fermenting colonies on MacConkey's agar plates, non-motile, negative indole test, negative Methyl red test, positive Voges- Proskauer test and positive citrate test [9].

Finally, the strains were kept for further processing at −20°C in tryptic soy broth medium supplemented with 15 % glycerol.

Antimicrobial susceptibility testing

Isolated strains were tested for antibiotic susceptibility using the conventional Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid, UK) and incubated for 16–18 hours at 37ºC according to the Clinical and Laboratory standard Institute (CLSI) guidelines [10]. The following antimicrobial agents (OXOID, UK) were included: Amoxicillin-clavulanate (20/10 µg), ampicillin-sulbactam (10/10µg), cefepime (30µg), ceftriaxone (30 µg), ciprofloxacin (5µg), levofloxacin (5µg), gentamycin (10 µg), amikacin (30µg), imipenem (10µg), meropenem (10µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), aztreonam (30µg) and nitrofurantoin (300µg).

Klebsiella pneumoniae isolates were subsequently categorized into MDR, XDR and PDR isolates as described by **Magiorakos, et al**. [11] as follows:

- MDR was identified as resistance to at least one agent in three or more antimicrobial groups.

- XDR was referred to as non-susceptibility to at least one agent in all but two or fewer antimicrobial groups.

- PDR was defined as resistance to all agents in all antimicrobial categories.

Phylogenetic typing by the RFLP-PCR of gyrA gene:

DNA extraction

DNA was extracted from the test isolates using QIAGEN DNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Amplification of *gyrA* gene by PCR [12]

The gene was amplified by PCR using a specific set of primers as follows*: gyrA-A* (F: 5`'-CGCGTACTATACGCCATGAACGTA-3′ and *gyrA-C* (R: 5′ACCGTTGATCACTTCGGTCAGG-3′) (fragment size, 441 bp).

The amplification reactions were performed in a total volume of 25 µl containing 2 µL (100 ng) of extracted DNA as a template, 2 µl forward primer (15 Pmol), 2 µl reverse primer (15 Pmol), 12.5 µl of 2× Master Mix (including 1.5 × PCR buffer, 0.5 mmol/L of dNTPs, 4 mmol/L of $MgCl_2$, and 0.08 IU of Taq DNA polymerase), and 6.5 µl nuclease free water.

The PCR amplifications were performed in thermocycler (Eppendorf-Mastercycler Gradient) using the following protocol: initial denaturation (94 ºC for 5 minutes), followed by 35 cycles of denaturation (94 ºC for 1 minute), annealing (54 ºC for 30 seconds) and extension (72 ºC for 30 seconds), with a single final extension for 5 minutes at 72 ºC.

Amplicons obtained from PCR reactions were analyzed by gel electrophoresis in 1.5 % agarose gel containing 0.1 µL/mL ethidium bromide and finally visualized with ultraviolet light. Amplicon size (bp) of the tested gene was identified and compared to a 100 bp molecular size standard DNA ladder (Axygen Biosciences). *K. pneumoniae* (ATCC BAA-1705) was used as a positive control in this study.

Genotyping of *KPC* isolates using RFLP-PCR [12,13]

The amplicons were submitted to RFLP-PCR using *TaqI* and *HaeIII* restriction enzymes (NEB, UK), separately. The reactions were carried out using 1 µg DNA, 5 µL of 10X rCutSmart Buffer, 1 µL of restriction enzyme and nuclease free water in a total volume of 50 µL. Incubation for *TaqI* was done at 65 ºC for 15 minutes, while incubation for *HaeIII* was done at 37 ºC for 15 minutes. Finally, the RFLP products were separated in 2.5 % agarose gel in 1 X TBE buffer, followed by staining with 0.5 µg/mL ethidium bromide stain and visualizing under ultraviolet light as follows:

- In *KpI* strains, four bands were produced by the *TaqI* enzyme (197 bp, 142 bp, 93 bp, and 9 bp) and four bands were produced by the *HaeIII* enzyme (175 bp, 129 bp, 92 bp, and 45 bp).

- In *KpII* strains, the *TaqI* enzyme created three bands (including 197 bp, 151 bp, and 93 bp) while the *HaeIII* enzyme created four bands (including 175 bp, 129 bp, 92 bp, and 45 bp).

- In *KpIII* strains, four bands were produced by the *TaqI* enzyme (197 bp, 142 bp, 93 bp, and 9 bp) and the *HaeIII* enzyme created three bands (including 175 bp, 174 bp, and 92 bp).

Statistical analysis

Collected data were entered into a database file. All statistical analyses were performed using Statistical Package for Social Science program (SPSS version 22 for windows) (SPSS, Chicago, IL, USA). Descriptive data was managed according to its type; mean, standard deviation and range summarized continuous data, while qualitative data

was summarized by frequencies. In analytical data, chi square and ANOVA tests were used to detect the difference between qualitative data. Data was analyzed and presented as numbers and percentages using tables and graphs with the confidence interval (CI) at 95%, *p value* of 0.05 was used as the limit of statistical significance**.**

Results

This study was conducted on 143 patients (52.4 % males and 47.6 % females) with mean age of 55.7 ± 12.82 years admitted to different wards at SCUHs. Seventy- six (53.1 %) non repetitive *K. pneumoniae* strains were isolated from these patients by standard microbiological methods and confirmed to be *K. pneumoniae* by amplification of *gyrA* gene by PCR. Among these isolates, 40 (52.6 %) were from females and 36 (47.4 %) from males with mean age of 53.2 ± 10.64 years.

These *K. pneumonia* isolates were collected from different hospital wards. The highest rate from ICU (29 %), followed by pediatric wards (19.7 %), surgery wards (18.4 %), urology wards (15.8 %), internal medicine wards (11.8 %) while the lowest rate (5.3 %) was from the burn unit.

They were isolated from different specimens including endotracheal aspirates (ETA) 21 (27.6 %), urine 14 (18.4 %), pus 13 (17.1%), blood 12 (15.8%), sputum 9 (11.9 %), wound swab 6 (7.9 %) and CSF 1 (1.3 %) **(table 1)** and were collected from patients suffering from different chronic illness as 48.6 % had DM, 17.1 % had chronic heart disease, 27.6 % had end stage renal failure and chronic obstructive lung disease each and 19.7 % had chronic liver disease.

Phylogenetic typing of the 76 *K. pneumoniae* isolates was done using the RFLP-PCR method as the *gyrA* gene amplicons were submitted to restriction enzymes *TaqI* and *HaeIII*. *TaqI* produced restriction profiles (197bp, 142 bp, 93bp and 9 bp fragments) for *KPI* and *KP III*, and restriction profiles (197bp, 151bp and 93bp fragments) for *KPII* as shown in **figure (1)**. *HaeIII* restriction profile (175 bp, 129 bp, 92bp and 45 bp fragments) was obtained for *KPI* and *KPII*, and restriction profile (175bp, 174bp, and 92bp fragments) for *KPIII* as shown in **figure (2)**. It was found that 62 isolates (81.6 %) were identified as *KP I*, 9 isolates (11.8 %) were identified as *KP II* and 5 isolates (6.6 %) were identified as *KP III*.

The distribution of different *K. pneumoniae* phylogenetic groups according to gender, specimen types and hospital wards was illustrated in **table (1).** There was no statistically significant difference concerning the distribution of the different phylogroups and the gender, specimen types or hospital wards.

Regarding the antibiotic susceptibility patterns of KPC isolates, the highest resistance rates were found to be related to amoxicillin-clavulanate (95.2%), ampicillin-sulbactam (90.3%) and ceftriaxone (80%). However, the most effective antibiotic against *K. pneumonaie* was aztreonam (88.9%), followed by meropenem and imipenem (80%) each as shown in **table (2)**.

Thirty-nine isolates (63%) among the 62 *KP I* isolate showed MDR, 12 isolates (19.3%) showed XDR and 11 isolates (17.7%) were neither MDR nor XDR. Regarding the 9 *KP II* isolates, 7 isolates (77.8 %) showed MDR, while only one isolate (11.1%) showed XDR, and one isolate (11.1%) was neither MDR nor XDR. Two isolates (40%) among the 5 *KP III* isolates showed MDR, one isolate (20%) showed XDR, and 2 isolates (40%) were neither MDR nor XDR.

Table 1. The distribution of different *K. pneumoniae* phylogenetic groups according to gender, specimen types and hospital wards.

	No. of *K. pneumoniae* isolates (76)	***KPI* n= 62**	***KPII* n= 9**	***KPIII* n= 5**	***p value***
Gender					
Male	36 (47.4%)	27(43.5%)	6 (66.7%)	3(60%)	0.33
Female	40 (52.6%)	35(56.5%)	3(33.3%)	2(40%)	
Specimens					
Urine	14 (18.4%)	9(14.5%)	4(44.4%)	1(20%)	0.41
Blood	12 (15.8%)	9(14.5%)	1(11.1%)	2(40%)	
Wound swab	6 (7.9%)	6 (9.7%)	0	0	
ETA	21 (27.6%)	17(27.4%)	3(33.3%)	1(20%)	
Sputum	9 (11.9%)	8(12.9%)	0	1(20%)	
Pus	13 (17.1%)	12(19.4%)	1(11.1%)	0	
CSF	1 (1.3%)	1(1.6%)	0	0	
Wards					
ICU	22 (29%)	19(30.6%)	3(33.3%)	0	0.32
Surgery	14 (18.4%)	11(17.7%)	2(22.2%)	1(20%)	
Pediatrics	15 (19.7%)	12(19.4%)	2(22.2%)	1(20%)	
Burn	4 (5.3 %)	3(4.8%)	1(11.1%)	0	
Internal medicine	9 (11.8 %)	9(14.5%)	0	0	
Urology	12 (15.8%)	8(12.9%)	1(11.1%)	3(60%)	

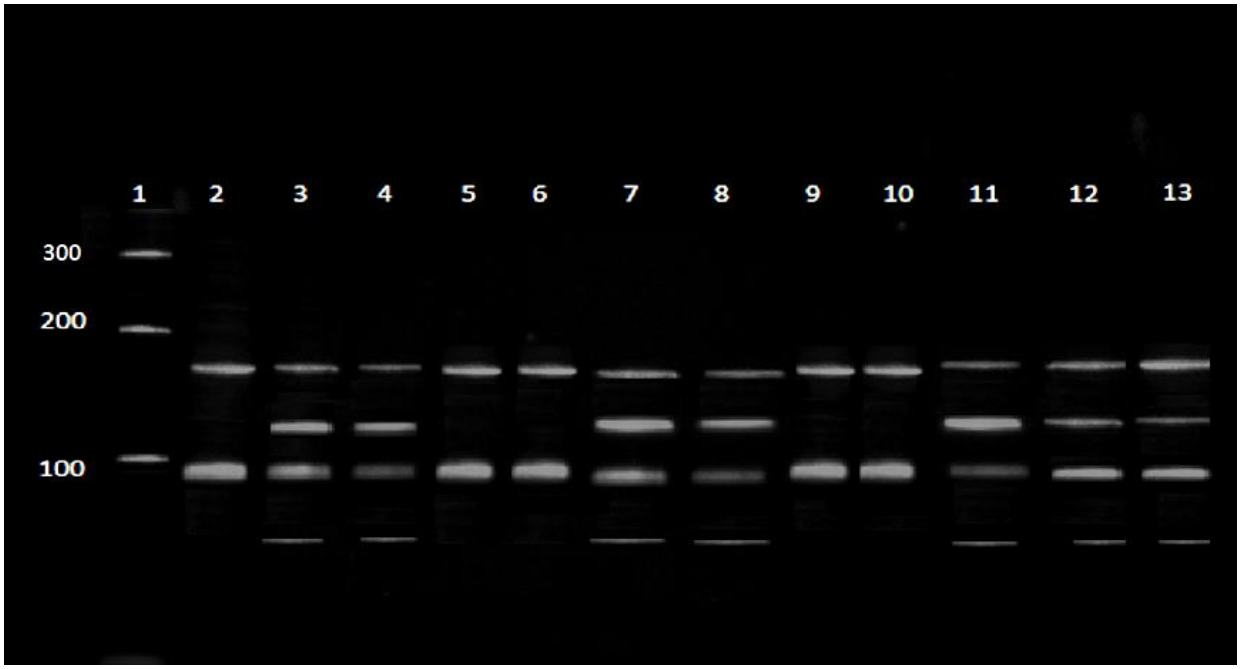
-ANOVA statistical test was used
-Statistically significant at $p \leq 0.05$

Table 2. Frequency distribution of the antimicrobial susceptibility patterns of the three *K. pneumoniae* phylogenetic groups.

	Sensitive N (%)			**Intermediate N (%)**			**Resistant N (%)**		
	***KPI* N=62**	***KPII* N=9**	***KP III* N=5**	***KPI* N=62**	***KP II* N=9**	***KP III* N=5**	***KPI* N=62**	***KPII* N=9**	***KPIII* N=5**
Amoxicillin-clavulanate	0(0)	1(11.1)	0(0)	3(4.8)	1(11.1)	1(20)	59(95.2)	7(77.8)	4(80)
Ampicillin-sulbactam	4(6.5)	2(22.2)	1(20)	2(3.2)	0(0)	0(0)	56(90.3)	7(77.8)	4(80)
Cefepime	29(46.8)	6(66.7)	1(20)	9(14.5)	3(33.3)	2(40)	24(38.7)	0(0)	2(40)
Ceftriaxone	12(19.4)	3(33.3)	1(20)	3(4.8)	2(22.2)	0(0)	47(75.8)	4(44.5)	4(80)
Ciprofloxacin	25(40.3)	5(55.6)	3(60)	5(8.1)	1(11.1)	1(20)	32(51.6)	3(33.3)	1(20)
Levofloxacin	23(37.1)	4(44.4)	1(20)	6(9.7)	1(11.1)	1(20)	33(53.2)	4(44.5)	3(60)
Gentamycin	13(21)	1(11.1)	2(40)	7(11.3)	2(22.2)	0(0)	42(67.7)	6(66.7)	3(60)
Amikacin	22(35.5)	2(22.2)	1(20)	3(4.8)	0(0)	2(40)	37(59.7)	7(77.8)	2(40)
Imipenem	47(75.8)	6(66.7)	4(80)	2(3.2)	1(11.1)	0(0)	13(21)	2(22.2)	1(20)
Meropenem	46(74.2)	6(66.7)	4(80)	2(3.2)	0(0)	0(0)	14 (22.6)	3(33.3)	1(20)
Trimethoprim-sulfamethoxazole	33(53.22)	7(77.8)	3(60)	1(1.6)	0(0)	1(20)	28(45.2)	2(22.2)	1(20)
Aztreonam	53(85.5)	8(88.9)	3(60)	3(4.8)	0(0)	0(0)	6(9.7)	1(11.1)	2(40)
Nitrofurantoin	11(17.7)	1(11.1)	1(20)	8(12.9)	3(33.3)	1(20)	43(69.4)	5(55.6)	3(60)

Figure 1. PCR-RFLP profiles of the *gyrA* gene using *TaqI* restriction enzyme. Lanes 2, 3, 4, 5, 7, 8, 11, 12, 13 for *KpI* (197 bp, 142 bp, 93 bp, and 9) and lanes 6, 9, 10 for *KpII* (197 bp, 151 bp, and 93 bp) . Lane 1, 100 bp molecular DNA marker (Axygen Biosciences).

Figure 2. PCR-RFLP profiles of the *gyrA* gene using *HaeIII* restriction enzyme. Lanes 3, 4, 7, 8, 11, 12, 13 for *KpI* isolates (175 bp, 129 bp, 92 bp, and 45 bp) and lanes 2, 5, 6, 9, 10 for *KpIII* (175 bp, 174 bp, and 92 bp). Lane 1, 100 bp molecular DNA marker (Axygen Biosciences).



Discussion

In recent years, WHO has listed *K. pneumoniae* as a critical priority microbe due to the high morbidity and mortality accompanied with its infection. For such a significant pathogen, genotyping is required. One molecular technique for determining the bacterial isolates genetic relationship is RFLP-PCR, which is mostly utilised for molecular epidemiology of pathogens of public health concerns [14].

A total of 143 specimens were collected from patients clinically diagnosed to have UTIs, wound infections, SSI, VAP, IAI, pneumonia and septicemia. Among them, *K. pneumoniae* showed a high frequency of isolation (53.1 %).

Similar results were reported from different sites in Egypt. **Ghonaim et al.** reported that the most commonly isolated bacteria causing septicemia in Menoufia hospitals was *K. pneumoniae* (31.6%) [15].

In addition, a recent large surveillance study conducted by **Sherif et al.** covering 91 ICUs in 28 hospitals found that *Klebsiella spp.* were the most commonly isolated pathogens (28.7%) [16]. The high frequencies of *Klebsiella spp.* in the HCAI might be because the majority of them are located in the intestinal system. They can also survive in wet places, inside mechanical respiration apparatuses, and inside bronchoscopes and laryngoscopes that are of difficult access to be cleaned and/or dried.

In our study, most of *K. pneumoniae* were isolated from ICU (29 %), followed by pediatrics wards (19.7%), surgical wards (18.4%), urology wards (15.8 %) and internal medicine wards (11.8%). The lowest rate was from the burn unit (5.3 %). Another study conducted at SCUHs by **El-Sweify et al.** also reported that ICU showed the highest percentage of clinical isolates (30%), followed by the neonatal intensive care unit (NICU) (22%), the surgical wards (16%), the urology wards (11%), the internal medicine wards (8%) and finally outpatient clinics showed the lowest rate (5%) [17]. The utilisation of invasive procedures, concomitant diseases, prolonged ICU stays, and strong antibiotic use pressure are the reasons for high prevalence of infections in ICUs.

Our results showed that most of the *K. pneumonaie* isolates were collected from ETA specimens (27.6 %), followed by urine (18.4 %), pus (17.1 %), blood (15.8 %), sputum (11.9 %), wound swab (7.9 %), while CSF showed the lowest rate (1.3 %). These findings agree with **Raheel et al.** study at SCUHs which showed that 46.6 % of the studied *K. pneumonaie* strains were isolated from ETA while other strains were isolated from blood (21.6 %), urine (9.4 %), sputum (8.6 %) and pus (13.8 %) [18].

A similar study in Egypt conducted by **Taha et al.** to investigate the prevalence of *K. pneumoniae* in Tanta University Hospitals reported that 50% of *K. pneumonaie* strains were isolated from urine, 25% from pus swabs, 12.5% from sputum and 6.25% from both ETA and blood [19]. On the other hand, **Sherif et al.** surveillance study covering 28 hospitals, reported that the most frequent sources of the isolates were blood (43.6%), respiratory tract samples (25.6%), whereas 15.4% were from hospital acquired wound infections and UTIs [16].

Currently, the distinct *KPC* phylogroups cannot be identified using specific biochemical tests in the standard clinical microbiology laboratories. Therefore, the usual identification of *Klebsiella spp.* is difficult and it is still challenging to identify *Klebsiella spp.* by a strong reliable marker. Applying an efficient typing technique, such the RFLP-PCR approach, could improve the treatment of *Klebsiella* infections because these species have unique pathogenic and epidemiological features.

In our study, phylogrouping of *Klebsiella* isolates was done by RFLP-PCR method using *TaqI* and *Hae III* restriction enzymes. Identification of the three groups was successfully done, the phylogroup I (81.6%) was the dominant group, followed by group II (11.8%) and group III (6.6%). A similar study conducted by **Baghbanijavid et al.** also reported that 96% of the *KPC* isolates were identified as *KpI*, 3% of isolates as *KpII*, and only 1 % of the isolates as *KpIII* using the RFLP-PCR method [6].

Similar findings were attained by **Pajand et al.** in a prior study conducted in Iran which showed that all the studied isolates using RFLP-pattern were grouped in three phylogroups, including 80.3% in group I, 16.4% in group II and 2.5% in group III [8]. Also, **Brisse et al.** determined the distribution of phylogenetic groups among *K. pneumoniae* isolates and reported that *KpI* had the most dominant phylogeny (82.1%), followed by *KpII* (6.9 %) and *KpIII* (11%) [13]. In addition, Phylogenetic groupings *KPC* isolated from community-based infections, nosocomial infections and natural microbiota have been analysed by **De Melo et al.** who reported that in total, 79.7% were

identified as *KpI*, 12.8% as *KpII*, and 7.5% as *KpIII*. The sources of the samples collected were urine (58%), blood (16%), wounds (9%), trachea (9%) and respiratory secretions (8%). Notably, internal medicine (27%), intensive care unit (22%), infectious disease (18%), surgical (13%), paediatric (11%), and emergency (9%) wards were the most common wards from which the samples were isolated [12].

On the other hand, some studies reported higher frequencies of *KPI*, for example, **Younes et al**. in Scotland reported that 100 % of *K. pneumonaie* was assigned to *KpI* [20]. Another study performed by **Pons et al.** in Mozambique demonstrated that all *KPC* isolates belong to the same phylogenetic group (*KpI*) using *gyrA* gene analysis, and a strong correlation was also discovered between the high incidence of *KpI* isolates and antibiotic resistance [21]. Interestingly, the rate of the phylogenetic groups is different from one population to another and this could be explained by variations in the host's genetic makeup, state of health, regional climate, and history of antibiotic usage [6].

The whole world is facing a global health threat due to increase and spread of the MDR *K. pneumoniae*, which has been detected as main cause of morbidity and mortality in the hospitalized patients. Added to that is the shortage of therapeutic options [22]. In the current study, it has been shown that the level of resistance for most of the antibiotics was highest among phylogroup I, intermediate in group II and lowest in group III. In terms of the modified Kirby–Bauer method, the highest resistance rates were found to be related to amoxicillin-clavulanate (95.2%), ampicillin-sulbactam (90.3%) and ceftriaxone (80%). However, the most effective antibiotic against *K. pneumonaie* was aztreonam (88.9%), followed by meropenem and imipenem (80%) each.

In our research, out of the total 62 *KPI* isolates, MDR was detected in 39 isolates (63%) and XDR was found in 12 isolates (19.3%). While out of the total 9 *KpII* isolates, 7 isolates (77.7%) were MDR and only one isolate (11.1%) showed XDR pattern. Concerning the 5 *KPIII* isolates, 2 isolates (40%) were MDR, while only one isolate (20%) showed XDR pattern.

Other studies in Egypt also revealed high rates of MDR; **Sherif et al.** revealed that 42.5% of *Klebsiella spp.* were ESBL producers and 48.1% were carbapenem resistant [16]. In addition, a study conducted by **Ghaith et al.** in the NICU at Cairo University hospital reported that *K. pneumoniae* isolates showed high resistance rates to carbapenems (imipenem, 43.5%; meropenem, 56.5%) [23]. Also, **El-Domany et al.** reported multiple resistance profiles among *K. pneumoniae* (MDR; 42.5%), (XDR; 35%), and (PDR; 5%) [24].

Variable results were obtained from global studies; in Iran, **Baghbanijavid et al.** showed that the highest resistance rates were found to be related to ceftazidime (88%) and piperacillin (84%). Fosfomycin was the most effective antibiotic against *K. pneumonaie* (85%), followed by amikacin (66%) and imipenem (50%) [6]. **De melo et al.** reported that the highest percentage of antibiotic resistance was observed in the *KpI*, *KpII*, and *KpIII* groups, respectively. Resistance to amoxicillin and ampicillin was present in the majority of the *K. pneumoniae* isolates. Only isolates from the *KpI* group showed resistance to imipenem, aztreonam, streptomycin, amoxicillin/clavulanate, and third-generation cephalosporins. 69.3 % of the *KpI* group isolates were MDR [12].

Similarily**,** The findings of **Brisse et al.** showed that *KpI* had a higher prevalence of antibiotic resistance than the other types, and that there was a significant correlation between antibiotic resistance and both *KpI* and *KpII* [13]. Furthermore, **Pajand et al.** studied the clonal relationship between *bla*NDM/OXA-48-producing strains and the phylogenetic type of *KPC*, results showed that most of the isolates producing NDM-1 (81%) belonged to *KpI* and the rest of them belonged to *KpII* and *KpIII* [8].

Klebsiella spp. have been a prominent cause of hospital and nursing facility outbreaks over the last ten years. Some researchers have argued that we are moving globally towards extended drug resistance based on a number of retrospective assessments and in vitro synergistic susceptibility testing with some reported cases of alarmingly pan-drug-resistant isolates [25].

In conclusion, restriction analysis of the *gyrA* gene (*gyrA* PCR– RFLP) allowed the distinction of three phylogenetic groups among *K. pneumoniae* isolates from Egypt. This ensures the value of genomic surveillance in research, clinical and public health settings. Moreover, the current study raised alarms about the concerning rise in HCAIs at SCUHs brought on by MDR and XDR *K.*

pneumonaie, which may start future outbreaks that could be difficult to manage by the available sets of antibiotics. This calls for increased focus on updating antibiotic stewardship, strengthening the implementation of infection control measures in our hospitals, and refocusing research to identify alternatives for countering this aggressive antibiotic resistance.

Limitations

Given that this study had a relatively small sample size, its findings must be interpreted in the context of its limitations. Furthermore, lack of financial facilities that disabled sequencing type determination. Notwithstanding these limitations, the study delivers valuable data that can be helpful in both execution of surveillance and spread control of resistance at a local level, however, further studies are needed to demonstrate other epidemiological features of *K. pneumoniae* strains in Egypt.

Consent to publish

Patients signed informed consent regarding publishing their data.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Conflict of interest

The authors report no conflicts of interest.

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Authors' contributions

All the authors were involved in the study conception and design, contributed to the methodology, writing the manuscript and analysis and interpretation of data. All authors read and approved the final version of the manuscript.

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