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ORIGINAL ARTICLE

Klebsiella pneumoniae: a Major Pathogen Causing Ventilator Associated Pneumonia at Suez Canal University Hospitals

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ABSTRACT

Background: *Klebsiella Pneumoniae (K. pneumoniae) is a serious emerging challenge among mechanically ventilated patients due to its disease severity, high virulence and antimicrobial resistance. Genotyping of such an aggressive pathogen is critical tool in restricting Klebsiella infections. Objective: This study aimed to characterize the diversity of Klebsiella pneumoniae complex (KPC) isolates causing ventilator associated pneumonia (VAP) for further reducing morbidity and mortality rates of intensive care unit (ICU) patients.* ***Methodology:*** *K. pneumoniae spp. were isolated from endotracheal tube aspirate specimens of patients admitted to ICU at Suez Canal University Hospitals (SCUHs). Species identification was done using conventional microbiological methods. Antimicrobial susceptibility testing was performed by disk diffusion method. Confirmation of K. pneumoniae was done by PCR amplification of gyrA gene. Genotyping was done by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method using TaqI and HaeIII restriction enzymes.* ***Results:*** *Out of 83 specimens, 32 Enterobacteriaceae strains were isolated, 65.6% were identified to be K. pneumoniae. PCR-RFLP identified 17 isolates(81%) as KPI, 3 (14.3%) as KPII and 1 (4.7%) as KPIII. High rates of MDR and XDR were reported. Among KPI, 8 isolates (47.1%) showed MDR and 8 isolates (47.1%) showed XDR. For KPII, 2 isolates (66.7%) showed MDR and 1(33.3%) showed XDR. The only KPIII isolate was XDR.* ***Conclusion:*** *GyrA PCR-RFLP provides a reliable tool for genotyping of KPC isolates. MDR and XDR KPC isolates are common causative pathogens for VAP at SCUHs and can initiate future outbreaks of nosocomial infections which obligates strengthening the infection control measures to combat such aggressive infections.*

INTRODUCTION

Gram negative bacteria, specially *K. pneumoniae*, are at the frontline of the causative agents of ventilator-associated pneumonia (VAP). VAP is the most serious intensive care unit (ICU) acquired infection of the lung parenchyma that develops 48 hours after endotracheal intubation and mechanical ventilation. The emergence of antimicrobial resistance and virulence in *K. pneumoniae* potentiates invasive and difficult-to-treat infections and the world health organization has listed *K. pneumoniae* as a crucial priority microorganism owing to its alarming morbidity and mortality rates at a time when there is a lack of promising new antimicrobial agents on the horizon[1].

Recently, improved sequencing capabilities and molecular epidemiology have classified *Klebsiella pneumoniae complex (KPC)* into three phylogroups*: Klebsiella pneumoniae (KpI), Klebsiella quasipneumoniae (KpII) and Klebsiella variicola (KpIII)* [2].

Precise species identification by the analysis of the *gyrA* gene sequence has rendered applicable for various clinical microbiology aspects[3]. This gene is not prone to frequent horizontal transfer among clusters and therefore represents reliable phylogenetic marker [4].

Previous studies reported that nearly 30% of routine clinical isolates initially identified as *KPI* were later recognized as *KPII* or *KPIII* based on genotypic identification[5]. The excessive misidentification has resulted in under estimation of the actual clinical significance of other phylogroups which present under recognized cause of fatal infections[6].

K. pneumoniae phylogroup (*KpI*) represents about 80% of opportunistic isolates. *KPI* is most frequently associated with a wide range of human infections due to existence of a large accessory genome approaching 30,000 protein-coding genes and more than 150 deeply branching lineages with numerous multi drug resistant (MDR) or hypervirulent clones[4].

K. quasipneumoniae phylogroup *(KpII*), is a new species discovered in recent years. *KpII* was originally thought to be largely confined to agriculture and the environment; however, it causes a wide spectrum of infections[5]. Several recent studies have demonstrated that it harbors virulence factors and acquires clinically relevant resistance genes[7]. *KPIII*, as an emerging human pathogen, had caused numerous infections worldwide

but with a lower frequency, it can display hypervirulent and hypermucoviscous phenotypes and has been reported as a cause for fatal cases of bacteremia[6].

Molecular typing is useful in terms of determination of dominant genotype among isolates and it is crucial to recognize the source of infection for nosocomial infections[8]. The advantages of PCR-RFLP over other molecular typing methods that it is able to distinguish between closely related bacterial strains as well as being a direct, rapid and reliable genotyping method[5].

Clearly, there is a compelling need for high-resolution genomic techniques for *Klebsiella* spp. identification and characterization, capable of discriminating *KPC* members. Therefore, the present study aimed to identify *KPC* phylogroups using PCR-RFLP based molecular typing of isolates from endotracheal tube aspirate (ETA) specimens in Suez Canal University Hospitals (SCUHs) to determine their relative prevalence in health care associated infections and to compare their levels of antimicrobial susceptibility.

METHODOLOGY

This cross-sectional descriptive study is a part of thesis submitted for doctorate degree that was performed at Medical Microbiology and Immunology Department and SCUHs, Faculty of Medicine, Suez Canal University, Ismailia, Egypt, during the period from May 2022 to April 2024.

Patients:

Enrolled cases were selected from patients admitted to the ICU in SCUHs. Patients were included if they were mechanically ventilated for more than 48 hours. Patients with evidence of chest infection prior to intubation were excluded. The study involved 83 patients of both sex and from all age groups.

Informed consents were obtained from the first-degree relatives of the study patients. A detailed history was taken with reference to name, age, occupation, date of admission and underlying chronic condition (diabetes mellitus, hypertension, chronic heart or liver disease, chronic renal failure and malignancy). History of prior hospitalization, recent surgery, antibiotic therapy, steroid and immunosuppressive drugs was taken.

Approval was obtained from the research Ethics Committee (Research 4905b#) at Faculty of Medicine, Suez Canal University.

Collection and processing of specimens:

The ETA specimens were collected under aseptic conditions from ICU patients clinically diagnosed to have VAP with the aid of intensive care physicians[9].

- Patients were pre-oxygenated and a standard 500 mm, 14-gauge tracheal aspiration catheter was attached to a 20 ml syringe filled with 20 ml sterile saline. The distal end was lubricated with sterile gel, introduced via the endotracheal tube, and advanced until significant resistance was encountered. The saline was instilled over 10–15 seconds, the tube was then withdrawn 10–20 mm, the saline was immediately re-aspirated and the catheter was then removed. In total, 5–10 ml fluid were recovered.
- Samples were transported to the microbiology laboratory and Gram-stained smears showing more than 25 neutrophils and less than 10 squamous epithelial cells/LPF was indicative of purulent lower respiratory secretions and possible infection. The most purulent portion of the secretion was liquefied with the addition of an equal volume of 1% N-acetyl cysteine and subsequently homogenized by vortexing for one minute at 3,000 rpm then inoculated onto blood and MacConkey agar plates (Oxoid, UK), which were aerobically incubated at 35±2 °C for 18-24 hours. For definite diagnosis of VAP, 10^5 CFU/mL was considered as threshold. Growth of any organism below this threshold was considered to be contamination.

Colonies suspected to be members of *Enterobacteriaceae* were identified up to the species level by the routine bacteriological methods including microscopic examination and biochemical reactions.

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing of isolates was performed by the standard Kirby-Bauer disc diffusion method on Mueller- Hinton agar (Oxoid, UK) incubated at 37ºC for 16-18 hours according to the Clinical and Laboratory standard Institute (CLSI) guidelines[10]. The following antimicrobial agents (OXOID, UK) were included: Amoxicillin-clavulanate (20/10µg), Ampicillin-sulbactam (10/10µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefepime (30µg), Ceftriaxone (30µg), Gentamycin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Aztreonam (30µg), Meropenem (10µg), Imipenem (10µg) and Trimethoprim-sulfamethoxazole (1.25/3.75µg).

K. pneumoniae isolates were subsequently categorized according to the standardized international document to MDR, extensively drug resistant (XDR) and pan drug resistant (PDR) isolates[11].

- MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. XDR was referred to as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. PDR was defined as non-susceptibility to all agents in all antimicrobial categories.

DNA preparation and phylogenetic typing by the PCR-RFLP 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:

GyrA gene amplification was carried out by PCR with a specific set of primers, *gyrA-A* (F: 5``-CGCGTACTATACGCCATGAACGTA-3′ and *gyrA-C* (R:5′ACCGTTGATCACTTCGGTCAGG-3′) **(fragment size, 441 bp)** [2].

The amplification reactions were prepared in a total volume of 25 µL, containing 1 ng of genomic DNA, 1.0 U of *Taq* DNA polymerase (Promega, USA), 200 µM of each dNTP (Promega, USA), 1.5 mM of $MgCl_2$, 2 µM of each primer and 1x reaction buffer (final concentration).

The PCR amplifications were performed in thermocycler (Eppendorf-Mastercycler Gradient) using the following protocol: initial denaturation (94 ºC for 5 min), followed by 30 cycles of denaturation (94 ºC for 45 sec), annealing (54 ºC for 30 sec) and extension (72ºC, for 1 min) 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 in 1x Tris-Borate-EDTA (TBE) buffer containing 0.1 µl/mL ethidium bromide at 120 volts for 45 minutes 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 strain in this study.

***KPC* isolates genotyping using RFLP [12]:**

The *gyrA* PCR amplicons were submitted to RFLP using restriction enzymes *TaqI* and *HaeIII* (New England Biolabs (NEB), UK) separately. The reactions were performed in a total volume of 50 µL containing 1 µg of DNA, 5 µL of 10x rCutSmart Buffer, 1 µL of restriction enzyme, completing the total volume with sterile nuclease free water. 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‘‘Molecular Screening’’ M.S.6 (Pronadisa, Madrid) gels, followed by staining with 0.5 µg/mL ethidium bromide stain and visualizing under ultraviolet light as follows [2].

In ***KpI*** strains, *TaqI* enzyme created four bands (197 bp, 142 bp, 93 bp and 9 bp) and *HaeIII* enzyme created four bands (175 bp, 129 bp, 92 bp and 45 bp).

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

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

Statistical analysis

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 was used to detect the difference between qualitative data. Data was analyzed and presented as numbers and percentages using tables and graphs with the CI at 95%, *p value* of 0.05 was used as the limit of statistical significance.

RESULTS

The study was carried out on 83 patients admitted to ICU in SCUHs and clinically diagnosed to have VAP. The percentage of males (n=45) was slightly higher than that of females (n=38) (54.2% versus 45.8%). The majority of specimens were collected from patients aged from 50 to 70 years (n=35) (42.2%) with mean age of 52.4 ± 10.24 years.

Gram-negative spp. were isolated at a frequency of 68.7%, while other organisms were isolated at a frequency of 24.1%. The frequencies of *Enterobacteriacae* spp., *Pseudomonas* spp., *Acienetobacter* spp., Gram-positive organisms and *Candida* spp. were 38.6%, 20.5%, 9.6%, 15.7% and 8.4% respectively. No growth was detected in 7.2% of the cases.

The isolation rate of different *Enterobacteriaceae* spp., *K. pneumoniae* was isolated at the highest frequency (21/32) (65.6%) followed by *Escherischia coli* (*E. coli*) (6/32) (18.9%), *Proteus mirabilis* and *Enterobacter cloacae* (2/32) (6.2%) each, while *Serratia marcescencs* showed the lowest frequency of isolation (1/32) (3.1%). Data and risk factors distribution among mechanically ventilated patients in ICU with *K. pneumonaie* isolates were illustrated in **tables 1 and 2** respectively.

Table 1: Data of *K. pneumoniae* isolates from mechanically ventilated patients in ICU:

Isolate	Patient Gender	Patient Age	Hospital stay	Cause of patient admission	Adonitol fermentation	Phylogroup
1	F	64	15 days	DKA	positive	*KP II*
2	M	69	12 days	Post operative	positive	*KP I*
3	M	75	23 days	Pulmonary embolism	positive	*KP I*
4	F	46	11 days	Malignancy	negative	*KP II*
5	F	85	18 days	Stroke	positive	*KP I*
6	F	77	5 days	Post operative	positive	*KP II*
7	F	49	15 days	Trauma	positive	*KP I*
8	M	70	3 days	Post operative	positive	*KP I*
9	F	64	17 days	DKA	negative	*KP I*
10	M	72	10 days	Intra cranial hemorrhage	positive	*KP I*
11	M	83	12 days	Stroke	positive	*KP I*
12	M	57	18 days	Renal failure	negative	*KP I*
13	M	34	3 days	Trauma	positive	*KP I*
14	F	75	16 days	Liver abcess	negative	*KPIII*
15	M	78	11 days	DKA	positive	*KP I*
16	F	76	15 days	Intra cranial hemorrhage	positive	*KP I*
17	M	54	6 days	Malignancy	positive	*KP I*
18	F	71	16 days	Stroke	positive	*KP I*
19	F	79	15 days	Post operative	positive	*KP I*
20	M	74	16 days	Malignancy	negative	*KP I*
21	F	60	18 days	Renal failure	positive	*KP I*

Table 2: Risk factors distribution among the patients with *K. pneumoniae* isolates:

		Risk factors
Chronic diseases	**N***	%
Diabetes mellitus	**11**	52.3
Chronic heart disease	**9**	42.8
End stage renal failure	**2**	9.5
Chronic liver disease	**1**	4.8
Chronic obstructive lung disease	**3**	14.2
Other conditions	**4**	19
Medical device use	**N**	%
Urinary catheter	**21**	100
Central venous line	**5**	23.8
Arterial stent	**3**	14.3
Prosthetics	**4**	19
Brain shunt	**1**	4.8
Drug use	**N**	%
Steroids	**6**	28.6
Chemotherapy	**3**	14.2
Immunosuppressive drugs	**1**	4.8
Exposure	**N**	%
Surgery	**4**	19
Major trauma	**2**	9.5
Hospital stay	**N**	%
More than 14 days	**12**	57.1
less than 14 days	**9**	42.8

* N = number. Other conditions included autoimmune diseases, tuberculosis, malignancies and HIV infection. Prosthetics included prosthetic heart valves and prosthetic hip and knee joints.

A total number of twenty-one non repetitive isolates (65.6%) were proved to be *K. pneumoniae* by amplification of *gyrA* gene by PCR. Amplification was carried out with the primers of *gyrA* gene, amplicons obtained from PCR reactions were analyzed by gel electrophoresis **(fragment size, 441 bp)** as shown in **figure 1**.

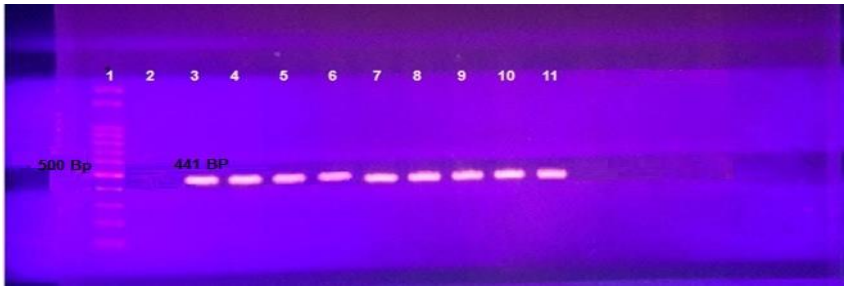

Fig. 1: The PCR product of the *gyrA* gene **(**441-bp**).** Lane 1, 100 bp molecular DNA marker (Axygen Biosciences); Lane 2, negative control, Lane 3 positive control for *K. pneumoniae* ATCC BAA-1705, Lanes 4-11 are clinical specimens positive for 441 bp PCR product of the *gyrA* gene.

Genotyping of the *K. pneumoniae* clinical isolates was performed using the PCR-RFLP method as *gyrA* gene amplicons were submitted to *TaqI* and *HaeIII* restriction enzymes. *TaqI* produced restriction profiles (197bp, 142 bp, 93bp and 9 bp) for both *KPI* and *KP III* and restriction profiles for *KPII* (197bp, 151bp and 93bp) as shown in **figure 2**. *HaeIII* produced (175 bp, 129 bp, 92bp and 45 bp) restriction profile for both *KPI* and *KP II*, while different *HaeIII* restriction profile was produced for *KPIII* (175bp, 174bp and 92bp) as shown in **figure 3**.

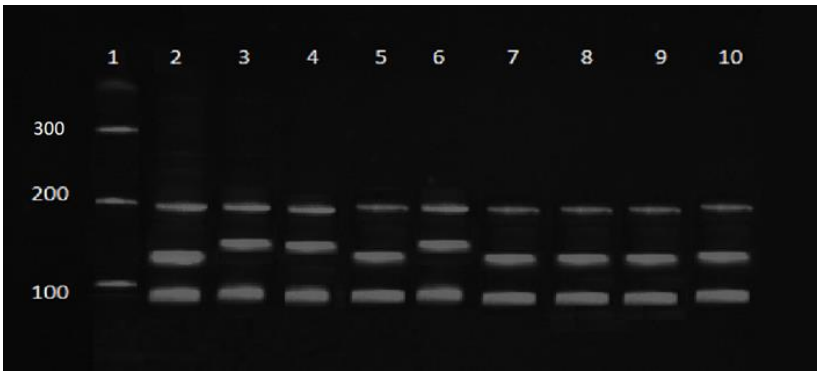

Fig. 2: PCR-RFLP profiles of the *gyrA* gene identified in the *K. pneumoniae* isolates using *TaqI* restriction enzyme. Lanes 2, 5, 7, 8, 9, 10 for *KpI* and lanes 3,4,6 for *KpII*. Lane 1, 100 bp molecular DNA marker (Axygen Biosciences). *TaqI* restriction profiles (197bp, 142 bp, 93bp and 9 bp fragments) for *KPI* and *KP III* and *TaqI* restriction profiles for *KPII* (197bp, 151bp and 93bp fragments).

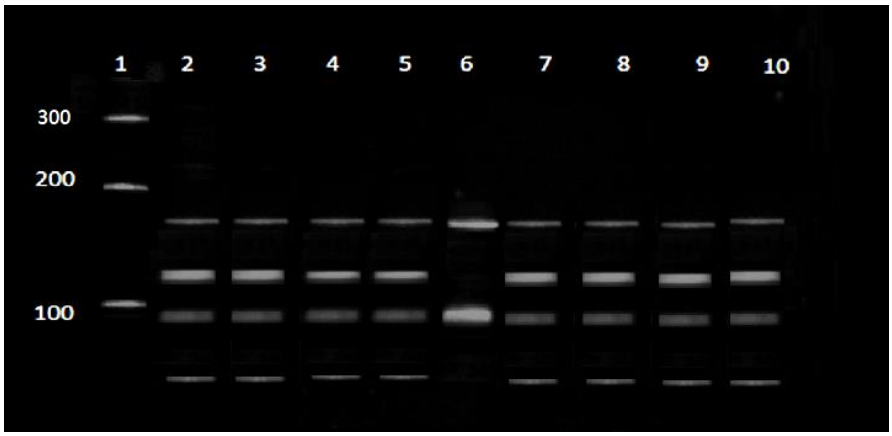

Fig. 3: PCR-RFLP profiles of the *gyrA* gene identified in the *K. pneumoniae* isolates using *HaeIII* restriction enzyme. Lanes 2,3, 4, 5,7,8,9, 10 for *KpI* isolates and lane 6 for *KpIII* isolate. Lane 1, 100 bp molecular DNA marker (Axygen Biosciences). *HaeIII* restriction profile (175 bp, 129 bp, 92bp and 45 bp fragments) for *KPI* and *KPII*. *HaeIII* restriction profile for *KPIII* (175bp, 174bp and 92bp fragments).

Regarding the isolation rates of different *K. pneumoniae* phylogenetic groups, 17 clinical isolates (81%) were identified as *KP I*, 3 isolates (14.3%) were identified as *KP II* and 1 isolate (4.7%) was identified as *KP III*.

Adonitol fermentation was positive in 14 (82.4%) of *KPI*, 2 (66.7%) of *KPII* and negative in *KPIII* isolate. Chi square test was used to test significance concerning the distribution of the phylogroups and adonitol fermentation, no statistically significant difference was seen (*p value* > 0.05).

In terms of the modified Kirby–Bauer method, the highest resistance rates of *KPI* were found to be related to amoxicillin-clavulanate (100%), ampicillin-sulbactam (100%). The most effective antibiotic against *KPI* was aztreonam (76.5%) followed by meropenem (64.8%) and trimethoprim sulfamethoxazole (58.9%) as shown in **table 3**.

Table 3: The antimicrobial susceptibility patterns of *K. pneumoniae* phylogroup I (*KP I*) isolates.

	***KP I* (N*=17)**					
	Sensitive		**Intermediate**		**Resistant**	
	N*	**%**	**N***	**%**	**N***	**%**
Amoxicillin-clavulanate	0	0	0	0	17	100
Ampicillin-sulbactam	0	0	0	0	17	100
Cefotaxime	0	0	1	5.8	16	94.1
Ceftazidime	1	5.8	0	0	16	94.1
Cefepime	1	5.8	0	0	16	94.1
Ceftriaxone	1	5.8	0	0	16	94.1
Ciprofloxacin	1	5.8	0	0	16	94.1
Levofloxacin	1	5.8	0	0	16	94.1
Gentamycin	1	5.8	0	0	16	94.1
Amikacin	1	5.8	0	0	16	94.1
Imipenem	8	47.1	0	0	9	53
Meropenem	11	64.8	1	5.9	5	29.5
Trimethoprim sulfamethoxazole	10	58.9	0	0	7	41.2
Aztreonam	13	76.5	0	0	4	23.5

N*= number.

High percentage of MDR and XDR was shown among *KPI*, 8 isolates (47%) among the 17 *KPI* isolates showed MDR, 8 isolates (47%) showed XDR and 1 isolate (5.9%) was neither MDR nor XDR.

In terms of the modified Kirby–Bauer method, the highest resistance rates of *KPII* were found to be related to amoxicillin-clavulanate, ampicillin-sulbactam, ceftazidime, ceftriaxone, ciprofloxacin, levofloxacin and gentamycin (100%) each, followed by meropenem, trimethoprim-sulfamethoxazole and cefotaxime (66.7%)each. The most effective antibiotics against *KPII* were amikacin, aztreonam and cefepime (66.7%) each as shown in **table 4**.

Table 4: The antimicrobial susceptibility patterns of *K. pneumoniae* phylogroup II (*KP II*) isolates.

	***KP II* (N*= 3)**					
	Sensitive		**Intermediate**		**Resistant**	
	N	**%**	**N**	**%**	**N**	**%**
Amoxicillin-clavulanate	0	0	0	0	3	100
Ampicillin-sulbactam	0	0	0	0	3	100
Cefotaxime	0	0	1	33.3	2	66.7
Ceftazidime	0	0	0	0	3	100
Cefepime	2	66.7	1	33.3	0	0
Ceftriaxone	0	0	0	0	3	100
Ciprofloxacin	0	0	0	0	3	100
Levofloxacin	0	0	0	0	3	100
Gentamycin	0	0	0	0	3	100
Amikacin	2	66.7	0	0	1	33.3
Imipenem	1	33.3	1	33.3	1	33.3
Meropenem	1	33.3	0	0	2	66.7
Trimethoprim-sulfamethoxazole	1	33.3	0	0	2	66.7
Aztreonam	2	66.7	0	0	1	33.3

N*= number.

High rates of MDR and XDR were reported among *KPII*, where 2 (66.7%) among the 3 *KPII* isolates showed MDR, while 1 (33.3%) showed XDR.

The only isolated *KPIII* strain was XDR as it showed resistance to all antibiotic groups and only sensitive for aztreonam.

DISCUSSION

Ventilator-associated pneumonia (VAP) has been reported to cause life-threatening healthcare-associated infection in ICUs. MDR strains causing VAP vary from one hospital to another and are affected by patient comorbidities and in low and middle income countries, it was found that the MDR Gram negative bacilli particularly strains of *KPC* predominate[6].

Our objectives were to identify isolates causing VAP and determine the most prevalent resistant strains and their antibiotic susceptibility pattern. Gram negative spp. were isolated at a frequency of 68.7%, while other organisms were isolated at a frequency of 24.1%. The frequencies of *Enterobacteriacae* spp., *Pseudomonas* spp., *Acienetobacter* spp. Gram positive organisms and *Candida* spp. were 38.6%, 20.5%, 9.6%, 15.7% and 8.4% respectively. No growth was detected in 7.2% of the cases.

Regarding the *Enterobacteriacae* spp. isolated in our study, the *K. pneumoniae* was isolated at the highest frequency (65.6%), followed by *E. coli* (18.9%), *P. mirabilis* and *Enterobacter cloacae* (6.2%) each, while *Serratia marcescencs* showed the lowest frequency of isolation (3.1%). A study conducted at SCUHs by Raheel *et al.* [13] showed a lower isolation rate of *Enterobacteriaceae* strains 48.4%, *K. pneumoniae* showed the highest frequency (45.1%), followed by *E. coli* (25.8%) with the lowest frequency for *Hafnia* (3.2%). Another related study at SCUHs, Khattab *et al.* [14] reported that *E. coli* and *klebsiella* spp. were isolated at a rate of 52.2%. The high prevalence of ICUs infections can be explained by prolonged hospital stay, comorbidities, invasive procedures, as well as antibiotic abuse[15,16].

The true clinical significance and epidemiological features of *KPC* phylogroups are of great value, however they may be underestimated, as they are not generally distinguished in routine testing in the clinical microbiology laboratories due to the inability of conventional microbiological methods to differentiate between *KPC* species resulting in high rates of misidentification[17].

Therefore, we need a strong marker to detect *KPC* species. In our study, phylogrouping of *KPC* was performed by PCR-RFLP method using *TaqI* and *HaeIII* restriction enzymes. Results showed that the *KPI* was isolated at the highest rate (81%) followed by *KPII* (14.2%) and *KPIII* (4.7%). Another study for clinical *KPC* conducted by Baghbanijavid *et al.* carried out by the PCR-RFLP method, showed that the dominant phylogroup was *KpI* (96%), while 3% of isolates were *KpII* and only 1% was identified as *KpIII* [18]. Similar results were reported in a study carried out by Pajand *et al.* categorized all the isolates according to their PCR-RFLP pattern, as 80.3% were *KPI*, 16.4% were *KPII* and 2.5% were *KPIII* [19]. Also, Brisse *et al.* [2] reported that *KpI* had the most dominant phylogeny (82.1%), while *KpII* were 6.9% and *KpIII* were 11%.

The greater prevalence of *KpI* isolates might be a consequence of ESBLs expression, since antimicrobial resistance is a critical parameter for the *KPC* transmission [12]. Interestingly, the rate of the phylogenetic groups is different from one population to another and this is attributed to variations in host genetic factors, health status, geographic climatic conditions, dietary factors and antibiotics use.

In the present study, 14 *KpI* and 2 *KpII* isolates analysed were adonitol positive, on the other hand, 3 *KPI*, 1 *KPII* and the only *KpIII* isolate were adonitol negative. So, adonitol fermentation does not seem to be a useful marker as no statistically significant difference was found concerning the distribution of the phylogroups and adonitol fermentation (*p value* > 0.05). Considering the application of adonitol fermentation as a differential test of *Klebsiella* spp., Brisse *et al.* [2,3] reported that *KpI*, *KpII* and *KpIII* are adonitol variable. Moreover, Rosenblueth *et al.* showed that isolates of *KPIII* did not ferment adonitol, a general characteristic of *K. pneumoniae* [17].

Highly drug resistant *Klebsiella* spp. especially *KPI*, have been a major concern due to the extensive spread. The association of resistance and virulence genes potentially end in emergence of untreatable invasive human and animal infections[6]. In terms of Kirby–Bauer method, the highest resistance rates in *KPI* were found to be related to amoxicillin-clavulanate (100%), ampicillin-sulbactam (100%) followed by ceftazidime, ceftriaxone, ciprofloxacin, levofloxacin, amikacin and gentamycin (94.1%). On the other hand, the most effective antibiotic against *KPI* was aztreonam (76.5%) followed by meropenem (64.8%) and trimethoprim-sulfamethoxazole (58.9%). For *KPII*, the highest resistance rates were found against amoxicillin-clavulanate, ampicillin-sulbactam, ceftazidime, ceftriaxone, ciprofloxacin, levofloxacin and gentamycin (100%) followed by meropenem, trimethoprim-sulfamethoxazole and cefotaxime (66.7%) each and the most effective antibiotics against *KPII* were amikacin, aztreonam and cefepime (66.7%) each.

Similarly, another study conducted by Azzab *et al.*,[20] in ICU in University Hospital in Egypt reported that out of culture-positive samples, 82.6% were identified as MDR. Among Gram negative isolates, 88.8% were found to be MDR. Among the *K. pneumonaie* isolates,

70.2% were resistant to imipenem, 64.9% were resistant to both ertapenem and meropenem.

Variable results were obtained from global studies; Ahmadi *et al.*,[21] who carried out their study on *K. pneumoniae* spp. The results indicated that the highest resistance levels were for ceftriaxone 65%, cefotaxime 64% and fosfomycin 60%. The lowest levels of resistance were reported for colistin 23%, imipenem 28% and azithromycin 32%. More than 50% of the strains were MDR.

Health crisis of MDR seems overwhelming. MDR Gram negative bacteria pose a serious threat to current medical practices[6]. In our research, the antimicrobial resistance patterns showed high rates of MDR and XDR. Among *KPI*, 8(47.1%) isolates showed MDR and 8(47.1%) showed XDR. While among *KPII*, 2(66.7%) showed MDR and 1 (33.3%) isolate showed XDR. The only isolated *KPIII* was XDR as it showed sensitivity only for aztreonam. Similar results were reported in study carried out by Saxenborn *et al.*,[22] that showed a total of 94% of the *KPC* isolates were genotypically MDR. More specifically, high rates of MDR were reported in *KPI* (94%) and *KPII* (80%) which carried the combination of β-lactam, quinolone and fosfomycin resistance genes. The high rates of antibiotic resistance reported for *Klebsiella* isolates may be explained by the rapid transmission of antibiotic resistance determinants. The extensive use of β-lactam antibiotics, including third-generation cephalosporins, for treating various infections is another factor that helps spread of resistant isolates, in addition to the lack of adherence to infection control standards[6].

CONCLUSION

This study highlights the significance of using high-resolution genotypic methods for clinical *Klebsiella* spp. identification and characterization. Our findings indicate that infections caused by both MDR and XDR *K. pneumonaie* at SCUHs are a more prevalent clinical issue than previously described, mainly due to high rates of misidentifications, which can initiate future outbreaks of nosocomial infections that could be difficult to manage by the available sets of antibiotics. This requires more attention for revising the antibiotic stewardship, strengthening the application of infection control precautions in our hospitals and redirecting research towards finding alternatives for combating such aggressive antibiotic resistance.

Limitations: The results of this study have to be considered in light of the limitations that include a relatively small study sample size and lack of financial facilities that disabled sequencing of the isolated strains. On the other hand, the study provides valued data that is helpful in surveillance and management of antimicrobial resistance, however, further research is required to explore other epidemiological prospects of the *K. pneumoniae* strains in Egypt.

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