Int.J.Curr.Microbiol.App.Sci (2016) *5*(7): 931-943

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 7 (2016) pp. 931-943**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.507.105

Phenotypic and Genotypic Diversity of Nosocomial Multi-Drug Resistant *Klebsiella pneumoniae* Isolated from Cancer Patients in Cairo, Egypt

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A B S T R A C T

Nosocomial *Klebsiella pneumoniae* infections are particularly a problem among cancer and immunocompromised patients worldwide. *K. pneumoniae* strains are widespread in nature thus typing is required to discriminate them in the epidemiological investigations. In this study, the diversity of 43 nosocomial multi-drug resistant (MDR) *K. pneumoniae* isolates recovered from different clinical specimens collected from cancer patients at National Cancer Institute, Cairo, Egypt, were phenotypically and genotypically analysed. These isolates were identified using conventional microbiological methods and the API 20E system. Investigation of the antimicrobial susceptibility patterns against 16 diverse antimicrobial agents revealed that all isolates are MDR. The phenotyping was performed using the API 20E-based biotyping and antibiogram typing which showed three different biotypes and 25 antibiogram types among the isolates. The genotyping using random amplified polymorphic DNA (RAPD) analyses revealed 39 different RAPD-based fingerprints and/or 43 different patterns among the isolates. In conclusion, *K. pneumoniae* infections in this institution have been caused by diverse MDR *K. pneumoniae* genotypes and/or phenotypes clone groups with isolates in the same phenotype group possess different genotypes. Biotyping and antibiogram typing of *K. pneumoniae* isolates have been shown to be well for preliminary screening of strain relatedness. The use of RAPD-PCR-based analyses is recommended, which has high discriminatory power providing definite information to evaluate the epidemic status of the nosocomial infections caused by MDR *K. pneumoniae*.

Keywords

Klebsiella pneumoniae, RAPD-PCR, multi-drug resistant, typing, genetic diversity.

Article Info

Accepted:
25 June 2016
Available Online:
10 July 2016

Introduction

K. pneumoniae, a member of the *Enterobacteriaceae* family, is one of the most common opportunistic Gram-negative pathogens (Zhao *et al.*, 2010). This bacterium has emerged worldwide as a

leading cause of nosocomial infections, including pneumonia, bacteremia, urinary tract infections and wound infections (Cao *et al*., 2015). *K. pneumoniae* infections are particularly a problem among the elderly, the immunocompromised persons and patients with underlying malignancy (Henao-Martínez *et al*., 2013; Holt *et al*., 2015). The main nosocomial reservoirs of *K. pneumoniae* include contaminated medical equipments, hands of hospital staff and the gastrointestinal tract of patients (Samra *et al*., 2007).

At present, the emergence of MDR *K. pneumoniae* strains represents an urgent important threat to human health; leaving only limited options for treatment. Thus, infections with MDR *K. pneumoniae* strains are usually associated with high morbidity and mortality, long hospital stay and high healthcare costs (Cao *et al*., 2015; Passet and Brisse, 2015). All mechanisms of antimicrobial resistance demonstrated in Gram-negative bacteria have been mostly manifested in *K. pneumoniae*, such as enzymatic hydrolysis, target mutation and reduced intracellular accumulation through reduced uptake and active efflux (Filgona *et al*., 2015). Besides extended-spectrum β-lactamase (ESBL) production, *K. pneumoniae* is frequently known to be resistant to multiple antimicrobial agents including fluoroquinolones, amino-glycosides and trimethoprim/ sulfa-methoxazole (Tan *et al.*, 2015). Considerably, the acquisition of carbapenemases-coding genes has depleted the last choice for treating infections caused by MDR *K. pneumoniae* (He *et al*., 2015).

Bacterial typing, including phenotyping and genotyping, are used for detecting the diversity among strains of the same species. Phenotyping is mainly based on the different biochemical reactions, serological reactions and antimicrobial susceptibility profiles. On the other hand, genotyping refers to the discrimination of bacterial strains based on their genetic construction (Li *et al*., 2009). Importantly, several outbreaks of infection caused by MDR *K. pneumoniae* strains have been reported (Cartelle *et al.*, 2004). In addition, various typing methods have been applied to recognize the transmission patterns for surveillance and prevention of the dissemination of MDR *K. pneumoniae* in a hospital setting. Pulsed-field gel electrophoresis (PFGE) analysis of genome has been shown to be a leading discriminatory technique for typing; however, it is technically demanding, time-consuming and requires specific equipment. Consequently, there is a need for less expensive and laborious methods that allow rapid evaluation of the relatedness of strains, on a local scale, to identify outbreaks with MDR *K. pneumoniae*. In recent studies, PCR-based typing techniques, such as randomly amplified polymorphic DNA (RAPD) analysis, and enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR) which are faster and easier to perform, have been successfully used for typing *K. pneumoniae* isolates (Cartelle *et al*., 2004; Sachse *et al*., 2014; Ashayeri-Panah *et al.*, 2014).

This study aimed to investigate the diversity among MDR *K. pneumoniae* isolated from different clinical specimens collected from cancer patients to determine the epidemiological status of this organism at National Cancer Institute, Cairo, Egypt.

Materials and Methods

Isolation, identification and biotyping of *K. pneumoniae* isolates

A total of 43 non-duplicated *K. pneumoniae* isolates were included in this study. These

isolates were recovered from various clinical specimens including blood (27), pus (10), sputum (4), urine (1) and stools (1), collected from cancer inpatients at National Cancer Institute, Cairo, Egypt, during the period from September 2015 to January 2016. Isolates were identified to species level using conventional microbiological methods, such as cultural characteristics on MacConkey's agar, Gram staining and biochemical testing. Identification was confirmed using the API 20E system (BioM´erieux, France). Both Luria-Bertani broth (LB) and agar (Lab M, UK) were used for growing isolates at 37°C. The isolates were subjected to biotyping on the basis of the biochemical profile produced by the API 20E system following the manufacturer's instructions.

Antimicrobial susceptibility testing and antibiogram typing

Antimicrobial susceptibilities of *K. pneumoniae* isolates to different antimicrobial agents were determined using Kirby-Bauer disk diffusion method on Mueller-Hinton agar following the Clinical and Laboratory Standards Institute (CLSI, 2015) guidelines. A number of 16 antimicrobial discs, representing different classes of antimicrobial agents, were included in this study. Discs were the product of Oxoid, UK: ampicillin (10 µg), amoxicillin/clavulanate (20/10 µg), cefazoline (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ertapenem (10 µg), imipenem (10 µg), gentamicin (10 µg), amikacin (30 µg), azithromycin (15 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (10 µg), colistin (10 µg), and nitrofurantoin (300 µg). Isolates that showed resistance to at least three different classes of antimicrobial agents were considered as MDR. Discs were stored at 4°C and allowed to reach room temperature before being used. Results were calculated by measuring the inhibition zones developed around the discs in millimetre (mm). Interpretation of results as susceptible (S), intermediate (I) or resistant (R) to a particular antimicrobial agent was performed according to CLSI (2015). For typing, isolates were grouped into different antibiotypes based on the antimicrobial susceptibility profiles (antibiograms).

RAPD analysis-based genotyping

RAPD-PCR fingerprinting was carried out to determine the genetic diversity among *K. pneumoniae* isolates.

Oligonucleotide primers used in RAPD-PCR

For RAPD analysis, preliminary PCR assays were performed to test fives primers synthesised by Eurofins Genomics, USA. Upon PCR analysis, the primer 1290 was selected based on the accuracy and reproducibility of the amplification profiles. The primers used were 10 bases long of arbitrary sequence. The details of primers used in this study are listed in Table 1.

PCR reactions and cyclic conditions

Genomic DNA was extracted from *K. pneumoniae* isolates using commercially available GeneJET Genomic DNA purification Kit (Thermo Scientific, USA). The PCR reactions were prepared in total volumes of 25 µl, contained ~ 10 ng of template DNA, 10 pmole of each primer and 12.5 µl MyTaq HS 2× mastermix (Bioline, UK). The amplifications were done in a Veriti 96 well Thermal Cycler (Applied Biosystems, USA) programmed for 5 min at 94°C, 40 cycles of denaturing at 94°C for 1 min, annealing at 36°C for 2 min and

extension at 72°C for 2 min, followed by a final extension at 72°C for 10 min.

TAE-agarose gel electrophoresis

RAPD-PCRs products were resolved through TAE agarose gel (1 %) electrophoresis prepared using molecular biology grade agarose (Bioline, UK) in 1× TAE buffer. DNA fragments, stained with ethedium bromide, were visualized by placing on a UV light source and photographed directly. For sizing of the separated DNA fragments, GeneRuler 1 kb DNA ladder (Thermo Scientific, USA) was used.

RAPD profiles analysis

RAPD patterns were analyzed and binary scoring was carried out using GelQuest computer software. UPGMA clusters showing the genetic similarity of the isolates were plotted using Numerical Taxonomy System software (NTSYS, Applied Biostatistics, Inc) based on Jaccard coefficient.

Discriminatory power of typing methods

The Simpson's diversity index was calculated to assess the discriminatory power of the biotyping, antibiogram typing and PCR-based RAPD typing methods performed in this study.

Results and Discussion

Identification and biotyping of *K. pneumoniae* isolates

A total of 43 *K. pneumoniae* isolates were isolated from different clinical specimens, collected from cancer patients in this study. The 43 isolates were identified by culture characteristics, standard biochemical procedures and the identification kit API 20E; that showed the typical profiles of *K. pneumoniae*. For biotyping, the API 20E system identified three different biochemical profiles (or index numbers) based on the differences in the ability of *K. pneumonia* isolates to utilize different carbon sources. The biochemical profiles were arbitrarily designated as B1, B2 and B3 that have three different profiles 5215773, 7215773, and 7214773, respectively, with biotype B1 (code number 5215773) was the most frequent biotype observed in 39 (91 %) of *K. pneumoniae* isolates (Table 2).

Antimicrobial susceptibility patterns and antibiogram-based phenotyping of *K. pneumoniae* isolates

The antimicrobial susceptibility patterns showed higher frequencies of resistance among *K. pneumoniae* isolates. All *K. pneumoniae* isolates (100 %) were resistant to each ampicillin, amoxicillin/clavulanic acid and cephazolin, in addition to 97.7 % of isolates was resistant to ertapenem and ceftriaxone, 95.3 % of isolates was resistant to trimethoprim-sulfamethoxazole and ceftazidim. Colistin showed lowest level of resistance 19 (44.2 %) isolates. Table.3 shows the antimicrobial susceptibility of *K. pneumoniae* isolates. All *K. pneumoniae* isolates (100 %) in this study were described as MDR as all isolates showed resistance to at least three or more classes of antimicrobial agents. Based on the antibiograms, the 43 *K. pneumoniae* isolates were grouped into 25 antibiogram types (antibiotypes), designated as A1 to A25, depending upon their resistance to the antimicrobial agents tested (Table.4).

PCR-based RAPD genotyping of *K. pneumoniae* isolates

K. pneumoniae isolates were analyzed by PCR-based RAPD fingerprinting technique using five primers (RAPD4, 640, 1247,

1252, 1290) with G/C contents ranging from 50 to 80 %. The primer 1290 containing a 60 % G/C content gave a good discriminatory result. Thus, based on the fingerprint clarity and discrimination obtained, primer 1290 was used for RAPD analysis of *K. pneumoniae* isolates throughout this study. RAPD-PCR amplifications with primer 1290 resulted in DNA fragments ranging from approximately 0.27 - 2.7 kbp. Notably, all DNA fragments were under 3 kbp, which is typical for RAPD profiles. Although many fragments appeared common to several isolates, the patterns were qualitatively sufficient for accurate isolates differentiation. The strains were considered to be within a pattern if the level of similarity was 70 % or more, thus the RAPD analysis revealed 39 distinct patterns (Figure 1B). The 39 RAPD genotypes were arbitrarily designated R1 to R39. Variant subtypes having a banding pattern similarity of 70 % or more were indicated by a letter suffix. Thus, R7 had three variant patterns (R7a, R7b, R7c), while R8 and R29 had two variant patterns (R8a, R8b and R29a and R29b, respectively).

GeneRuler 1 kb DNA Molecular weight marker (Thermo Scientific, USA). (B) Corresponding dendrogram generated with Jaccard's coefficient and the UPGMA clustering method.

Discriminatory power of typing methods

The Simpson's diversity index for biotyping, antibiogram typing and PCR-based RAPD typing methods were 0.17, 0.92 and 0.99, respectively, where RAPD profiles exhibited the highest discriminatory power. The discriminatory power of each typing method is shown in Table.5. Typing of all isolates included in this study is summarized in Table.6. *K. pneumoniae* is an important opportunistic pathogen causing serious hospital-acquired as well as community-acquired infections (Cryz *et al.*, 1984; Nordmann *et al*., 2009). Notably, patients with underlying malignancy presenting with bacteraemia are more likely to be infected with *K. pneumoniae*. This may be explained partly by *K. pneumoniae* virulence factors, such as capsule, pili, lipopolysaccharide and siderophore, which can give an adaptive advantage in patients with underlying malignancy and increase the potential for gastrointestinal translocation or biofilm formation in indwelling intravascular catheters; thus resulting in *K. pneumoniae* bacteraemia over other intestinal colonizers (Henao-Martínez *et al*., 2013). In a cohort study, underlying malignancy was identified in 63 % of *K. pneumoniae* nosocomial bacteraemia (Henao-Martínez *et al*., 2013).

The prevalence of infections caused by MDR *K. pneumoniae* has increased during the last decade, reflecting the selective pressure posed by the extensive and misuse of antimicrobial drugs. Thus, MDR *K. pneumoniae* is considered as an important health problem due to limited options for antimicrobial therapy resulting in higher morbidity and mortality rates (Correa *et al*., 2013; Hou *et al*., 2015). In the current study, the microbial diversity among MDR *K. pneumoniae* isolated from cancer patients was investigated using phenotypic and RAPD-based molecular typing methods for homology analyses. A total of 43 *K. pneumoniae* isolates, recovered from different clinical specimens, collected from cancer patients at National Cancer Institute, Cairo, Egypt, were included in the present study. For biotyping, the biochemical system profile number of the API 20E system (BioMerieux, France) clustered *K. pneumoniae* isolates in this study into three biotypes designated B1, B2, and B3 with three different biochemical profiles of 5215773, 7215773 and 7214773, respectively. The biotype B1 was the most

predominant one among isolates comprise 39 (90.7 %) isolates, followed by B2 in three (7 %) isolates and B3 represented by only one isolate (2.3 %). In another study, the biochemical profile 5215773 represented 37.5 % among *K. pneumoniae* isolates from blood, urine and sputum (Khattak and Fraise, 2011). In addition, the antibiogram typing based on antibiograms (susceptibility patterns to different antimicrobial agents tested) obtained in this study grouped the *K. pneumoniae* isolates into 25 antibiotypes (antibiograms) designated A1 to A25.

MDR bacteria are defined as the bacteria resistant to at least one agent in three or more classes of antimicrobial agents (Magiorakos *et al.*, 2012). Following this definition, all *K. pneumoniae* isolates included in this study were described as MDR as all 43 (100 %) isolates showed resistance to at least three or more classes of antimicrobial agents. *K. pneumoniae* is naturally resistant to ampicillins and early cephalosporins due to the production of the chromosomal mediated extended-spectrum β-lactamases (ESBLs) in this organism. However, the acquisition of resistance to amoxicillin/clavulanic acid and broad-spectrum cephalosporins has become a global phenomenon showing variable occurrence rates worldwide (Bouzenoune *et al*., 2009).

The antimicrobial susceptibility testing showed highest resistance frequency of 100 % among *K. pneumoniae* isolates to each ampicillin, amoxicillin/clavulanic acid and cephazolin, followed by resistance frequencies of 97.7 % and 95.3 % to ceftriaxone and ceftazidim, respectively, suggesting that these drugs are unreliable for the routine treatment of *K. pneumoniae* infections in this institution. Effective antimicrobial drugs, such as aminoglycosides, fluoroquinolones and carbapenems, have been used to treat ESBL-producing *K. pneumoniae* infections (Nordmann and Mammer 2007; He *et al*., 2015). Although, in the current study, *K. pneumoniae* isolates showed high resistance to gentamicin and amikacin of 69.8 % and 55.8 %, respectively. *K. pneumoniae* showed high resistance rates to the tested fluoroquinolones drugs ciprofloxacin and levofloxacin of 81.4 % and 65.1 %, respectively. *K. pneumoniae* had intrinsic sensitivity to fluoroquinolones which is commonly used for empirical treatment of urinary tract infections. Because of extensive use of fluoroquinolones as an alternative medication to treatment failure with other routine drugs, this might be responsible for the high non-susceptibility to quinolones in *K. pneumoniae* nowadays (Nordmann and Mammer 2007). Resistance rate to ciprofloxacin was 33 % in the Rabat region (Morocco) (Bouzenoune *et al.*, 2009). Although, carbapenems have been considered as last option treatments against infections caused by MDR Gram-negative organisms. *K. pneumoniae* has developed an efficient carbapenem resistance mechanism, known as KPC (*Klebsiella pneumoniae* carbapenemase) (Naas *et al.*, 2008). KPC enzyme-producing *K. pneumonia* is generally susceptible to few antimicrobial agents, and it is associated with a high mortality rate among patients with bloodstream infections (Vuotto *et al.*, 2014).

In this study, carbapenem drugs including imipenem and ertapenem, showed higher resistance rates of 74.4 % and 97.7 %, respectively. In this study, trimethoprim /sulfamethoxazole showed significant higher resistance rate of 95.3 %. Trimethoprim /sulfamethoxazole combination has been used extensively for the treatment of urinary tract infections (UTIs), particularly that caused by *K. pneumoniae* which led to higher resistance levels. The resistance

profile to trimethoprim/sulfamethoxazole was reported to be 61 % in Morocco (Bouzenoune *et al.*, 2009). With growing resistance of *Enterobacteriaceae* to the commonly used antimicrobial agents, nitrofurantoin has become increasingly important in the treatment of UTIs. They are known to have less potential for promoting resistance and therefore should be used preferentially.

Table.1 Nucleotide sequences of oligonucleotides used in RAPD-PCR analysis

Primer	Sequence 5' to 3'	GC (%)	Reference
RAPD4	AAGACGCCGT	60	Sachse *et al*. (2014)
640	CGTGGGGCCT	80	Eftekhar and Nouri (2015)
1247	AAGAGCCCGT	60	Samra *et al*. (2007)
1252	GCGGAAATAG	50	
1290	GTGGATGCGA	60	

Table.2 Biotypes of *K. pneumoniae* isolates based on API 20E analytical profiles.

Biotype	Code No.	No. of isolates (%)*
B1	5215773	39 (90.7)
B2	7215773	3 (7)
B3	7214773	1 (2.3)

*Percentage correlated to the total number of isolates.

Table.3 Frequency of the antimicrobial susceptibilities among *K. pneumoniae* isolates.

Antimicrobial Agent	Sensitive No. of isolates (%[1])	Resistant No. of isolates (%[2])
Colistin (CT)	24 (55.8)	19 (44.2)
Amikacin (AK)	19 (44.2)	24 (55.8)
Gentamycin (CN)	13 (30.2)	30 (69.8)
Ciprofloxacin (CIP)	8 (18.6)	35 (81.4)
Levofloxacin (LEV)	15 (34.9)	28 (65.1)
Tertracycline (TE)	11 (25.6)	32 (74.4)
Azithromycin (AZM)	6 (14)	37 (86)
Nitrofurantoin (F)	5 (11.6)	38 (88.4)
Trimethoprim/sulfamethoxazole (SXT)	2 (4.7)	41 (95.3)
Imipenem (IMP)	11 (25.6)	32 (74.4)
Ertapenem (ETP)	1 (2.3)	42 (97.7)
Ceftazidime (CAZ)	2 (4.7)	41 (95.3)
Ceftriaxone (CRO)	1 (2.3)	42 (97.7)
Ampicillin (AMP)	0 (0)	43 (100)
Amoxicillin/clavulanic acid (AMC)	0 (0)	43 (100)
Cephazolin (KZ)	0 (0)	43 (100)

[1,2]Percentages correlated to the total number of isolates.

Table.4 Antibiotypes of *K. pneumoniae* isolates based on antibiogram patterns.

Antibiotype	Antibiogram pattern	No. of isolates (%)*
A1	Resistant to all antimicrobial agent classes tested	10 (23)
A2	Resistant to all antimicrobial agent classes used except CT	7 (16)
A3	Resistant to all antimicrobial agent classes used except CN	1 (2)
A4	Resistant to all antimicrobial agent classes used except CT and AK	3 (7)
A5	Resistant to all antimicrobial agent classes used except CT and CN	1 (2)
A6	Resistant to all antimicrobial agent classes used except CT and TE	2 (5)
A7	Resistant to all antimicrobial agent classes used except CT and AZM	1 (2)
A8	Resistant to all antimicrobial agent classes used except CN, AK and LEV	1 (2)
A9	Resistant to all antimicrobial agent classes used except CN, LEV and IMP	1 (2)
A10	Resistant to all antimicrobial agent classes used except CT, AK, LEV and F	1 (2)
A11	Resistant to all antimicrobial agent classes used except CT, AK, TE and AMP	1 (2)
A12	Resistant to all antimicrobial agent classes used except CT, CN, AK and LEV	1 (2)
A13	Resistant to all antimicrobial agent classes used except CT, CN, AZM and F	1 (2)
A14	Resistant to all antimicrobial agent classes used except AK, TE, LEV, IMP, and CIP	1 (2)
A15	Resistant to all antimicrobial agent classes used except AK, TE, LEV, IMP, and F	1 (2)
A16	Resistant to all antimicrobial agent classes used except CN, AK, LEV, IMP, and CIP	1 (2)
A17	Resistant to all antimicrobial agent classes used except CT, CN, AK, TE, and AZM	1 (2)
A18	Resistant to all antimicrobial agent classes used except CN, AK, LEV, AZM, CAZ and SXT	1 (2)
A19	Resistant to all antimicrobial agent classes used except CT, AK, LEV, IMP, CIP, and F	1 (2)
A20	Resistant to all antimicrobial agent classes used except CN, AK, TE, LEV, IMP and CIP	1 (2)
A21	Resistant to all antimicrobial agent classes used except CT, AK, TE, LEV, IMP, and F	1 (2)
A22	Resistant to all antimicrobial agent classes used except CT, AK, TE, LEV, IMP and CIP	1 (2)
A23	Resistant to all antimicrobial agent classes used except CT, CN, AK, TE, LEV, IMP and CIP	1 (2)
A24	Resistant to all antimicrobial agent classes used except CT, CN, AK, LEV, IMP, CIP, AZM and ETP	1 (2)
A25	Sensitive to all antimicrobial agent classes used except AMP, ETP, F, CT, KZ and AMC	1 (2)

*Percentages correlated to the total number of isolates. AMP, ampicillin; CT, colistin; CN, gentamicin; AK, amikacin; TE, tetracycline; AZM, azithromycin; LEV, levofloxacin; IMP, imipenem; F, nitrofurantoin; AMP, ampicillin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CAZ, ceftazidime; ETP, ertapenem; AMC, Amoxacillin/clavulanic acid; KZ, cephazolin.

Table.5 Simpons's index of diversity for *K. pneumoniae* isolates in this study.

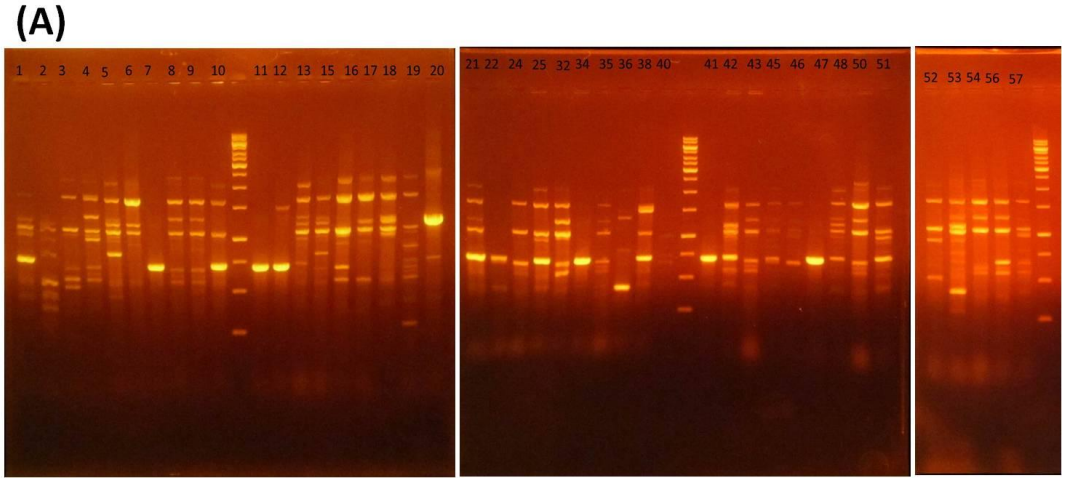
Typing method	No. of diverse types	Simpons's index of diversity
RAPD-PCR	39	0.9945
Antibiogram typing	25	0.9225
Biotyping	3	0.176

Table.6 Summary of biotyping, antibiogram typing (antibiotyping) and RAPD-PCR typing of *K. pneumoniae* isolates relative to source of specimens.

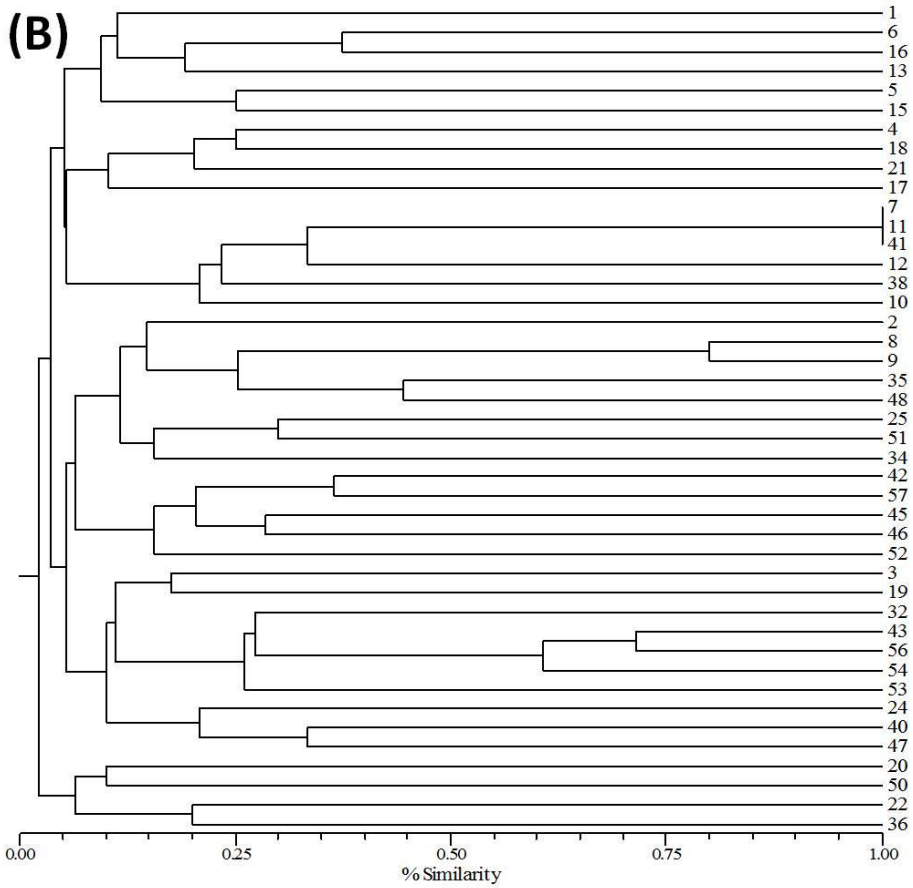
Specimen	No. of isolates	Phenotype		RAPD-based genotype (No.)*
		Biotype (No.)*	Antibiotype (No.)*	
Blood	27	B1 (24), B2 (2), B3 (1)	A1 (2), A2 (4), A3 (1), A4 (3), A5 (1), A6 (1), A9 (1), A10 (1), A12 (1), A13 (1), A14 (1), A15 (1), A16 (1), A17 (1), A18 (1), A19 (1), A22 (1) A23, (1), A24 (1), A25 (1)	R4 (1), R5 (1), R6 (1), R7a (1), R7c (1), R8a (1), R8b (1), R21 (1), R22 (1), R23 (1), R24 (1), R25 (1), R26 (1), R27 (1), R28 (1), R29a (1), R29b (1), R30 (1), R31 (1), R32 (1), R33 (1), R34 (1), R35 (1), R36 (1), R37 (1), R38 (1), R39 (1)
Pus	10	B1 (9), B2 (1)	A1 (4), A6 (1), A7 (1), A8 (1), A11 (1), A20 (1), A21 (1)	R1 (1), R2 (1), R3 (1), R7b (1), R9 (1), R10 (1), R11 (1), R12 (1), R13 (1), R15 (1)
Sputum	4	B1 (4)	A1 (2), A2 (4)	R16 (1), R17 (1), R18 (1), R19 (1)
Stool	1	B1 (1)	A2 (1)	R14 (1)
Urine	1	B1 (1)	A1 (1)	R20 (1)

*****No. of isolates represent each type.

Fig.1 PCR-based RADP RAPD patterns of *K. pneumoniae* isolates with primer 1290. (A) Agarose gel (1 %) electrophoresis of amplification products; GeneRuler 1 kb DNA Molecular weight marker (Thermo Scientific, USA).



(B) Corresponding dendrogram generated with Jaccard's coefficient and the UPGMA clustering method.



However, the susceptibility of *K. pneumoniae* isolates in this study to nitrofurantoin was low (11.6 %). Although tetracyclines have decreased susceptibility to develop resistance (Pieboji *et al.*, 2004), in our study 74.4 % of our isolates were non-susceptible to tetracyclines. These findings of increasing the resistance profiles to tested antimicrobials suggested that these antimicrobial agents may not be appropriate for initiation of empirical therapy of infections caused by *K. pneumoniae* in this institution of study and/or in a developing country like Egypt. However, the highest susceptibility profile was shown in this study to colistin as 55.8 % of isolates were sensitive, which could be attributed to the limited use of this antimicrobial agent and may suggest using it in the empirical therapy for *K. pneumoniae* infections.

In the current study, the genetic diversity of *K. pneumoniae* isolates with similar biotypes and/or multidrug resistance profiles was investigated using arbitrarily primed RAPD analysis. The RAPD patterns obtained was clustered by dendrogram generated with Jaccard's coefficient and the UPGMA clustering method. PCR-based RAPD fingerprinting of the 43 *K. pneumoniae* isolates revealed a significant molecular heterogeneity of *K. pneumoniae* isolated within this hospital indicated by 39 different RAPD-based groups designated R1 to R39

and/or 43 different patterns were observed among isolates. That demonstrates the high discriminatory power of RAPD with the primer 1290. Fortunately, the existence of all isolates within distinguished RAPD patterns indicated that there was no occurrence of bacterial spread among patients. In addition, pathogenic *K. pneumoniae* isolated from the institute comprise a genetically variable group of organisms. These results are consistent with Lai *et al*., (2000) observation that pathogenic *K. pneumoniae* population is highly heterogeneous, based on the distribution of different nucleotide sequences.

The biochemical profiles and antibiograms are usually not reliable to show microbial diversity and more discriminatory epidemiological marker, such as molecular methods, should be used for microbial typing (Limansky *et al*., 2004). In the current study, the Simpons's index of diversity showed a higher discriminatory power of PCR-based RAPD (0.995) over antibiogram typing (0.922) and biotyping (0.176). Although, the antibiogram typing showed an outstanding discriminatory power comparable to that of PCR-based RAPD; suggesting the possibility of combining the two methods for detection of microbial diversity of *K. pneumoniae* isolates. The epidemiological investigation based on API-based biotyping may not accurately predict relatedness of the strains as shown in the present study. Although, both biotyping and antibiogram typing of *K. pneumoniae* isolates could be well for preliminary screening of strain relatedness.

In conclusion, in this study, MDR *K. pneumoniae* is becoming a serious problem in cancer patients due to limited choice for treatment. The epidemiological typing of 43 MDR *K. pneumoniae* clinical isolates was performed using phenotypic and molecular typing methods. *K. pneumoniae* infections in this institute were caused by a variety of bacterial genotypes and/or phenotypes. However, RAPD clearly prevailed among the other typing methods (biotyping, antibiotyping) and proved to be a useful technique in distinguishing related and unrelated *K. pneumoniae* clinical isolates. Continuous studies should be carried out to investigate the antimicrobial resistance and to try the use of antimicrobial combinations to overcome these resistances. We propose that infection control measures and strict antimicrobial stewardship policies should be applied to reduce the selective pressure that favours the emergence and epidemic of MDR bacteria.

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How to cite this article:

Mahmoud M. Tawfick, Samira M. Hamed, Hadeel M. Darwich and Hadir A. El-Mahallawy. 2016. Phenotypic and Genotypic Diversity of Nosocomial Multi-Drug Resistant *Klebsiella pneumoniae* Isolated from Cancer Patients in Cairo, Egypt. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 931-943. doi: http://dx.doi.org/10.20546/ijcmas.2016.507.105