Microbes and Infectious Diseases 2023; 4(1): 168-181

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Microbiological profile of blood stream infection: Genetic relatedness of carbapenemase producing *Klebsiella pneumoniae* isolates using ERIC PCR

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ARTICLE INFO

Article history:
Received 16 November 2022
Received in revised form 15 December 2022
Accepted 19 December 2022

Keywords:
BSIs
K. pneumoniae
ERIC PCR
Carbapenem resistant Gram-negative bacilli

ABSTRACT

Background: There has been an expansion in antibiotic resistance among *Enterobacteriaceae* over the previous ten years raising serious questions about global health. **Our objectives** were to isolate the carbapenem-resistant Gram-negative bacilli that cause blood stream infections and investigate the genetic relatedness between carbapenemase producing *Klebsiella pneumoniae (K. pneumoniae)* isolates. **Methods**: A total of 255 blood specimens were withdrawn into blood culture bottles and incubated, pathogenic microorganisms were identified, antibiotic susceptibility was done for Gram-negative isolates. Carbapenemase producers were screened for molecular detection of carbapenemase and extended spectrum β-lactamase genes. **Results:** One hundred and thirty-three (133) pathogens were isolated, of these 77 Gram-negative isolates (including 32 *K. pneumoniae*,18 *E. coli*, 12 *Acinetobacter*, 9 *Pseudomonas* and 6 *Enterobacter cloacae).* Out of them, 55 were carbapenem resistant by phenotypic method. 23/55 of them were harboring carbapenemase genes by PCR ERIC, results showed that Anesthesia intensive care unit (ICU) isolates showed >90% genetic similarity while isolates from Chest and Internal medicine ICUs showed <70% similarity. **Conclusion:** Increased awareness, persistent observation, and strict adherence to the recommendations for antibiotic stewardship should be used to limit spread of carbapenem resistant Gram-negative bacilli.

Introduction

Over the past ten years, the global concern over antibiotic resistance among *Enterobacteriaceae* has intensified [1]. Extended spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-PE) are responsible for 19% of hospital-acquired infections (HAIs), according to the US Centers for Disease Control and Prevention's 2013 report [2]. The percentage of patients who die from blood stream infections (BSIs) caused by ESBL-PE is 57% greater than for patients with BSIs caused by bacteria that don't produce ESBL [3].

Carbapenems are regarded as the preferred treatment for BSIs brought on by ESBL-PE due to their strong antibacterial activity and decreased toxicity [4,5].

DOI: 10.21608/MID.2022.175107.1414
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Unfortunately, increased carbapenem use has caused Gram-negative bacteria like *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. to develop carbapenem resistance (CR), along with evolution of pathogens carrying up to three separate carbapenemase genes, which might spread both inside and outside hospitals, resulting in a serious global health hazard [6,7].

The rapid spread of carbapenem resistance has produced a problem with global public health because of the lack of innovative antibiotics that may be used as a last treatment option [8,9].

Rapid detection of genes correlated with antimicrobial resistance could aid in monitoring the appropriate use of antibiotics, enhancing patient outcomes, and promoting antibiotic stewardship. This could reduce hospitalization costs, morbidity, and death of hospitalized patients [10]. Multiplex polymerase chain reaction (PCR) is affordable, ensures the detection of numerous genes in a single reaction, and verifies the coexistence of numerous genes in a single isolate while conventional detection methods take time and don't adequately characterize the pattern of drug resistance [11,12].

Exact and rapid detection of clonal relatedness or variabilities among resistant bacteria is important to enhance their management and stop their spread in hospitals [13,14]. Numerous significant genotypic methods have been developed such as microarray technology, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and ERIC -PCR (enterobacterial repetitive intergenic consensus- PCR) [15].

Even though PFGE is the preferred subtyping technique for determining how closely related bacterial strains from various sources are, it is labor and time consuming [16]. Whereas, DNA-based fingerprinting method, such as ERIC, is simple to use, quick, and sensitive for distinguishing closely related types [17].

The incidence of Gram-negative bacilli that are resistant to carbapenem has noticeably increased at Tanta University hospitals, Egypt. These bacteria were derived from different patient specimens. Accordingly, this study's main goals were to isolate the Gram-negative bacilli that cause BSIs that are carbapenem-resistant and to employ ERIC PCR DNA fingerprinting to examine the relationships between these strains.

Materials and methods

Study design and setting

A cross-sectional study was conducted at the Microbiology department, Clinical pathology department and different intensive care units (ICUs) of Tanta university hospitals - a tertiary care hospital in Egypt - during a year from May 2021 to May 2022. All patients with suspected BSI (organism cultured from blood that is not related to any infection at another site of the body) in ICUs were enrolled in the study. From patient's records, demographic information, clinical information, and admission date were gathered.

Inclusion criteria:

- Patients having clinical signs of HAIs (infections that developed after 48 hours of admission).
- Patients show poor response to antibiotic treatment.

Exclusion criteria:

- Patients having infections that developed less than 48 hours of admission (community-acquired infections).

Ethical considerations

The ethical committee of Tanta University - Faculty of Medicine, Egypt has approved this investigation (No: 35712/9/2022). All enrolled subjects in the study provided signed, informed consent.

Samples collection

Before starting antibiotic therapy, all feverish patients had their peripheral veins completely aseptically sampled twice for blood cultures. If applicable, additional blood cultures were taken from the central venous catheter for a particular set of cases. The blood samples were taken out of the tubes and placed in BACT/ALERT blood culture bottles to be incubated in the BACT/ALERT system (BioMerieux, France). Blood cultures that show positive results were exposed to conventional laboratory methods for pathogens identification through subculture on MacConkey, blood, chocolate aga (Oxoid UK) followed by a 24-hour incubation period at 5 to 10% CO2, 37°C, and verification using the VITEK-2 system (BioMerieux, France).

Antimicrobial susceptibility testing

In accordance with the Clinical and Laboratory Standards Institute's (CLSI) recommendations, the test for antibiotic susceptibility was performed using the Kirby Bauer modified disc diffusion method [18]. The following antibiotic discs (Oxoid UK)

were utilized: ampicillin/sulbactam (10/10 µg), amoxicillin / clavulanic acid (20/10 µg), piperacillin/ tazobactam (100/10 µg), ceftazidime (30 µg), cefepime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), imipenem (10 µg), meropenem (10 µg), trimethoprim-sulfamethoxazole (25µg), gentamicin (10 µg), amikacin (30 µg), tigecycline (15 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg). The antibiotic discs were placed on top of each bacterial isolate after it had been prepared using a 0.5 McFarland density suspension and swabbed onto Muller-Hinton agar. This was then incubated at 37°C, for 18 to 24 hours.

Phenotypic detection of carbapenemase activity

According to CLSI guidelines 2020, bacterial isolates with intermediate or high resistance to imipenem or meropenem were identified as possible carbapenemase producers and subsequently screened using the Modified Carbapenem Inactivation Method (mCIM). A 2 ml trypticase soy broth (TSB) suspension of the tested isolate was placed within a meropenem disc, and it was then incubated at 37 °C for 4 hours. The disc was then placed in the center of an (Oxoid UK) Mueller-Hinton agar plate that had already been tipped with *E. coli* ATCC® 25922 inoculum. The inhibition zone surrounding the disc was assessed after an overnight incubation. Carbapenemase producers were defined as isolates with a zone diameter of 6 to 15 mm or those with pinpoint colonies inside a 16 to 18 mm zone.

Detection of carbapenemase and extended spectrum β-lactamase genes using molecular techniques

The genes tested were Oxacillinase-48 (OXA-48), New Delhi metallo-beta-lactamase-1 (NDM-1), and *Klebsiella pneumoniae* carbapenemase (KPC). From the ESBL genes bla_{TEM} and bla_{SHV} were examined.

DNA extraction

Mini kit from Qiagen (Germany, GmbH) was used for DNA extraction from the samples in accordance with the manufacturer's instructions, the eluted DNA was stored in sterile Eppendorf tubes at -20ºC until amplification.

Multiplex PCR assay

Two separate multiplex PCRs were used. The tested carbapenemase genes were detected using multiplex PCR assays according to **Poirel et al.** [19]. The PCR system cycler from Creacon (Holland, Inc.) used the following program for amplification: initial denaturation at 94 °C for 5 min, then 30 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min.

The ESBL genes were detected using multiplex PCR assays according to **Colom et al.** [20]. The PCR program for amplification was as follows: initial denaturation at 94 °C for 5 min, followed by 32 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 54 °C, extension at 72 °C for 1min, and a final extension at 72 °C for 10 minutes. The list of primers used is given in **table (1)** [21].

Agarose gel electrophoresis and detection of amplification products

After doing PCR, 7 µl of the products were removed and electrophoresed in 1 % agarose gel stained with ethidium bromide and images were taken with UV illumination.

Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) for typing of carbapenemase producing *K. pneumoniae* isolates

PCR amplification

The process was carried out in an Applied biosystem 2720 thermal cycler. **Table 2** summarizes the cycling parameters [22].

Examining the ERIC- PCR products

The products were separated using 5V/cm gradient electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH). To measure the DNA fragment size, 100-3000 bp DNA ladder from Genedirex (Taiwan) was used. The gel was photographed computer software was used to analyze the information.

Fingerprints of ERIC-PCR

Depending on the bands pattern, the ERIC fingerprinting data were translated into a binary code. The Ward's hierarchical clustering procedure and the unweighted pair group technique with arithmetic average (UPGMA) were used to create dendrograms. Using SPSS, version 22, we conducted a cluster analysis and created a dendrogram (IBM 2013) [23].

Statistical analysis

With the help of the (SPSS) 26 programs, all data were examined. While qualitative factors were given as percentages and figures, the results for quantitative variables were presented as mean SD.

Results

Patient characteristics & distribution of isolates from different ICUs

The 255 individuals who were admitted to the adult ICUs with suspected BSIs resulted in the isolation of a total of 133 microorganisms. Of these 77 (57.8%) Gram-negative isolates (including 32 *K. pneumoniae*, 18 *E. coli*, 12 *Acinetobacter*, 9 *pseudomonas* and 6 *Enterobacter cloacae*). Other found isolates were 45 (33.8%) Gram-positive bacteria & 11 (8.2%) fungal infection.

The 77 non duplicate Gram-negative isolates were retrieved from diverse ICUs. Isolates were mostly recovered from Anesthesia ICU 38 (49.3%) followed by Neuropsychiatry ICU 21 (27.2%), then Chest ICU 12(15.5%). The least isolates were isolated from Internal medicine ICU 6 (7.7%). Demographic characteristics of the patients infected with Gram-negative organisms are shown in **table (3)**, regarding age, gender and included ICUs.

Phenotypic detection of ESBL & carbapenem resistance

Regarding disc diffusion method, out of 77 Gram-negative isolates, 61 were ESBL, while 55 were carbapenem resistant (CR), CR was detected as follows (25/32) *K. pneumoniae* isolates (75.7%), 11/18 *E. coli* isolates (61.1%), *Acinetobacter* & *Pseudomonas* showed 10/12 (84.3%), 9/9 (100%) resistance respectively, while all *Enterobacter cloacae* isolates were 100% sensitive.

Regarding Modified Carbapenem Inactivation Method (mCIM), (52/77) Gram-negative isolates were carbapenemase producer as follows (24/33 *K. pneumoniae* isolates (72.7%), 10/18 *E. coli* isolates (55.5%), *Acinetobacter* & *Pseudomonas* showed 10/12 (84.3%), 8/9 (88.8%) resistance respectively.

Genotypic detection of ESBL and carbapenemase genes by multiplex PCR

The Gram-negative isolates were tested for detection of carbapenemase genes and ESBL genes by multiplex PCR assay, the occurrence of the assessed genes was 23/55 (41.8%) of the genomic DNA extracts harboring carbapenemase genes. The distribution of carbapenemase genes were as follows: *K. pneumoniae* (10 bla_{NDM},6 bla_{OXA-48} & 5 bla_{KPC}); *E. coli* (3 bla_{NDM} ,1 bla_{KPC} &1 bla_{OXA-48}); *A. baumannii* (3 bla_{OXA-48} & 2 bla_{NDM} positives) & *P. aeruginosa* (2 bla_{KPC} and 1 bla_{OXA-48}) **(Figure 1, Table 4).**

The result of ESBL genes shows that 96.4% (53/55) of the phenotypic CR isolates co-harboured both TEM-1 and SHV-1 genes. However, only the TEM-1 gene was present in two of the isolates (one *E. coli* and one *Acinetobacter*). Both ESBL genes (TEM-1 and SHV-1) were present in each of the 23 carbapenemase-positive gene bearing isolates **(Figure 2).**

ERIC-PCR Analysis

The ERIC-PCR classified the 10 tested *K. pneumoniae* isolates into two branches (BI and BII) and three clades (C 1-3) with a total of 8 distinct ERIC genotypes (E 1-8). The band patterns ranged from 2 to 5 bands with a size range from 200 to 2000 bp, and a discrimination index of 0.93. Isolates from the same source clustered together (i.e., Neuropsychiatry ICU isolates in C1, Anesthesia ICU isolates in C2, and Chest ICU isolates in C3). This particularly clear for Anesthesia ICU isolates which showed >90% genetic similarity. Three of these isolates belonged to the same ERIC genotype (E3) and two of them shared identical antibiotic resistance genetic profile. Isolates from Chest and Internal medicine ICUs belonged to BI showed <70% similarity with isolates from Anesthesia and Neuropsychiatry ICUs (belonged to BII) **(Figure 3,4).**

Table 1. Sequences of the primers and products of amplification.

Gene	Primer sequence	Amplicon size (bp)	Reference
bla **OXA-48**	**F:** 5′ GCGTGGTTAAGGATGAACAC 3′ **R:** 5′ CATCAAGTTCAACCCAACCG 3′	438	[19]
bla **KPC**	**F:** 5′ CGTCTAGTTCTGCTGTCTTG 3′ **R:** 5′ CTTGTCATCCTTGTTAGGCG 3′	798	[19]
bla **NDM**	**F:** 5′ GGTTTGGCGATCTGGTTTTC 3′ **R:** 5′ CGGAATGGCTCATCACGATC3	621	[19]
bla **TEM**	**F:** 5′-TCAACATTTCCGTGTCG-3′ **R:** 5′-CTGACAGTTACCAATGCTTA-3′	860	[21]
bla **SHV**	**F:**5′-ATGCGTTATATTCGCCTGTG-3′ **R:** 5′AGATAAATCACCACAATGCGC-3′	780	[21]

F:forward, R: reverse

Table 2. Primers sequences, target genes, amplicon sizes and cycling conditions

Target	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
ERIC	F:5′AAGTAAGTGACTGGGGTGAGCG3′ R: 5′ATGTAAGCTCCTGGGGATTCAC3′	Variable	94˚C 5 min.	94˚C 30 sec.	52˚C 1 min.	72˚C 1 min.	72˚C 10 min.	[22]

F:forward, R: reverse; ERIC, enterobacterial repetitive intergenic consensus min, minutes; sec, seconds

Table 3. Descriptive data of patients infected by isolated Gram-negative organisms.

Data		Gram-negative organisms N = 77
Age	Range (years)	18 -75
	Mean (±SD)	33.46 (±19.4)
Gender	Male	45 (58.4%)
	Female	32 (41.5%)
ICU types	Anesthesia	38 (49.3%)
	Neuropsychiatry	21 (27.2%),
	Chest	12 (15.5%)
	Internal medicine	6 (7.7%)
Organisms	*Klebsiella pneumoniae*	32 (41.5%)
	Escherichia coli	18 (23.4%)
	Acinetobacter baumannii	12 (15.6%)
	Pseudomonas aeruginosa	9 (11.7%)
	Enterobacter cloacae	6 (7.8%)

N, Numbe ; SD, standard deviation; ICU, intensive care unit ; Data presented as n(%)

Table 4. Frequency of carbapenemase genes detected in CR Gram-negative isolates.

	PCR results				
Carbapenemase genes positive (Total N=23)	**N**	***K. pneumoniae* N= 10/25**	***E. coli* N=5/11**	***Acinetobacter* N=5/10**	***Pseudomonas* N= 3/9**
bla$_{KPC}$	10	5	3	0	2
bla$_{NDM}$	13	10	1	2	0
bla$_{OXA-48}$	11	6	1	3	1
Both **bla**$_{OXA-48}$ + **bla**$_{NDM}$	5	5	0	0	0
Both **bla**$_{KPC}$ + **bla**$_{NDM}$	4	4	0	0	0
Both **bla**$_{OXA-48}$ + **bla**$_{NDM+KPC}$	1	1	0	0	0
Carbapenemase genes negative (Total N=32)		15	6	5	6

CR, carbapenem resistance; PCR, polymerase chain reaction; *E. coli, Escherichia coli; K. pneumoniae, Klebsiella pneumonia*; N,number

Figure 1. Agarose gel electrophoresis of multiplex PCR for carbapenemase genes detection including *bla*$_{\text{oxa-48}}$ (438bp), *bla*$_{\text{NDM-1}}$(621bp), and *bla*$_{\text{KPC}}$(798bp), lane 1 DNA ladder100:1000bp, lane 2, 3, 4: +ve for *bla*$_{\text{KPC}}$ and *bla*$_{\text{NDM}}$, lane 5 positive for *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$ and *bla*$_{\text{oxa-48}}$, lane 6 positive for *bla*$_{\text{NDM}}$ and *bla*$_{\text{oxa-48}}$

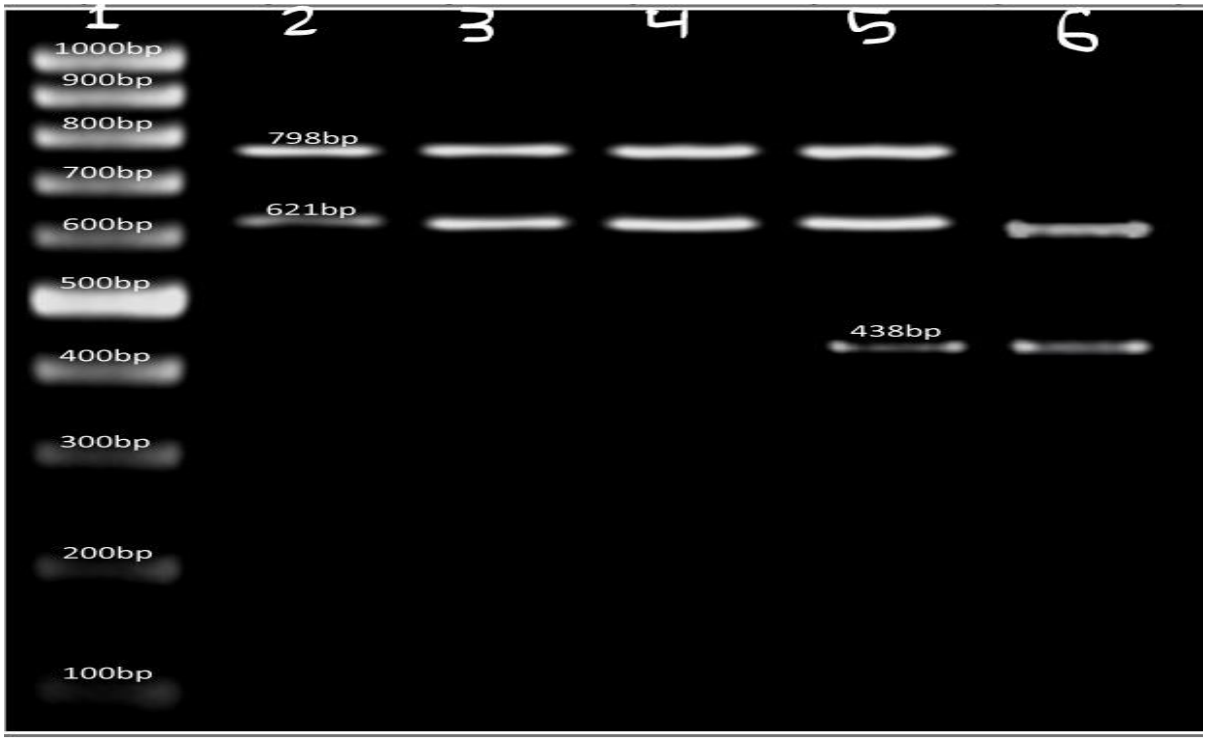


Figure 2. Agarose gel electrophoresis of multiplex PCR for ESBLs genes detection including *bla*$_{\text{TEM}}$(860bp) and *bla*$_{\text{SHV}}$(780 bp), lane 1 bp DNA ladder100:1000bp, lane 2,3,4,5,6: +ve for *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$

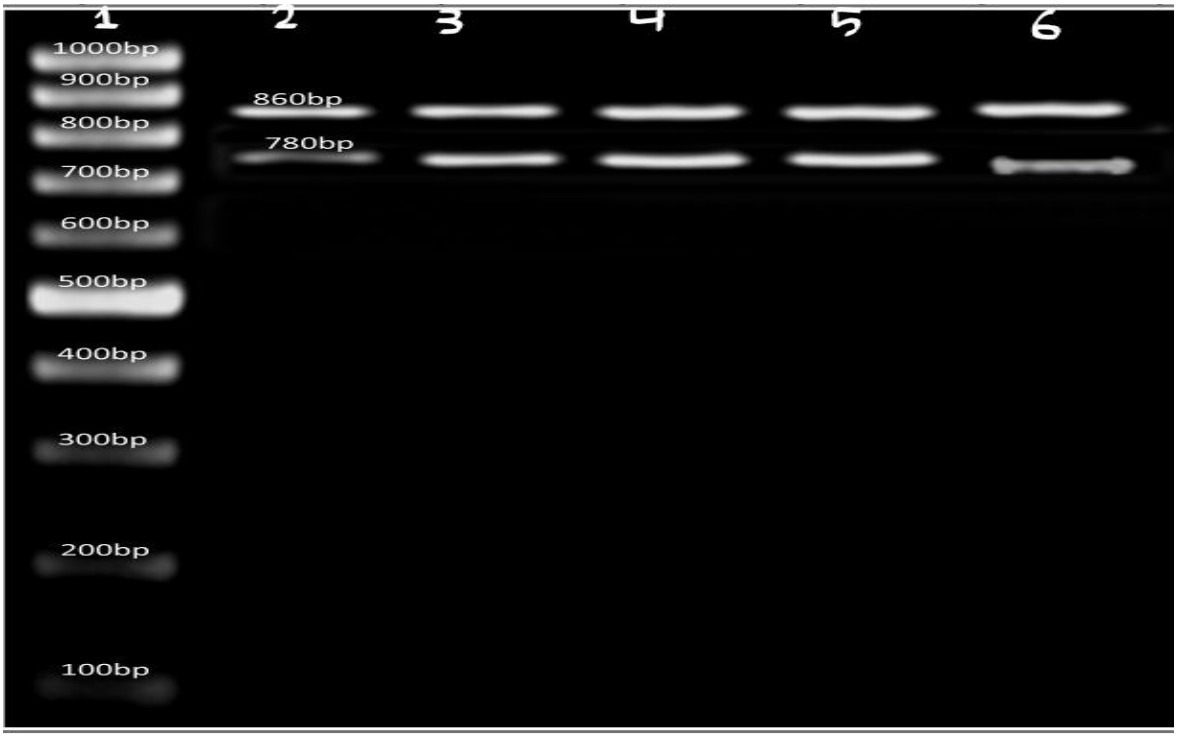


Figure 3. DNA fingerprinting of ERIC-PCR of *K. pneumoniae* isolates. Note: L: lane 100:3000 bp DNA ladder; lane 1 to 10: *K. pneumoniae* isolates.

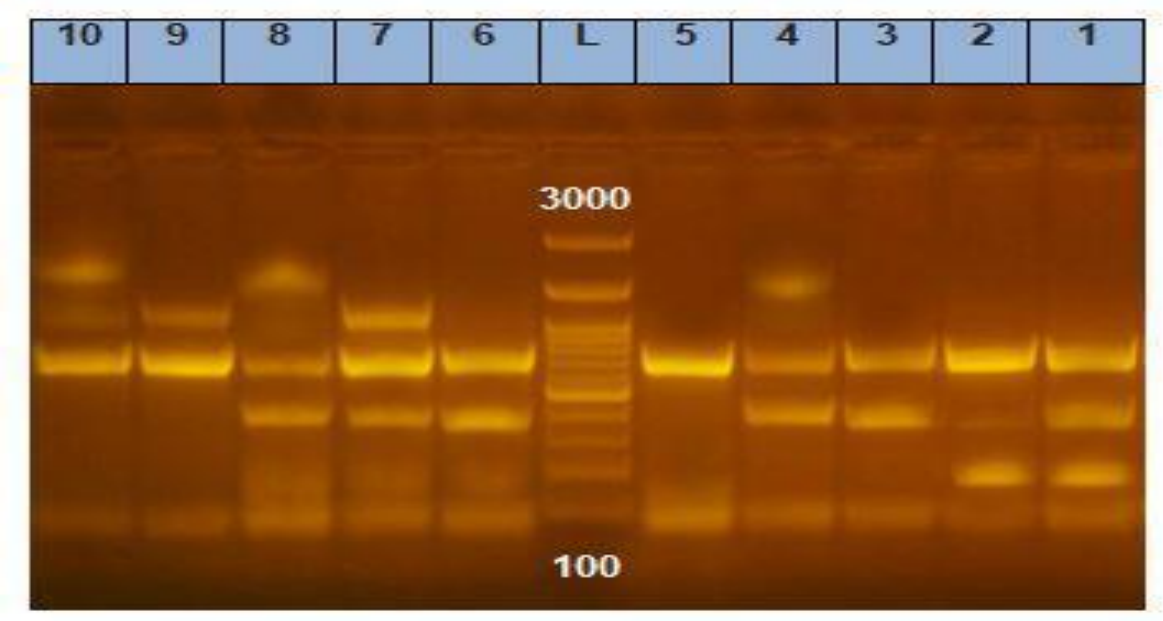
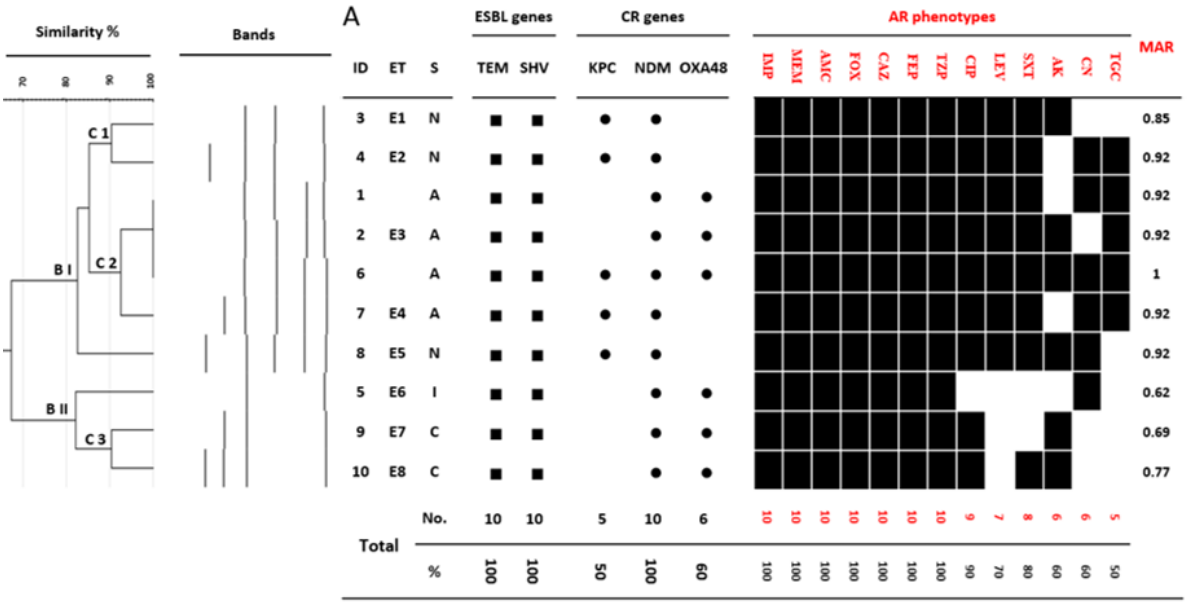


Figure 4. ERIC typing dendrogram of *K. pneumoniae* isolates and their associated genetic and phenotypic antimicrobial resistance patterns. The 10 tested isolates were classified into two branches (BI and BII) and three clades (C 1-3) with a total of 8 distinct ERIC genotypes (ET: ERIC type 1-8).



S: speciality; N: neuropsychiatry; A: anaesthesia ; I: internal medicine ; C: chest ;black dot: positive for antibiotic resistance gene; black square: positive for antibiotic resistance phenotype; MAR: antimicrobial resistance; TGC: tigecycline; CN: gentamicin; AK: amikacin; SXT: trimethoprim-sulfamethoxazole; LEV: levofloxacin; CIP: ciprofloxacin; TZP: piperacillin/ tazobactam; FEB: cefepime; CAZ: ceftazidime; FOX: cefoxitin; AMC: amoxicillin-clavulanic acid; MEM: meropenem; IMP: imipenem

Discussion

Among the most common infections in critically ill ICUs cases are bloodstream infections with a case-fatality rate ranging from 35% to 50% [24]. Rapid detection of the causative pathogen and the source of infection enables prompt therapy with the appropriate antibiotic to enhance patient outcomes, shorten hospital stays, and reduce associated medical expenses. Therefore, the goals of this study were to detect the carbapenem-resistant Gram-negative bacilli that cause BSIs and to explore the relationship between *K. pneumoniae* strains

which harbor greater number of carbapenemase genes using ERIC-PCR DNA fingerprinting.

In this research, the enrolled cases of Gram-negative BSIs presented a mean age of 33.46 (±19.4) and were commonly males 58.4% in comparison to females 41.5%. These results are concordant with those of **Leal et al., Amanati et al.,** and **Onorato et al.** who reported the male gender predominated among BSI patients [25-27]. In contrast to our results **Cortés Ortíz et al.** detected 44.04% of isolates from male patients and 55.95% of isolates from female patients [28]. Also, **Kumalo et al.** informed that females are more likely to have BSI than males [29].

In the current study, Gram-negative isolates were the most prevalent pathogens of BSIs accounting for 57.5%. which was more or less consistent with **Amanati et al.** [26] and **Vincent et al.** [30] who notified that Gram-negative etiology in BSI is conveyed to be about 50% and 63.3% respectively .While **wu et al.** described that Gram-negative BSIs were 40.84% [31].

Moreover, the preponderance of Gram-negative isolates has been documented in previous studies [32-35]. On the other hand, **Banik et al.** found that BSIs are primarily caused by Gram-positive cocci 60.37%, Gram-negative bacilli 36.29% and yeasts 3.33% [36].

In the current study, *K. pneumoniae* 41.5% were the predominant Gram-negative isolate in cases of BSIs followed by *E. coli* 23.4%. These results align with those of other studies [31,37]. Additionally, it was noted that CR-*K. pneumoniae* predominated in cases of BSIs [38,39].

In contrast to our results, **Amanati et al.** [26] and **Zhu et al. [**40] found that *E. coli* was the main pathogen in BSIs. Furthermore, **Ergönül et al.** [41] and **Qu et al.** [42] reported that *A. baumannii* was the most frequently isolated microorganism 31% and 58.6% respectively. Also, **Diekema et al.** reported that *Staphylococcus aureus* 20.7%, followed by *E. coli* 20.5% were the predominant pathogen in BSIs [43].

This discrepancy may be explained by the microorganism prevalence in cases of BSIs and their susceptibility patterns change over time both geographically and even within the same hospital [36].

In this report, most of the Gram-negative organisms were isolated from Anesthesia ICU 38 (49.3%) followed by Neuropsychiatry ICU 21 (27.2%), then Chest ICU 12(15.5%). The least isolates were isolated from Internal medicine ICU 6 (7.7%). Similarly, **Gandor et al.** found that 37% of Gram-negative isolates were from Anesthesia ICU [44]. According to **Negm et al.** report, most Gram-negative isolates (20.46%) were found in the Emergency ICU**,** followed by Surgical ICU (17.58%), The least isolates were isolated from Pulmonary ICU (0.69%), Coronary care unit (0.58%), and Cardiothoracic ICU (0.18%) [45].

Regarding carbapenem resistance, the current study found that 71.4% of Gram-negative isolates were carbapenem resistant by disc diffusion method and 67.5% were carbapenemase producers phenotypically detected by Modified Carbapenem Inactivation method among them *K. pneumoniae* were the most predominant**.** According to research carried out by **Villegas et al.** *K. pneumoniae*, was the most frequently identified carbapenemase producer, these results coincide with our findings [46].

Carbapenemase producing *K. pneumoniae* were 100% resistant to piperacillin/tazobactam, ceftazidime and cefepime, Moreover, they were extremely resistant to ciprofloxacin 90%, sulfamethoxazole/trimethoprim 80%, levofloxacin 70%, aminoglycosides (gentamicin, amikacin 60%) while the least resistance was to tigecycline 50 %. Our results were in line with **Gandor et al.** [44].

Concerning the carbapenemase genetic determinants, 41.8% of the genomic DNA extracts were harboring carbapenemase genes. For the ESBL, 96.4% (53/55) of the phenotypic CR isolates were positive for TEM-1 and SHV-1 genes. The two ESBL genes were present in each of the isolates that were carbapenemase-positive. While in **Codjoe et al.** study, carbapenemase genes were detected in 23.4% (26/111) of the genomic DNA extracts [47]. Additionally, 96.4% of the CR isolates co-harbored both TEM-1 and SHV-1 genes. Also, each of the 26 isolates that carried the carbapenemase-positive gene also carried the ESBL genes in agreement with our results.

According to our study, bla_{NDM} was the most frequently detected gene, followed by $bla_{OXA\text{-}48\text{-}}$ and bla_{KPC}. These results are going along with results reported by **Gandor et al.** and **Nabarro et al.** [44,48]. However**,** our findings were inconsistent with **Leal et al.** [25] and **Onorato et al.** [27] who reported that the bla_{KPC} was the most frequently

detected gene while *bla$_{NDM}$* were the least frequently noted.

In the current study, both ***bla$_{KPC}$*** and ***bla$_{NDM}$*** were coharbored in five *K. pneumonaie* isolates. while, both ***bla$_{NDM}$*** & ***bla$_{OXA-48}$*** were found coincidentally in six *K. pneumonaie* isolates. The results closely match those obtained by **Gandor et al., Emira et al.** and **El-Domany et al.** who found that many isolates contained multiple carbapenemase-encoding genes [44,49,50].

Unexpectedly, the three carbapenemase-encoding genes (***bla$_{NDM}$***, ***bla$_{KPC}$***, and ***bla$_{OXA-48}$***), were coharbored in one *K. pneumonaie* isolate. The unusual presence of the three carbapenemase-encoding genes (***bla$_{NDM}$***, ***bla$_{KPC}$***, and ***bla$_{OXA-48}$***) in one isolate also reported in a recent study in Egypt [44].

The ICU is one of the hospital sectors with the uppermost occurrence of hospital outbreaks [51]. The main sources of pathogens are cross-contamination by staff and the use of contaminated equipment [52].

Hospital outbreaks caused by carbapenem resistant *K. pneumoniae* have occurred in several different nations, placed a significant financial burden on hospitals and healthcare facilities due to the cost of containing their spread, necessitating the early identification of carbapenemases in infected individuals and/or carriers to stop outbreaks from occurring [53].

In the present study. ERIC-PCR genotyping showed genetic diversity between K. pneumoniae isolates from different sources. This was in line with previous reports in Egypt [54,55], and elsewhere [56]. However, isolates from anesthesia ICU showed high genetic similarity which was in line with another report in Mexico [28]. These findings suggest clonal dissemination of these isolates between patients in the Anesthesia ICU and may highlight improper hygienic practices within the unit. So, we need strong adherence to standard precautions including better compliance with hand hygiene and the use of personal protective equipment.

Conclusion

This study demonstrates that Gram-negative bacilli causing blood steam infections have become widely dispersed and resistant to carbapenems, one of the antibiotic groups used as a final resort, in our tertiary care hospital, Tanta, Egypt. This kind of resistance spread horizontally to different bacteria, posing restrictions and difficulties in the ability to cure bacterial illnesses. Therefore, in cases of significant illness, high caution should be practiced when prescribing carbapenems to be limited only to strict indications. Also, heightened attention, ongoing observation, and stringent execution of antibiotic stewardship guidelines should be applied.

Human rights

All procedures adhered to the ethical requirements of the institutional and national competent committee on human testing as well as the tenets of the Declaration of Helsinki (1975), as amended in (2013).

Funding: None.

Conflict of interest

The authors declare that they have no financial or other conflicts of interest.

Authors' contribution

Conceptualization: Marwa Taha & Eman Hegazy; **investigation and methodology**: Marwa Taha, Eman Hegazy & Sarah Shouib; **resources:** Marwa Taha, Eman Hegazy & Sarah Shouib; **supervision:** Marwa Taha, Eman Hegazy & Sarah Shouib; **writing – original draft:** Marwa Taha; **writing – review and editing:** Marwa Taha, Eman Hegazy, Sarah Shouib, Walid ELmonir & Shaimaa Zahraa. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [M.S.T]. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Acknowledgements: Declared none.

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