



Carbapenem-resistant Gram-negative bacteria associated with catheter-related bloodstream infections in three intensive care units in Egypt

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Abstract

We aimed to identify the carbapenem-resistant Gram-negative bacteria (GNB) causing catheter-related bloodstream infections (CRBSI) in intensive care units (ICU) in a tertiary care Egyptian hospital, to study their resistance mechanisms by phenotypic and genetic tests, and to use ERIC-PCR for assessing their relatedness. The study was conducted over 2 years in three ICUs in a tertiary care hospital in Egypt during 2015–2016. We identified 194 bloodstream infections (BSIs); 130 (67.01%) were caused by GNB, of which 57 were isolated from CRBSI patients (73.84%). Identification of isolates was performed using conventional methods and MALDI-TOF MS. Antimicrobial susceptibility testing (AST) was done by disc diffusion following CLSI guidelines. Phenotypic detection of carbapenemases enzymes activity was by modified Hodge test and the Carba-NP method. Isolates were investigated for the most common carbapenemases encoding genes *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48} using multiplex PCR. Molecular typing of carbapenem-resistant isolates was done by ERIC-PCR followed by sequencing of common resistance genes. The overall rate of CRBSI in our study was 3.6 per 1000 central venous catheter (CVC) days. Among 57 Gram-negative CRBSI isolates, *Klebsiella pneumoniae* (*K. pneumoniae*) was the most frequently isolated (27/57; 47.4%), of which more than 70% were resistant to Meropenem. Phenotypic tests for carbapenemases showed that 37.9% of isolates were positive by modified Hodge test and 63.8% by Carba-NP detection. Multiplex PCR assay detected the *bla*_{NDM} in 28.6% of the isolates and *bla*_{KPC} in 26.8%, *bla*_{NDM} and *bla*_{KPC} were detected together in the same isolate in 5.6%, while *bla*_{OXA-48}-like were not detected. ERIC-PCR detected limited genetic relatedness between *K. pneumoniae* isolates. Elevated resistance rates were observed to all antibiotics including carbapenems among *K. pneumoniae* isolates causing CRBSI. ERIC-PCR showed that the resistant isolates were mainly polyclonal. Our results call for reinforcement of antimicrobial stewardship and measures to prevent CRBSI.

Keywords Carbapenem resistance · Gram-negative bacteria · Catheter-related bloodstream infections · ERIC-PCR

Introduction

Catheter-related bloodstream infections (CRBSIs) are among the leading nosocomial infections worldwide, with highest risks among the elderly, immunocompromised, and patients in intensive care units (ICUs) [1]. The risk factors for CRBSIs in patients with a central venous catheter (CVC) include inadequate asepsis, inexperience of the operator, colonization of the insertion site, contamination of the catheter hub, and duration of catheter use. Besides, inappropriate administration of antimicrobials and prolonged hospitalization increase the risk of developing infections caused by multi-drug resistant (MDR) microorganisms [2].

Escherichia coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are particularly

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important pathogens in the hospital setting and in CRBSIs [3]. Regrettably, pathogens associated with nosocomial BSIs are usually equipped with higher numbers of antimicrobial resistance traits [4]. The resistance to fluoroquinolones has become common worldwide, which limits therapeutic options for infections caused by Gram-negative bacteria [5]. Resistance to carbapenems is of special concern since these agents have been considered the last resort for effective therapy for MDR Gram-negative bacteria [6]. Accordingly, active surveillance of MDR pathogens causing CRBSI and studying their phenotypic and genetic characteristics are essential measures to identify genetic relatedness of bacteria to improve their control and interrupt their transmission in ICUs [7]. As the causative MDR pathogens are institution-specific and show variable temporal trends, we aimed to identify the carbapenem-resistant Gram-negative pathogens causing CRBSI in ICUs of a tertiary care Egyptian hospital, study their phenotypic and genetic characteristics, and use Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR to test for relatedness.

Materials and methods

The study was conducted in a tertiary care hospital in Cairo, which is also a major transplantation and hemato/oncology center, from 1/1/2016 to 31/12/2016 after approval by the Institution Review Board. We enrolled all patients with CVCs in three ICUs, documented their demographic and clinical data, dates of ICU admission, CVC insertion and removal, and their outcome. Surveillance for device-associated infections was conducted as described before [8].

CRBSI and central line-associated bloodstream infection case definitions

Both CRBSI and central line-associated bloodstream infection (CLABSI) are primary BSI in a patient who had a central line within the 48-h period before the development of the BSI and is not related to infection at another site [9]. Common skin contaminants were considered when cultured from at least two blood samples withdrawn on separate occasions, in the context of signs and symptoms of sepsis and in the absence of infection at another site [10]. While CLABSI is a term used by CDC's National Healthcare Safety Network (NHSN) for surveillance purposes, CRBSI is a clinical definition that identifies the catheter as the source of the BSI. In this study, we used both definitions interchangeably [9].

Calculation of CRBSI rate

CRBSI rate was calculated by dividing the number of CRBSI by the total number of CVC days and multiply by 1000.

CRBSI was expressed as the number of infections per 1000 CVC days [8].

Laboratory methods

According to the ICU protocol, at least two sets of blood cultures were collected from all febrile patients before antibiotic administration. For a group of patients, additional blood cultures were collected from the CVC. The blood samples were withdrawn into BACT/ALERT blood culture bottles and incubated in the BACT/ALERT system (BioMerieux, France). The positive blood cultures were subjected to identification of pathogens and their antimicrobial susceptibility (AST) by conventional laboratory methods and confirmed by the VITEK-2 system (BioMerieux, France). Identification was confirmed by MALDI-TOF MS (BioMerieux, France) in a reference laboratory.

Phenotypic detection of carbapenemase activity

We tested isolates that showed reduced susceptibility to meropenem (10 µg) disk for carbapenemase production by Modified Hodge test (MHT) [11]. The clover leaf-like appearance between the test streaks near the disc was taken as positive for carbapenemase production. The Carba-NP test was performed for suspected carbapenemase producers according to Nordmann et al. [12].

PCR amplifications

PCR primers synthesis and DNA sequencing

The PCR primers were synthesized by Invitrogen (UK) as listed in Table 1. The primers were prepared by adding the required volumes of nuclease-free water and stored in a concentration of 100 pmol/µl at -20 °C. The concentration of each primer was adjusted to 10 pmol/µl using nuclease-free water (Promega, USA). DNA sequencing was carried out at Clinilab Reference Laboratory (Cairo, Egypt) using the Applied Biosystems 3500 Genetic Analyzer (Thermo-Fisher Scientific, USA) for Sanger sequencing. In silico PCR amplification software available at <http://insilico.ehu.es/PCR/> and NCBI/Primer-BLAST was used to simulate and examine the specificity of the primers used in multiplex PCR assay against up-to-date sequenced prokaryotic genomes as described before [13].

DNA extraction

For PCR-based detection of carbapenemase encoding genes, total DNA was obtained directly from bacterial colonies by a boiling method. A large single colony, picked from a freshly grown plate using a sterile pipette tip, was transferred to 50 µl of sterile nuclease-free water in a microfuge tube. The tube

Table 1 Nucleotide sequences of PCR oligonucleotide primers

Target gene	Sequence (5'–3')	PCR amplicon(bp)	Source
<i>bla</i> _{OXA-48}	F: 5' GCGTGGTTAAGGATGAACAC 3' R: 5' CATCAAGTTCAACCCAACCG 3'	438	[13]
<i>bla</i> _{KPC}	F: 5' CGTCTAGTTCTGCTGTCTTG 3' R: 5' CTTGTCATCCTTGTAGGCG 3'	798	
<i>bla</i> _{NDM}	F: 5' GGTTTGGCGATCTGGTTTTTC 3' R: 5' CGGAATGGCTCATCACGATC3'	621	

was then heated to 95 °C for 5 min in a heat block then centrifuged at 14,000 rpm for 2 min. After that, 2 µl of supernatant was used as template in PCR. For ERIC-PCR analyses and DNA sequencing, bacterial genomic DNA was extracted using the GeneJET Genomic DNA purification Kit (Thermo Scientific, USA).

PCR cycling conditions

Multiplex PCR assay

The genes *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48} were detected using multiplex PCR assays according to Poirel et al. [13]. DNA fragments of sizes 798, 621, and 438 bp from *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA-48}, respectively, were simultaneously amplified. The PCRs were prepared in total volumes of 25 µl containing 2 µl of total DNA extract, 12.5 µl GoTaq® Green Master 2× Ready Mix (Promega, USA), and 6 pmol PCR primers (1 pmol of each forward and reverse primers), and the volume was completed to 25 µl by adding 4.5 µl of nuclease-free water. The PCR program for amplification was as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min.

ERIC-PCR

Molecular typing of carbapenem-resistant isolates was performed by ERIC-PCR [14]. The PCRs were prepared in total volumes of 25 µl, including ~10 ng of template DNA,

10 pmol of ERIC-1 primer (5'-ATGTAAGCTCCTGGGGATTAC-3'), 12.5 µl of PCR Master Mix (Promega Corporation, Madison, USA) and the volume was completed to 25 µl by adding nuclease-free water. The amplifications were done in a Veriti 96-well Thermal Cycler (Applied Biosystems, USA) programmed for an initial denaturation at 95 °C for 5 min and 40 cycles of denaturation at 95 °C for 1 min, primer annealing at 45 °C for 1 min, and extension at 72 °C for 8 mins, followed by final extension at 72 °C for 10 min.

ERIC-PCR profiles analysis

ERIC patterns were analyzed using Similarity index Dice coefficient and the UPGMA clustering method using dendrogram construction utility at http://insilico.ehu.es/dice_upgma/. Agarose gel electrophoresis was done after each PCR type.

Results

We conducted a prospective surveillance study including 1210 patients admitted for a total of 17,785 patients' ICU days and 11,014 central line days. CVC were inserted for 645 (53.30%) patients admitted to the ICUs (Table 2). We identified 194 BSIs, 130 (67.01%) caused by GNB and 34 (17.52%) by Gram-positive bacteria. CRBSI caused by GNB was detected in 57/130 (73.84%) and the number of secondary bloodstream infection was 73/130 (56.15%). The total rate of CRBSI in our

Table 2 Susceptibility pattern of Gram-negative bacteria associated with CRBSI

Type of antibiotic	<i>E. coli</i> (8)	<i>K. pneumoniae</i> (27)	<i>E. cloacae</i> (9)	<i>P. aeruginosa</i> (6)	<i>A. baumannii</i> (7)
Amox/clav (AMC) (20/10 µg)	0	0	0	0	0
Piperacillin/tazobactam (30 µg)	14.3	15.2	22.2	66.7	14.3
Cefotaxime (CTX) (30 µg)	0	0	11.1	0	0
Gentamicin (CN) (10 µg)	28.6	25.9	44.4	50	14.3
Ciprofloxacin (CIP) (5 µg)	57.1	14.8	11.1	50	0
Meropenem (MEM) (10 µg)	85.7	29.6	66.7	66.7	28.6
Polymixin (PB) (300 U)	100	100	100	100	100
Colistin (CT) (50 µg)	100	100	100	100	100

Table 3 Frequency and percentages of the *bla*_{KPC}, *bla*_{NDM}, and *bla*_{OXA} in GNB

Organism	Total no. (57)	<i>bla</i> _{KPC} no. (%)	<i>bla</i> _{NDM} no. (%)	<i>bla</i> _{OXA} no. (%)
<i>K. pneumoniae</i>	27	9 (33.3%)	13 (48.1%)	0 (0%)
<i>E. coli</i>	8	0 (0%)	1 (12.5%)	0 (0%)
<i>E. cloacae</i>	9	3 (33.3%)	1 (11.1%)	0 (0%)
<i>P. aeruginosa</i>	6	1 (16.6%)	0 (0%)	0 (0%)
<i>A. baumannii</i>	7	2 (28.6%)	1 (14.3%)	0 (0%)
Total	56 (100%)	15 (26.8%)	16 (28.6%)	0 (0%)

study was 3.6/1000 device utilization days (DU); medical (MICU)/ coronary (CCU) had the highest rate (4.9/1000 CL days) followed by NICU (4.1/1000 CL days), and surgical intensive care unit (SICU) (3/1000 CL days).

Among CRBSI GNB pathogens *K. pneumoniae* 27/57 (47.4%) was the commonest. AST demonstrated 100% susceptibility to colistin and polymyxin B. GNB showed a very low rate of susceptibility to third generation cephalosporins. In addition, *A. baumannii* and *K. pneumoniae* isolates showed decreased susceptibility to meropenem (Table 2).

Among the bacterial isolates, 38.6% (22/57) were positive for MHT, while carba-NP detected 64.9% (37/57) of carbapenemase producers. Multiplex PCR for *K. pneumoniae* isolates revealed that the *bla*_{NDM} gene was the most predominant (48.14%) followed by *bla*_{KPC} gene (33.33%) but none of the isolates contained *bla*_{OXA-48} (Table 3).

ERIC-PCR analysis was performed to determine the genetic relatedness of *K. pneumoniae* isolates harboring carbapenemase genes *bla*_{KPC} and *bla*_{NDM} (Fig. 1). Twenty-five percent (5/20) of KPC-positive *K. pneumoniae* isolates

showed a closer genetic relatedness, and only 18.2% (2/11) of NDM-positive *K. pneumoniae* same cluster group. Cluster groups with $\geq 85\%$ similarity were identified as identical pattern types [15].

Discussion

The overall rate of CRBSI was 3.6/1000 CVC days and the highest was in medical ICU (4.1/1000 CVC days), followed by neonatal ICU (4.9/1000 CVC days) and was least in surgical ICU (3/1000 CVC days).

The rate of CRBSI in limited-resource countries such as Argentina, Brazil, India, Iran, Mexico, Thailand, Tunisia, Turkey, and Peru ranged from (1) 6–44.6/1000 CVC days in adult and pediatric ICUs and from (2) 6–60/1000 central line days in neonatal ICUs with an average of 4.1/1000 CVC days [16]. Our CRBSI rate is in agreement with that reported by Talaat et al., 2016 [8] from Egypt who reported total CRBSI 2.6/1000 CVC days. This is in spite of our higher CVC utilization ratio (0.6 CVC utilization ratio) compared to 0.4 in their study [8], and our higher-risk transplantation and hematology/ oncology patients.

We studied GNB responsible for CRBSI and detected very high rates of antimicrobial resistance compared to previous reports from the same hospital and from Egypt [14]. In the older study, susceptibility to carbapenems was 99–100% among *Enterobacteriaceae* and 90% among *Acinetobacter* isolates, and susceptibility to third-generation cephalosporins ranged between 35 and 71%. This marked decrease in susceptibility is due to the lack of national antimicrobial stewardship program; misuse and overuse of antibiotics in human, animal, and plant care; and the inconsistency of implementation of national infection control guidelines [17]. The antimicrobial susceptibility in our study is less than in other reports [14–18]. This reflects temporal and geographic differences and variation in patients groups. Our patients were dominated by

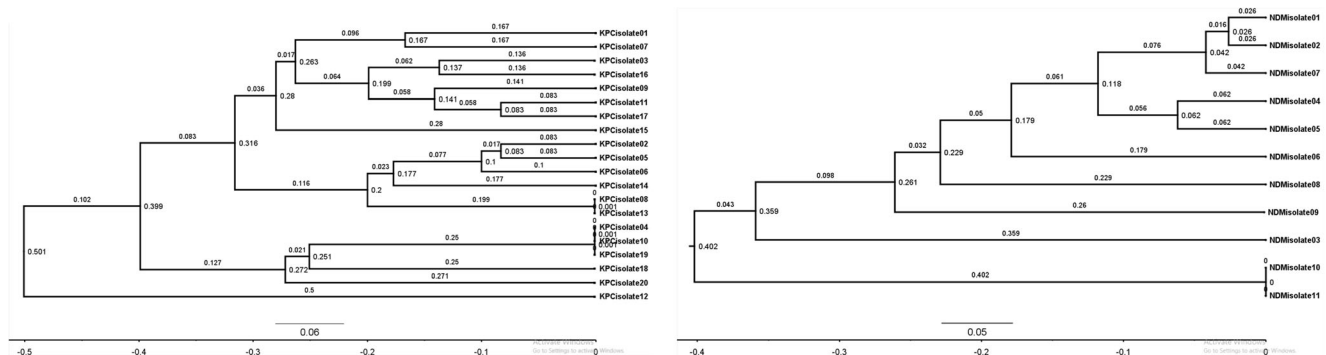


Fig. 1 Genetic relatedness of *K. pneumoniae* isolates by PCR-based ERIC patterns showed 25 and 18.2% of similarity of *bla*_{KPC} and *bla*_{NDM} producing isolates. Corresponding dendrogram generated with

similarity index dice coefficient and the UPGMA clustering method using dendrogram construction utility at http://insilico.ehu.es/dice_upgma

transplant recipients and hematological malignancy patients. Saltin [18] reported that approximately 3–10% of solid organ transplant recipients in carbapenem-resistant *Enterobacteriaceae* (CRE)-endemic areas developed CRE infection, and the infection site correlated with the transplanted organ. Mortality rates were very high in this group of patient.

The predominance of Gram-negative pathogens was reported from the USA [19], Saudi Arabia, [20], and Brazil [21]. The International Nosocomial Infection Control Consortium (INICC) hospitals reported an average of 43.2% carbapenem resistance among *K. pneumoniae* [16]. Our study also revealed that *K. pneumoniae* was the most common pathogen causing CRBSI (47.4%), followed by *E. coli*, *E. cloacae*, *A. baumannii*, and *P. aeruginosa* (12.5, 16.1, 12.5, and 10.8% respectively). This is in keeping with recent reports that multidrug-resistant *K. pneumoniae* strains were a common colonizer in the hospital settings and main bloodstream infection pathogens in immunocompromised patients [18].

We identified 64.9% carbapenemase-producing GNB by carba-NP test compared to 38.6% by MHT. This result confirmed the improved sensitivity of carba-NP for detection of carbapenemases as reported before [11].

Although KPC was identified in many countries as the predominant carbapenem resistance gene [18], we detected KPC producers in only 26.8% of our isolates and identified *bla_{NDM}* as the predominant gene in 48.1% of *K. pneumoniae*, and in 14.3% of *A. baumannii*. This is in agreement with previous studies that reported the predominance of *bla_{NDM}* in Egypt and Middle East [18, 22, 23].

We used ERIC-PCR to study the epidemiology of carbapenem-resistant *K. pneumoniae* being the most predominant bacterial species isolated from our CRBSI patients. No clonal relatedness was detected among 90% of the tested isolates. Only five isolates of KPC-positive *K. pneumoniae* and two isolates in NDM-positive isolates showed genetic relatedness. These clonally related isolates were collected from different ICUs and within different time frames negating any horizontal transfer. This polyclonality is in accordance with a previous study in Egypt [24]. In contrast, Gozalan [25] reported high clonal relatedness between *K. pneumoniae* isolates that suggested cross-contamination and spread of the resistance genes in their hospital settings.

Conclusion

Our study revealed an alarming rate of antimicrobial resistance among CRBSI isolates. This calls for a strict hospital-wide and nationwide antimicrobial stewardship program. Rapid detection of pathogens, monitoring antimicrobial resistance trends and. Strict adherence to infection control practices are urgently required.

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