



Prevalence and antibiotic resistance patterns of carbapenemase-producing organisms in burn and wound infections

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Abstract

Background: Carbapenems are broad-spectrum β -lactam antibiotics, often reserved as last-line treatment for infections caused by multidrug-resistant (MDR) Gram-negative bacteria. Carbapenemase producing organisms (CPOs) pose a serious public health threat, contributing to severe healthcare-associated infections and increased mortality rates. This study aimed to determine the prevalence of CPOs and their antibiotic resistance patterns in isolates from burn and wound infections.

Methods: A total of 250 clinical samples (140 wound swabs and 110 burn swabs) were collected from hospitalized patients in Kirkuk and Sulaimaniyah hospitals between January and July 2023. Specimens were cultured on MacConkey agar and cetrimide agar and incubated at 37°C for 18−24 hours. Bacterial identification and antimicrobial susceptibility testing were performed using the BD Phoenix[™] M50 system, while carbapenemase production was confirmed using the BD RAPIDEC® CARBA NP assay.

Results: Among the isolates, 27 (38.02%) were confirmed as carbapenemase-producing and exhibited multidrug resistance. The distribution was as follows: *Pseudomonas aeruginosa* (44.44%, 12 isolates), *Escherichia coli* (33.33%, 9 isolates), *Enterobacter cloacae* (18.51%, 5 isolates), and *Klebsiella pneumoniae* (3.7%, 1 isolate). Notably, CP-*P. aeruginosa* and CP-*K. pneumoniae* showed the highest resistance, being resistant to 15 antibiotics across seven different classes.

Conclusion: This study reveals a high prevalence of MDR CPOs in burn and wound infections, likely due to antibiotic misuse or overuse. The findings highlight the urgent need for novel therapeutic strategies to combat carbapenem-resistant pathogens, which are associated with increased global morbidity and mortality.

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Introduction

Carbapenems are β -lactam antibiotics that have multiple bactericidal activities (1). These antibiotics are usually considered the last-choice drugs in treating infections with multidrug-resistant (MDR) Gramnegative bacteria. However, according to Patel and Bonomo (2011), (2) reported that carbapenemase-producing organisms (CPOs) are associated with serious healthcare-associated infections, making the mortality rate higher. Some of these bacteria contribute significantly to the prevention and treatment of infections.

The issue of carbapenem-resistant pathogens, which is a global concern these days, requires appropriate strategies at the national and international levels (3). The basic reason for carbapenem resistance is mostly achieved by the synthesis of carbapenemase enzymes, which hydrolyse carbapenem drugs (A group of β -lactam antibiotics) (4). Different genes are involved in carbapenem resistance via the production of carbapenemases. As a result, those genes that code for carbapenemases are associated with various types of mobile genetic elements (5).

Different kinds of β -lactamases hydrolysis carbapenem like metallo β -lactamases (MBLs) which include: β -lactamase of New Delhi metal (NDM), Verona imipenemase (VIM) and Impipenemase (IMP); in addition to class A Ambler member, *Klebsiella pneumoniae* carbapenemase (KPC), and class D member, oxacillinase-48 (OXA-48) (6).

The transmissible carbapenemase genes in Gram negative bacteria was considered as the greatest threat to the public health across the globe, due to the fact that these pathogens limit the effective antibiotic treatments and cause high mortality rate among patients (7), these carbapenemase genes are transmitted, not only among *Acinetobacter*

baumannii but also other nosocomial pathogens like Enterobacteriaceae family members or *Pseudomonaerous aeruginosa* (8).

The world faces an emergency that demands the development of new antimicrobial agents which can be applied against CPOs with MDR bacteria pattern. This study is the first in Iraq to determine the prevalence and multidrug resistance patterns of CPOs isolated from burn and wound infections.

Methods

Sample collection and culturing

A total of 250 clinical samples (140 wound swabs and 110 burn swabs) were collected aseptically from hospitalized patients in Kirkuk and Sulaimaniyah hospitals between January and July 2023. All samples were immediately transported to the microbiology laboratory under sterile conditions to prevent contamination. Upon arrival, specimens were cultured on MacConkey agar for Gram-negative selection and cetrimide agar for Pseudomonas isolation, followed by incubation at 37°C for 18-24 hours. Colony morphology, including color, shape, and texture was examined for preliminary identification.

Bacterial identification and antimicrobial susceptibility testing

Bacterial isolates were identified using the BD PhoenixTM M50 automated system. Antimicrobial susceptibility testing was performed against 18 antibiotics representing seven classes: aminoglycosides (Amikacin, gentamicin), carbapenems (Ertapenem, imipenem, meropenem), cephalosporins (Cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime), beta-lactam combinations (Ceftolozanetazobactam, amoxicillin-clavulanate, piperacillin-tazobactam), penicillins (Ampicillin), fluoroquinolones (Ciprofloxacin, levofloxacin), and other agents (Trimethoprim-sulfamethoxazole,

tigecycline). Testing procedures followed the manufacturer's standardized protocols.

Carbapenemase detection

Carbapenemase production was detected phenotypically using the BD RAPIDEC® CARBA NP assay according to the manufacturer's instructions. This chromogenic test specifically identifies carbapenemase activity in Gram-negative bacterial isolates.

Statistical analysis

Data analysis was performed using Microsoft Excel 2016 to determine prevalence rates and resistance patterns.

Results

From the 250 clinical samples analyzed, 71 (28.4%) grew Gramnegative bacterial isolates. The distribution of pathogens was as follows: 31 isolates (43.66%) were *Pseudomonas aeruginosa*, 16 isolates (22.53%) were *Escherichia coli*, 13 isolates (18.3%) were *Enterobacter cloacae*, and 11 isolates (15.49%) were *Klebsiella pneumoniae* (Figure 1).

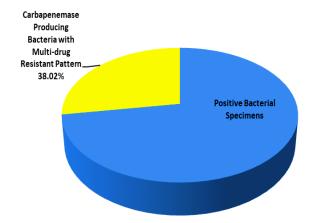


Figure 1. Prevalence of carbapenemase producing bacteria with multi-drug resistant pattern among the positive bacterial isolates

Table 1. Carbapenemase-producing Gram-negative isolates and their multi-drug resistant patterns

Isolate	Total No. of CR- MDR isolates	No. and resistance type	Antibiotic resistance profile	No. of antibiotics/ No. of classes
Pseudomonas aeruginosa	12	1CR-MDR	Amikacin, Gentamicin, Ertapenem, Imipenem, Meropenem, Cefazolin, Cefuroxime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin, Tigecycline.	15/7
		1CR-MDR	Gentamicin, Ertapenem, Imipenem, Meropenem, Cefazolin, Cefuroxime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin, Tigecycline	14/7
		1CR-MDR	Ertapenem, Imipenem, Meropenem, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Ceftolozane- Tazobactam, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin, Tigecycline.	14/6
		1CR-MDR	Gentamicin, Ertapenem, Cefazolin, Cefuroxime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Tigecycline.	11/7
		1CR-MDR	Ertapenem, Cefazolin, Cefuroxime, Ceftriaxone, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin, Tigecycline.	10/6
		3CR-MDR	Imipenem, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Tigecycline.	9/5
		1CR-MDR	Ertapenem, Cefazolin, Cefuroxime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Tigecycline.	9/5
		3CR-MDR	Ertapenem, Cefazolin, Cefuroxime, Ceftriaxone, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Tigecycline.	8/5
Escherichia coli	9	1CR-MDR	Ertapenem, Imipenem, Meropenem, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ceftolozane-Tazobactam, Ampicillin, Amoxicillin-Clavulanate, Piperacillin-Tazobactam, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin.	15/5
		2CR-MDR	Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin.	9/4
		1CR-MDR	Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ceftolozane-Tazobactam, Ampicillin, Ciprofloxacin, Levofloxacin.	9/3
		1CR-MDR	Gentamicin, Cefazolin, Cefuroxime, Ceftolozane-Tazobactam, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin.	8/4
		1CR-MDR	Cefazolin, Cefuroxime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin	7/4
		1CR-MDR	Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin.	7/3
		2CR-MDR	Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin, Trimethoprime-Sulfamethoxazole	6/2
Enterobacter cloacae	5	1CR-MDR	Gentamicin, Imipenem, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin	12/6
		1CR-MDR	Gentamicin, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin.	11/5
		1CR-MDR	Gentamicin, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Ciprofloxacin.	10/4
		2CR-MDR	Gentamicin, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ampicillin, Amoxicillin-Clavulanate, Trimethoprime-Sulfamethoxazole.	10/4
Klebsiella pneumoniae	1	1CR-MDR	Gentamicin, Ertapenem, Cefazolin, Cefuroxime, Ceftazidime, Ceftriaxone, Cefepime, Ceftolozane-Tazobactam, Ampicillin, Amoxicillin-Clavulanate, Piperacillin-Tazobactam, Trimethoprime-Sulfamethoxazole, Ciprofloxacin, Levofloxacin, Tigecycline.	15/7

CR-MDR: Carbapenem Resistant-Multi-Drug Resistant

Carbapenemase-producing Gram-negative isolates and their corresponding MDR patterns are detailed in Table 1. Among these isolates, 27 (38.02%) were identified as CPOs with MDR patterns. The CPO distribution showed that 12 isolates (44.44%) were carbapenemase-producing Pseudomonas aeruginosa, 9 isolates (33.33%) were carbapenemase-producing Escherichia coli, 5 isolates (18.51%) were carbapenemase-producing Enterobacter cloacae, and 1 isolate (3.7%) was carbapenemase-producing Klebsiella pneumoniae.

The antibiotic resistance profiles of carbapenem-resistant isolates revealed significant MDR patterns. Among the carbapenem-resistant Pseudomonas aeruginosa isolates, the most resistant strain demonstrated resistance to 15 antibiotics spanning 7 classes. Other P. aeruginosa isolates exhibited resistance to 14 antibiotics (7 classes), 14 antibiotics (6 classes), 11 antibiotics (7 classes), 10 antibiotics (6 classes), and multiple isolates showed resistance to either 9 antibiotics (5 classes) or 8 antibiotics (5 classes). The carbapenem-resistant Escherichia coli isolates displayed variable resistance patterns, with one isolate resistant to 15 antibiotics across 5 classes, while other isolates showed resistance ranging from 6 to 9 antibiotics covering 2 to 4 classes. Notably, the single carbapenem-resistant Klebsiella pneumoniae isolate exhibited extensive resistance to 15 antibiotics from 7 classes. The carbapenem-resistant Enterobacter cloacae isolates demonstrated resistance patterns ranging from 10 to 12 antibiotics, covering 4 to 6 different antibiotic classes, with multiple isolates showing similar resistance profiles of 10 antibiotics across 4 classes.

Discussion

CPOs have been increasingly reported worldwide among Enterobacteriaceae (9,10). However, data on carbapenem-resistant Gram-negative bacteria in Iraq remain limited. This study investigated the prevalence and resistance patterns of CPOs isolated from burn and wound infections in Iraqi hospitals.

Our findings revealed Pseudomonas aeruginosa as the most prevalent Gram-negative pathogen, consistent with its known ability to develop MDR and survive in diverse environments, leading to increased mortality and prolonged hospitalizations (11). Using the reliable RAPIDEC CARBA NP test and BD PhoenixTM M50 system, we identified 27 (38.02%) carbapenemase-producing isolates exhibiting MDR patterns. This aligns with studies from Spain, Italy, and Iraq, confirming carbapenemase production as a major resistance mechanism (12-14). Globally, CPOs pose a severe threat due to three primary resistance mechanisms: porin loss, efflux pump overexpression, and carbapenemase production. Notably, carbapenemase genes are often plasmid- or transposon-borne, facilitating horizontal gene transfer across bacterial species (15).

All studied pathogens showed high resistance to β-lactams (Penicillins and cephalosporins), likely due to β-lactamase production, altered penicillin-binding proteins, and membrane permeability changes (16). CR-P. aeruginosa and CR-K. pneumoniae exhibited the highest resistance (15 antibiotics across 7 classes). Variations in resistance patterns across studies may reflect regional differences, hospital hygiene, infection types, and detection methods (17,18). The rapid spread of MDR pathogens is exacerbated by mobile genetic elements (e.g., plasmids, transposons), which disseminate resistance genes and promote extreme drug resistance (19-21). Self-medication, antibiotic misuse, and inadequate laboratory surveillance in critical units (e.g., ICUs, burn centers) further compound this issue. As emphasized by Sadeghi et al. (22), robust surveillance programs and novel therapeutics are urgently needed to combat CPOs, which significantly increase global morbidity and mortality (23).

Conclusion

In the current study, the CPOs were highly MDR against most classes of antibiotics due to misuse or overuse of antibiotics. It is recommended that the Iraqi Ministry of Health include a new routine method in the health laboratory system for detecting the carbapenem-resistant bacteria. The wide prevalence of these organisms is in need to continuous monitoring and includes recent strategies for antibacterial resistance control and infection treatment, furthermore the discovery of new drugs.

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Ethical statement

The medical institution in Kirkuk city\Iraq agreed and approved this study (Approval no. 102, approval date 5/2/2023). During this study, the research adhered to the guidelines of the Ministry of Health, Government of Iraq.

Conflicts of interest

The authors state no conflict of interest.

Author contributions

All the medical works in the current study were conducted by the author, Sarah Ahmed Hasan, and supervised by Waad Mahmood Raoof and Khaled Khalil Ahmed.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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