Original Article

Phenotypic and Molecular Characterization of Carbapenemase-Producing Gram-Negative Isolates Detected from Wound Infections

Mashael W. Alruways

ABSTRACT

Objective: To characterize the carbapenemase-producing Gram-negative (CPGN) bacteria, carbapenemase genes and to report the antimicrobial resistance (AMR).

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the College of Applied Medical Sciences, Shaqra, Saudi Arabia from November 2021 to November 2022.

Materials and Methods: A total of 109CPGN were identified from wounds by API 20E and 20NE. CPGN isolates were screened phenotypically by modified carbapenem inactivation (mCIM), and gene variants were amplifiable using multiplex PCR. AMR was performed using the Kirby-Bauer disk diffusion method

Results: CPGN isolates were higher (67.7%) in male wound infections but did not appear to be significantly associated ($p = 0.52$). CPGN strains were significantly associated ($p = 0.04$) with cases admitted to surgery. The most common CPGN bacteria isolated were Pseudomonas aeruginosa (38.7%) and E. coli (25.8%). The carbapenemase gene variants predominantly include 24 (77%) bla_{OXA-23}, 17 (54%) bla_{NDM-1}, 14 (45%) bla_{VIM}, 9 (29%) bla_{OXA-24}, 9 (29%) bla_{NDM-5}, 5 (16%) bla_{NDM-7}, and 3 (9%) bla_{IMP} gene variants. In all cases, the CPGN strains were resistant to carbapenems. Most isolates were resistant to co-amoxiclav, co-trimoxazole, cephalosporins, and fluoroquinolones

Conclusion: It is concerning that a greater number of CPGN isolates are exhibiting high antibiotic resistance and harboring carbapenemase gene variants, which leaves us with few therapeutic options.

Key Words: Wound infections, Gram-negative bacteria, carbapenemase genes, antibiotic resistance

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INTRODUCTION

A wound is an injury in the skin that exposes the subcutaneous tissue, causing the skin to lose its integrity. Wounds can result from surgery, burns, accidental traumas, or diabetic ulcers.[1] Wound infections continue to pose a worldwide health concern despite advances in medicine and methods of preventing them.[2] It is possible for microbial colonization, proliferation, and infection to occur on wounds because they provide a moist, warm, and nutritive environment.[3]

Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Shaqra University, Saudi Arabia.

Correspondence: Dr. Mashael W. Alruways, Assistant Professor of Clinical Laboratory Sciences, College of Applied Medical Sciences, Shaqra University, Shaqra 15273, Saudi Arabia
Contact No: +966 55 4084083
Email: m.alruways@su.edu.sa

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Infections in wounds impede healing in various ways, including delaying healing and resulting in the breakdown of the wound.[4] It has been widely recognized that wound infections are the most prevalent nosocomial infection.[5]

Several bacteria are most likely to cause an infection in wounds, including Staphylococcus aureus, Escherichia coli, Proteus species, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii.[6,7] In most cases, wounds become infected with Gram-negative bacteria (GNB), treated with carbapenems, considered the best antibiotics.[8,9] There is an increase in antibiotic resistance in GNB due to the production of carbapenemases, which can hydrolyze a wide variety of beta-lactam antibiotics, including carbapenem, which are widely used in medicine.[10,11] Carbapenemases belong to the Ambler classification, which is based on structural similarities, and they have a strong propensity to spread.[12]A number of plasmid or chromosomal mediated genes, including bla_{OXA}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and $blaG_{ES}$, play a role in the development of multidrug-resistance in GNB.[13]

The prevalence of multidrug-resistant (MDR) carbapenemase-producing Gram-negative bacteria (CPGN) has increased worldwide, emphasizing the

need for judicious use of antimicrobials.[14] Due to the escalation of MDR CPGN, patients have faced limited treatment options, which has resulted in substantial increases in therapeutic costs and prolonged morbidity.[15] The identification of CPGN as epidemiologically significant, understanding their presence in a particular area, and implementing preventative measures against their spread is vital to wound infection prevention.[7,14] This study aimed to identify GNB pathogens capable of producing carbapenemase using phenotypic and molecular methods. Furthermore, the prevalence of carbapenemase gene variants and antimicrobial resistance (AMR) in CPGN was studied to identify potential therapeutic approaches.

MATERIALS AND METHODS

This cross-sectional study was conducted on wound swabs from November 2021 to November 2022. The study does not involve human or animal participants and is performed according to the ethical considerations of the WMA Declaration of Helsinki. The wound specimens were collected on Amies transport media swabs without the contamination of skin microbiota. The specimens were cultured on Blood, Chocolate, and Mac Conkey agar plates and were incubated overnight at 35 – 37ºC for 24 – 48 hours.

The colony morphology and biochemical characteristics were performed to identify bacterial isolates. The Gram-positive bacteria were excluded from the study, and Gram-negative bacterial isolates were further confirmed by API 20E and 20 NE.[16,17] The isolates were processed for carbapenamase production, and a copy of each isolate was preserved at – 70 °C for future use.[11]

CPGN isolates were screened phenotypically by modified carbapenem inactivation (mCIM) technique using 10 µg meropenem disc and E. coli ATCC 25922 as described by the CLSI manual.[18,19] Phenotypically isolated CPGN isolates were processed to detect carbapenemase genes using the earlier techniques.[20] A multiplex PCR was conducted to amplify the carbapenemase gene variants bla_{OXA-23}, bla_{NDM-1}, bla_{VIM}, bla_{OXA-24}, bla_{NDM-5}, bla_{NDM-7}, and bla_{IMP}using primers previously reported in the literature.[20,21]

Spectrum AMR was observed against various antibiotics such as aminoglycosides, cephalosporins, fluoroquinolones, carbapenems, and beta-lactam combinations. AMR profile against the different antibacterial drugs was observed using the Kirby-Bauer disk diffusion technique,[22] and the broth microdilution technique was used to determine the AMR against colistin. AMR results were interpreted as described by the CLSI.[19] The data was analyzed using SPSS v.25 and GraphPad Prism 9.3, and a p-value of < 0.05 was taken as a significant association.[23]

RESULTS

The demographic characteristics of the cases infected with Gram-negative bacterial wound infections showed a higher number of male patients (62.4%) who suffered from wound infections than female patients. No significant association was found ($p = 0.52$) based on sex distribution among carbapenemase-producing and non-producing bacterial strains. The highest number of CPGN strains was 15 (32.3%) isolated from the surgery ward and 6 (19.4%) from the intensive care unit. The CPGN strains were significantly associated ($p = 0.04$) with the cases admitted to the surgery ward (Table 1).

Table No.1: Demographic characteristics of cases infected with carbapenemase and non-carbapenemase-producing Gram-negative bacteria

Variables		Total n = 109 n (%)	Carbapenemase producers n = 31 n (%)	Non-carbapenemase producers n = 78 n (%)	p-value
Gender	Male	68 (62.4)	21 (67.7)	47 (43.1)	0.52
	Female	41 (37.6)	10 (32.3)	31 (28.4)	
Ward	Surgery	36 (33)	15 (48.4)	21 (26.9)	0.04
	Outpatient department	23 (21.1)	2 (6.5)	21 (26.9)	
	Orthopedic	19 (17.4)	5 (16.1)	14 (17.9)	
	Medical ward	17 (15.6)	3 (9.7)	14 (17.9)	
	Intensive care unit	14 (12.8)	6 (19.4)	8 (10.3)	

The highest number of CPGN bacteria was Pseudomonas aeruginosa (38.7%) and Escherichia coli (25.8%). Few other CPGN bacteria were isolated from wounds compared to the isolates mentioned above (Fig. 1). Phenotypic and molecular characterization revealed several carbapenemase gene variants present in CPGN bacteria. A total of 31 CPGN bacterial strains harbored 24 (77%) bla_{OXA-23}, 17 (54%) bla_{NDM-1}, 14 (45%) bla_{VIM}, 9 (29%) bla_{OXA-24}, 9 (29%) bla_{NDM-5}, 5 (16%) bla_{NDM-7}, and 3 (9%) bla_{IMP} gene variants (Fig. 2).

The AMR profile of CPGN bacteria presented 100% to carbapenem drugs and variables resistance to other antibacterial drugs.

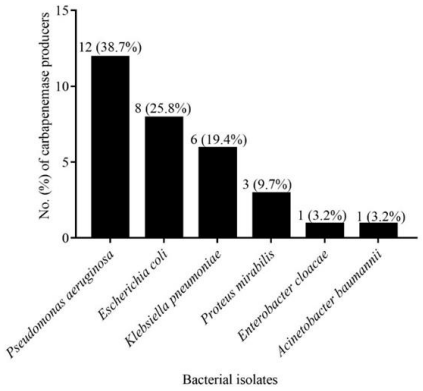

Figure No.1: Detection of carbapenemase producers isolated from wound infections (n = 31)

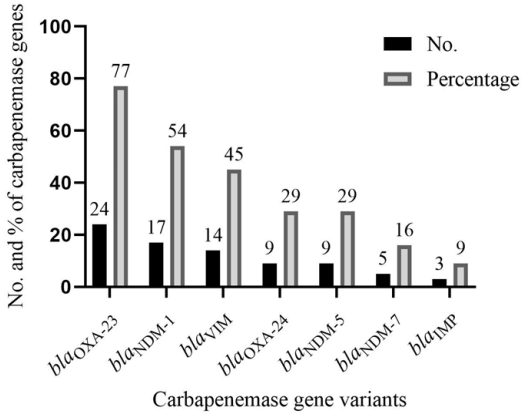

Figure No.2: Carbapenemase genes variants among the clinical strains isolated from wound infections

Table No.2: Antimicrobial resistance of carbapenemase-producing Gram-negative strains (n = 31)

Antibiotic	P. aeruginosa n = 12	E. coli n = 8	K. pneumoniae n = 6	P. mirabilis n = 3	E. cloacae n = 1	A. baumannii n = 1
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Amikacin	7 (58.3)	3 (37.5)	3 (50)	1 (33.3)	0 (0)	0 (0)
Co-amoxiclav	11 (91.7)	5 (62.5)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Cefepime	10 (83.3)	4 (50)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Cefixime	10 (83.3)	4 (50)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Cefotaxime	10 (83.3)	4 (50)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Ceftazidime	8 (66.7)	4 (50)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Ceftriaxone	9 (75)	4 (50)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Cefuroxime	10 (83.3)	4 (50)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Ciprofloxacin	9 (75)	4 (50)	4 (66.7)	2 (66.7)	0 (0)	1 (100)
Colistin	0 (0)	0 (0)	4 (66.7)	3 (100)	0 (0)	1 (100)
Co-trimoxazole	11 (91.7)	4 (50)	4 (66.7)	1 (33.3)	1 (100)	1 (100)
Ertapenem	12 (100)	8 (100)	6 (100)	3 (100)	1 (100)	1 (100)
Gentamicin	8 (66.7)	3 (37.5)	5 (83.3)	2 (66.7)	1 (100)	1 (100)
Imipenem	12 (100)	8 (100)	6 (100)	3 (100)	1 (100)	1 (100)
Levofloxacin	8 (66.7)	3 (37.5)	3 (50)	1 (33.3)	0 (0)	0 (0)
Meropenem	12 (100)	8 (100)	6 (100)	3 (100)	1 (100)	1 (100)
Moxifloxacin	9 (75)	4 (50)	4 (66.7)	2 (66.7)	0 (0)	1 (100)
Piperacillin-tazobactam	8 (66.7)	1 (12.5)	3 (50)	1 (33.3)	0 (0)	0 (0)

Most of the isolates were MDR and were highly resistant to co-amoxiclav, co-trimoxazole, cephalosporins, and fluoroquinolones. Pseudomonas aeruginosa, E. coli, and E. cloacae showed no resistance to colistin, while these isolates exhibited 58.3%, 37.5%, and 0% resistance to amikacin, respectively. There was 1 (12.5%) E. coli isolate resistant to piperacillin-tazobactam, while none of the E. cloacae and A. baumannii showed resistance to this combination. A. baumannii also showed no resistance to amikacin and levofloxacin. K. pneumoniae strains were extensively resistant among the isolated CPGN strains and presented a minimal resistance of 50% to each piperacillin-tazobactam, amikacin, and levofloxacin. P. mirabilis isolates were also MDR and showed a minimal resistance of 33.3% to each piperacillin-tazobactam, amikacin, levofloxacin, and co-trimoxazole. A detailed AMR of CPGN strains has shown in Table 2.

DISCUSSION

Hospitals face a serious problem with bacterial contamination of wounds, especially during surgical procedures when sterile areas are contaminated and become infected.[24] In recent years, Gram-negative bacteria have become increasingly resistant to antibiotics, largely due to carbapenemases, which have a wide range of hydrolytic activity against β-lactams. It is well known that the genes encoding the acquired

carbapenemases are likely to spread quickly. CPGN bacteria in the human body can also lead to high morbidity and even mortality.[11,20] Increasing resistance to pathogens has made AMR a global problem that threatens public health around the globe.[10,11]

This study reports the occurrence of more CPGN in wounds among males than females. The patients showed a statistically significant association with the surgery ward. This finding agrees with the previous report from Ethiopia, which reported a wound culture positivity rate of 70.5%, with a higher incidence among men.[25] Similar findings have also been reported in a report from Pakistan, which supports the present study's findings.[26] Another study found that male patients (59.1%) had a higher incidence of wound infections than female patients (40.9%).[27] Due to the nature of the work or accidental exposure, the male is more likely to suffer wound injuries. The open wounds in surgical wards make patients more vulnerable to bacterial wound infections, which can develop for days or months after surgery.[28]

This study detected a high number of Pseudomonas aeruginosa and E. coli, which is consistent with previous reports.[11,20]Wound infections are generally more associated with the GNB.[26] There is an increase in carbapenemase-mediated antibacterial resistance among enterobacterial species, Pseudomonas aeruginosa, and Acinetobacter baumannii.[29] Based on the molecular characterization of carbapenemases, seven gene variants have been identified, including bla_{OXA-23}, bla_{NDM-1}, bla_{VIM}, bla_{OXA-24}, bla_{NDM-5}, bla_{NDM-7}, and bla_{IMP} in this research. A German study identified bla_{VIM}, bla_{NDM-1}, bla_{GES}, and bla_{IMP}carbapenemase gene variants among Pseudomonas aeruginosa strains.[30] However, an Indian study report only bla_{VIM} and bla_{NDM-1} variants.[31] There is a wide variation in carbapenemase gene variants among GNB strains according to geographic location.

AMR profile of CPGN bacteria found in this study showed complete bacterial resistance carbapenems highly resistant to co-amoxiclav, co-trimoxazole, cephalosporins, and fluoroquinolones. The isolates showed variable resistance to piperacillin-tazobactam, amikacin, and levofloxacin, while colistin was a better therapeutic option. In the past decade, carbapenems have been recognized as a powerful class of antibiotics for treating bacteria that produce extended-spectrum β-lactamases. Additionally, they are often used in treating MDR GNB when systemic infections are present.[32]It has been shown that the overuse of these medications can increase the spread of carbapenem-resistant Enterobacterales.[33]A rise in the number of cases of AMR has been attributed to the spread of resistant bacteria.In order to reduce the risk associated with MDR organisms, an active surveillance system, a good policy, and appropriate preventive measures are essential. The work is limited in that it has focused on carbapenemase gene variants and other drug resistance mechanisms that could also have contributed to the AMR of wound pathogens.

CONCLUSION

Carbapenamase-producing Pseudomonas aeruginosa and E. coli were the main culprits among the cases of wound infections. The most commonly detected carbapenemase gene variants were bla_{OXA-23}, bla_{NDM-1}, bla_{VIM}, bla_{OXA-24}, and bla_{NDM-5}. In addition to carbapenems, CPGN has the potential to present MDR to aminoglycosides, cephalosporins, and fluoroquinolones.The in vitro efficacy of colistin was the highest, while some strains of bacteria responded to piperacillin-tazobactam, amikacin, and levofloxacin in vitro. In order to monitor antimicrobial drug resistance actively, bacteriological investigation of clinical specimens is crucial. It allows targeted antimicrobial therapy, limits ineffective antibiotics, and avoids unnecessary antimicrobial pressure on susceptiblestrains.

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Author's Contribution:

Concept & Design of Study:	Mashael W. Alruways
Drafting:	Mashael W. Alruways
Data Analysis:	Mashael W. Alruways
Revisiting Critically:	Mashael W. Alruways
Final Approval of version:	Mashael W. Alruways

Conflict of Interest: The study has no conflict of interest to declare by any author.

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