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Original Article

Prevalence and molecular determinants of carbapenemase-producing *Klebsiella pneumoniae* isolated from Jazan, Saudi Arabia

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Abstract

Introduction: The World Health Organization (WHO) designated Carbapenem-resistant Enterobacterales (CRE), formerly *Enterobacteriaceae*, among the global priority list of antibiotic-resistant bacteria. The rate of CRE in Arabian countries, including Saudi Arabia has increased. Here, we report the prevalence of carbapenemase-producing *Klebsiella pneumoniae* (CPKP) in the Jazan region, a southern coastal province of Saudi Arabia.

Methodology: Eighty-six non-repetitive clinical isolates of *K. pneumoniae* that showed resistance to at least one of the carbapenem drugs were collected from three tertiary hospitals in the Jazan region from March 2020 to April 2021. The identification and antimicrobial susceptibility testing (AST) of isolates were performed using various automated systems. Molecular detection of carbapenemase genes was conducted using a multiplex PCR.

Results: Out of the 86 tested CRKP isolates, 64 (74.4%) were carbapenemase-producing isolates. The *bla*OXA-48 gene was the most predominant carbapenemase gene, detected in 65.1% (n = 56) of isolates. The *bla*NDM gene was detected in only 9.3% (n = 8) of isolates; three were found to be co-harbored with blaVIM. Interestingly, one isolate of CRKP was found to have carbapenemase genes (*bla*NDM, *bla*VIM and *bla*KPC), which was associated with COVID-19 patient.

Conclusions: The incidence of carbapenemase-producing *K. pneumoniae* in Jazan hospitals seemed to be high, confirming the continued prevalence of carbapenem resistance in Saudi Hospitals. We report *K. pneumoniae* strain with triple carbapenemase genes in southern Saudi Arabia. The emergence of such an isolate could threaten patients and healthcare workers and requires great attention to rapid interventions to avoid further dissemination, particularly during the COVID-19 pandemic.

Key words: Carbapenem-resistant; *K. pneumoniae*; carbapenemase; OXA-48; Saudi Arabia.

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Introduction

The increasing occurrence of antimicrobial resistance and the lack of innovation in the development of new antimicrobial agents have gradually limited the therapeutic choices for bacterial infections [1]. Carbapenems are a group of broad-spectrum antibacterial drugs used as the last choice to treat severe healthcare-associated infections caused by multidrug-resistant (MDR) *Enterobacteriaceae*. The development of resistance mechanisms by *Enterobacteriaceae* has led to a reduction in the therapeutic efficacy of carbapenems. Currently, Carbapenem-resistant *Enterobacterales* (CRE) represent a significant threat to the healthcare community. Consequently, the World Health Organization (WHO) designated CRE among the global priority pathogen list of MDR bacteria [2]. *Klebsiella pneumoniae* is a major member of *Enterobacteriaceae* which causes a wide range of healthcare-associated infections and shows increasingly frequent antimicrobial resistance [3].

Carbapenem resistance can be commonly developed by the production of carbapenemase enzymes. Moreover, additional less frequent mechanisms of carbapenem resistance are via the combined effect of other beta-lactamases, reduced cell membrane permeability, and/or increased activity of bacterial efflux pump. Most carbapenemases are genetically encoded on mobile elements, facilitating gene transfer between bacterial isolates [4-6]. The major carbapenemase genes that are commonly acquired by clinical *K. pneumoniae* isolates include *Klebsiella pneumoniae* carbapenemases (bla_{KPC}), Oxacillinase-48 (bla_{OXA-48}), New Delhi metallo-B-lactamases (bla_{NDM}), imipenemase (bla_{IMP}), and Verona integron-encoded metallo-B-lactamases (bla_{VIM}) [7]. The dissemination of these major carbapenemases among *K. pneumoniae* has been described globally. In 2001, the first OXA-48 enzyme was detected in a *K. pneumoniae* isolate from Turkey [8], which was described as an endemic region in 2015 [9]. Currently, particularly among *Enterobacteriaceae*, OXA-48 is endemic in Turkey, North African countries, the Middle East, and India [10]. The first detection of NDM was from India in 2008 [11], and since then, the Indian subcontinent countries have been considered the main reservoirs of NDM-producing isolates. Recently, the Balkan countries and the Middle East have served as a second reservoir of NDM producers [12]. Moreover, since the initial discovery of VIM-1 in Italy in 1997 [13], VIM-producing isolates of *Enterobacteriaceae* have been identified worldwide, mainly in South European countries, such as Greece, Spain, Hungary, and Italy [14]. The first detection of *K. pneumoniae* harboring the bla_{KPC} gene was in 1996 in North Carolina, United States (US) [15]. Currently, the US, Latin America, and China are the major endemic regions of the KPC producers [16].

In the Arabian Gulf countries, molecular surveillance revealed the widespread prevalence of OXA-48 and NDM producers in these regions [17]. In the last decade, the prevalence and regional distribution of carbapenemase-producing *K. pneumoniae* (CPKP) in different Saudi regions were described in several reports, with little information documented on their genomic characteristics. Most of these reports were from Riyadh, with few data reported from other regions of Saudi Arabia [18]. The most predominant carbapenemases among CPKP clinical isolates were OXA-48, with less prevalence of NDM and VIM [18]. In contrast, the existence of KPC producers is rare in Saudi Arabia and the first detection of clinical KPC-producing *K. pneumoniae* was from Riyadh, in 2020 [19]. Moreover, unique clinical isolates of *K. pneumoniae* with triple carbapenemase genes (KPC/NDM/OXA-48) were recently reported in the Western region [20]. Only one study from the Southern regions reported the emergence of CPKP in the Aseer region with a highly endemic level of OXA-48 [21]. Building on available data, this study reports the prevalence of CPKP and its genetic determinants in the Jazan region, a southern coastal province of Saudi Arabia.

Methodology

Study area and ethical approval

This cross-sectional study was conducted in the period from March 2020 to April 2021 in three main tertiary hospitals (King Fahad Hospital (500 beds), Prince Mohammed Hospital (200 beds), and Armed Forces Hospital (70 beds)), Jazan region; a southern coastal province setting more than 1000 km from the capital city and 100 km from Yemen's northern borders. Ethics approval of this study was approved by the standing committee for scientific research at Jazan University, reference number: REC-43/03/042.

Isolates Collection, Identification, and Antimicrobial Susceptibility Testing

As part of routine testing that was conducted in the microbiology laboratories of the three hospitals, non-repetitive clinical isolates of *K. pneumoniae* showing resistance to at least one of the carbapenem drugs (meropenem, imipenem and ertapenem) were collected.

A total of 86 clinical *K. pneumoniae* isolates were identified using various semi-automated systems that are available in hospitals' laboratories, including MicroScan WalkAway plus system (Beckman Coulter, Inc., USA), the VITEK-2 system (BioMerieux, France), and BD Phoenix M50 instrument (Becton Dickinson, USA). The same automated systems were also used to perform antimicrobial susceptibility testing (AST), and the interpretation of results was carried out conforming to the Clinical and Laboratory Standards Institute (CLSI) M100-S28 [22]. The tested antimicrobial agents include carbapenems (Imipenem, meropenem, and ertapenem) and nine additional agents, including amoxicillin-clavulanate, amikacin, ceftazidime, cefepime, ciprofloxacin, gentamicin, piperacillin-tazobactam, tigecycline, and trimethoprim-sulfamethoxazole. All identified isolates were transferred from pure culture to 1.5 ml tubes containing Luria-Bertani (LB) broth (HiMedia Labs, India) and 20% (v/v) glycerol and were stored at -80 °C for further analysis.

Molecular detection of carbapenemase genes

Bacterial genomic DNA was extracted by emulsifying a loop full of pure bacterial culture into a microcentrifuge tube containing 300 µL sterile deionized water. The tube was then incubated on a heat block at 98 °C for 15 minutes and then centrifuged at 13,000 *g* for 10 minutes. The PCR reaction used two microliters of the supernatant as a DNA template. The presence of five prevalent carbapenemase genes (bla_{OXA-48}, bla_{VIM} bla_{NDM}, bla_{KPC}, and bla_{IMP}) among *K. pneumoniae* isolates were examined using multiplex polymerase chain reaction (PCR). Multiplex-PCR was performed using a series of primers previously described by Al-Zahrani and Alasiri (2018). Primers were supplied by Macrogen (Seoul, South Korea). Each PCR reaction (50 µL) consisted of 25 µL of Dream Taq PCR master mix (Thermo Fisher Scientific, USA), 13 µL sterile-RNase-free water, one microliter (1µM final concentration) of each forward and reverse primers, and two microliter of DNA template. PCR steps were carried out according to the previously reported protocol of Zarakolu *et al*. (2016) and Al-Zahrani and Alasiri (2018) [23,24]. The amplified PCR fragments were separated by gel electrophoresis on 2% agarose and visualized using a gel documentation system (Bio-Rad Laboratories, USA). The resulting PCR products with more than one gene in a single isolate were confirmed by singleplex PCR. Positive controls included the following strains: *K. pneumoniae* NCTC 13443 strain for NDM, for NCTC 13438 KPC, *E. coli* NCTC 13476 for IMP, NCTC 13440 strain for VIM, and NCTC 13442 strain for OXA-48. While *E. coli* (NCTC 10418) was utilized as a negative control.

Statistical analyses

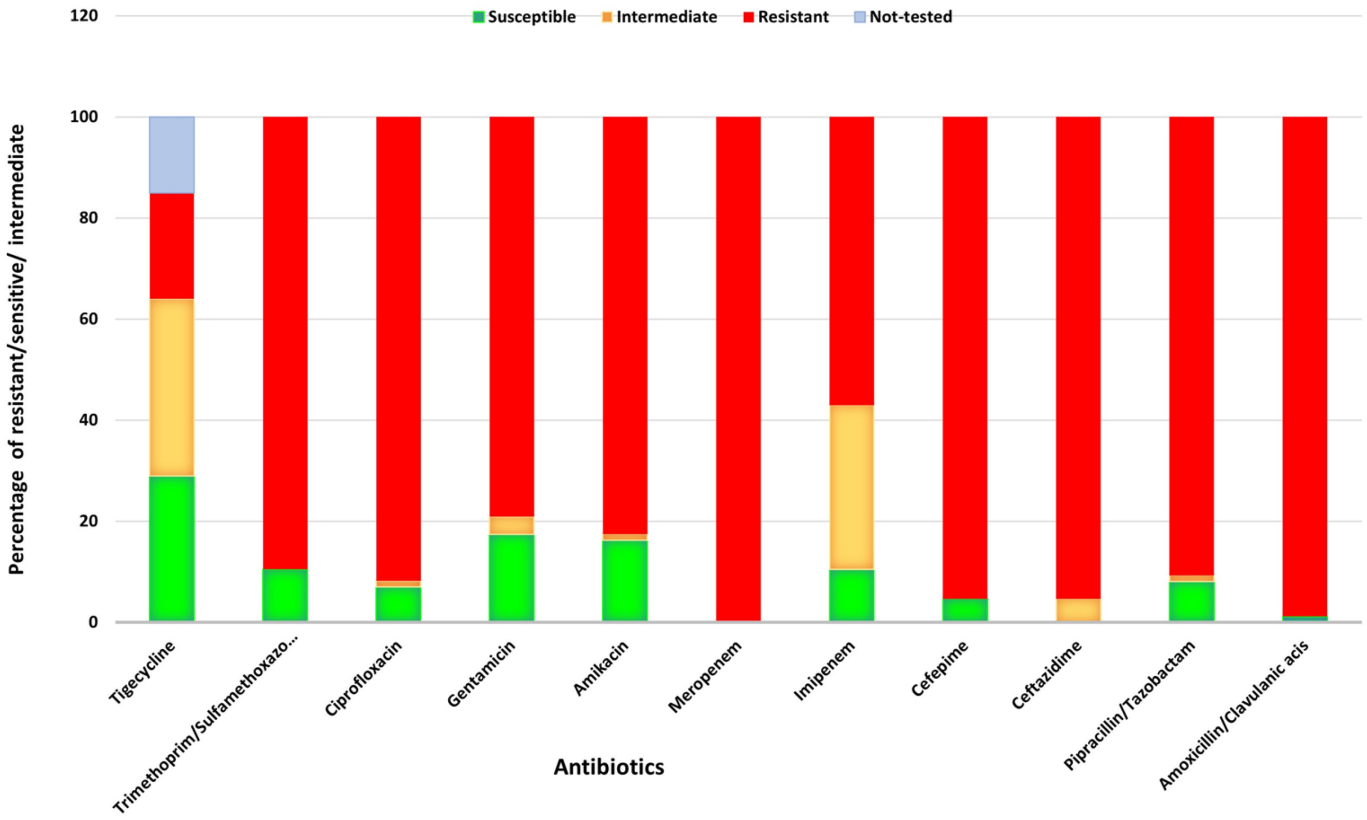
All data was analyzed using SPSS software Version 23. The chi-square test or Fisher's exact test were used for identifying the differences and association between categorical variables with consideration of significant results at a $p < 0.05$.

Results

Clinical data of carbapenem-resistant K. pneumoniae (CRKP) isolates

A total of 86 CRKP isolates were collected from hospitalized patients of three hospitals: most CRKP isolates (n = 46; 53.5%) were collected from prince Mohammed Hospital followed by King Fahad Hospital and Armed Forces Hospital with 23 (26.7%), and 17 (19.8%) isolates respectively. Fifty-nine (68.6%) isolates were obtained from male patients and a majority of isolates (n = 59; 68.6%) were from ICU

Figure 1. The antimicrobial susceptibility results for all 86 CRKP isolates.



patients. Fifty percent (n = 43) of isolates were collected from elderly patients (> 60 years old). The highest number of isolates was obtained from urine (n = 25; 29.1%), followed by sputum (n = 21; 24.4.%), blood (n = 16; 18.6%), wound (n = 13; 15.1%), then intravascular tip culture (n = 4; 4.7%), two (2.3%) isolates each from bedsore and endotracheal aspirate and one (1.16%) isolate each from high vaginal swab, peritoneal fluid and endotracheal tube tip.

Antimicrobial resistance profile of K. pneumoniae isolates

The distribution of antimicrobial resistance of our CRKP isolates was demonstrated in Figure 1. The isolates were most frequently resistant to amoxicillin-clavulanate (98.8%), piperacillin-tazobactam (90.7%), ceftazidime (95.3%), cefepime (95.3%), and ciprofloxacin (91.9%). Additionally, high resistance rates were reported for trimethoprim-sulfamethoxazole (89.5%), amikacin (82.6%), and gentamicin (79.1%), while the resistance rate against imipenem and tigecycline was 57% and 20.9%, respectively.

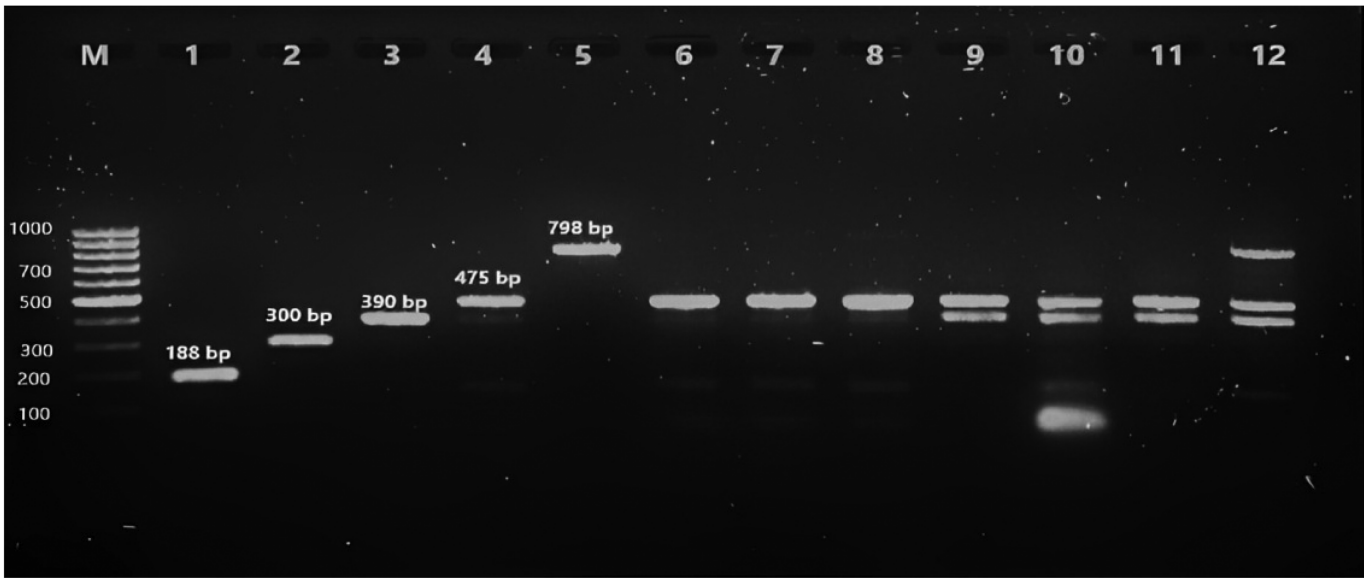
Carbapenemase genes detection

Out of the 86 CRKP isolates, multiplex PCR revealed that 74.4% (n = 64) of isolates were positive for the tested carbapenemase genes (Table 1). The $bla_{\text{OXA-48}}$ gene was the most prevalent gene 81.2% (n = 56) followed by the bla_{NDM} gene (n = 8; 11.6%), bla_{VIM} gene (n = 4; 5.8%), and bla_{KPC} gene (n = 1; 1.4%). Three isolates were found to co-harbor the bla_{VIM} and bla_{NDM} genes, while only one isolate carries triple carbapenemase genes (bla_{VIM}, bla_{NDM} and bla_{KPC}) (Table 1, Figure 2).

Descriptive analysis of carbapenemase-producing K. pneumoniae (CPKP) isolates

All CPKP isolates (n = 64) belonged to patients from prince Mohammed Hospital (n = 28; 43.8%), King Fahad Hospital (n = 21; 32.8%), and Armed Forces Hospital (n = 15; 23.4%). These differences in distribution were statistically significant ($p < 0.01$). Forty-three (67.2%) CPKP isolates were collected from male patients. Most isolates (n = 33; 51.6%) were recovered from elderly patients (> 60 years). However, no significant difference according to sex and age was found ($p > 0.05$). The rate of CPKP isolates from ICU was 75%, which was significantly higher than in other hospital wards (**$p < 0.05$**). A large number of CPKP isolates were obtained from sputum (n = 20; 31.3%), followed by urine (n = 14; 21.9%), blood (n = 12; 18.8%), and wound (n = 10; 15.6%). Despite the high proportion of these samples, no significant differences were found between various specimen types ($p > 0.168$). All CPKP isolates exhibited resistance to meropenem (100%), and only 33 (n = 51.6%) CPKP isolates were non-susceptible to imipenem. These differences in the rate of carbapenem resistance were not significantly associated with CPKP isolates ($p > 0.497$). Furthermore, the distribution of carbapenemase

Figure 2. Electrophoresis image showing the molecular detection of carbapenemase genes.



Lane M: 100bp DNA ladder, Lane 1: control strain of blaIMP, Lane 2: control strain of blaOXA-48, Lane 3: control strain of blaVIM, Lane 4: control strain of blaNDM, Lane 5: control strain of blaKPC, Lanes (6-8): representative strains of blaNDM, Lanes (9-11): isolates with double carbapenemase genes (blaVIM and blaNDM), Lane 12: isolate with triple carbapenemase genes (blaVIM, blaNDM & blaKPC).

genes based on clinical data of patients is demonstrated in Table 1. Generally, there were no significant differences between carbapenemase genes based on clinical data of patients ($p > 0.05$), while a significant difference was observed according to the gender of patients ($p < 0.05$). The association between imipenem resistance and the presence of *bla*$_{OXA-48}$ gene was observed ($p < 0.014$). Nevertheless, there was no correlation detected between the resistance to carbapenems and the other examined genes ($p > 0.05$).

Discussion

In the last two decades, the rate of carbapenem resistance in Arabian countries, including Saudi Arabia, has increased among *Enterobacteriaceae* species from 30.5 to 64.4%. Meanwhile, the prevalence of

Table 1. Clinical information and carbapenemase-producing *K. pneumoniae* (CPKP) isolates recovered from Jazan hospitals, Saudi Arabia.

No	Isolate	Patient			Source			IPM	MEM	ETP	AST of carbapenems (MICμg/mL)	Antibiotic-resistance profile
		Age	Sex	PN	Specimen	Unit	Hospital					
1	PM-K4	40	M	SA	Wound Swab	MSW	PM	I (2)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
2	AR-K6	48	M	SA	Sputum	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
3	PM-K7	64	M	SA	Wound Swab	MMW	PM	I (2)	R (8)	R (> 1)	AMC, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
4	PM-K8	87	M	SA	Urine	ICU	PM	I (2)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
5	KF-K9	34	M	SA	Blood	ICU	KF	R (≥ 16)	R (8)	N/T	AMC, AMK, CAZ, CFE, GEN, TZP, SXT	OXA-48
6	AR-K11	56	F	SA	Urine	FMW	AR	R (> 8)	R (> 8)	R (> 4)	AMC, CAZ, CFE, CIP, TZP, SXT	OXA-48
7	PM-K12	38	F	SA	Sputum	ICU	PM	I (2)	R (8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
8	PM-K13	82	M	SA	Sputum	ICU	PM	I (2)	R (8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
9	PM-K14	52	M	SA	Urine	ICU	PM	R (8)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
10	PM-K15	24	M	SA	Wound Swab	MSW	PM	R (8)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
11	PM-K16	87	M	SA	Blood	ICU	PM	R (> 8)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
12	PM-K18	27	F	SA	Urine	FMW	PM	R (8)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
13	KF-K20	58	M	SA	Blood	MSW	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
14	KF-K21	61	F	SA	Sputum	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
15	PM-K23	63	F	SA	Sputum	ICU	PM	I (2)	R (8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
16	PM-K24	68	M	SA	Wound Swab	ICU	PM	R (> 8)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	NDM
17	PM-K25	59	M	SA	Blood	MMW	PM	R (4)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
18	KF-K26	57	M	SA	Sputum	ICU	KF	R (4)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
19	PM-K28	72	M	SA	Blood	ICU	PM	S (≤ 1)	R (4)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GNT, TZP, SXT	OXA-48
20	PM-K29	86	M	SA	Sputum	ICU	PM	R (4)	R (8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
21	AR-K33	53	M	SA	Sputum	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, CAZ, CFE, CIP, GEN, TZP	OXA-48
22	KF-K34	29	M	NS	Tip Culture	ICU	KF	I (2)	R (8)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
23	KF-K35	60	M	SA	Blood	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
24	KF-K36	60	M	SA	Wound Swab	ICU	KF	R (8)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
25	KF-K37	60	M	SA	Sputum	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
26	KF-K38	74	F	SA	Urine	ICU	KF	R (4)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
27	AR-K39	79	M	SA	Sputum	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
28	AR-K40	67	M	SA	Urine	ICU	AR	R (8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
29	PM-K41	60	F	SA	Sputum	ICU	PM	S (≤ 4)	R (8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
30	PM-K44	53	M	SA	Bed sore	ICU	PM	I (2)	R (8)	R (> 1)	AMC, TGC, SXT	NDM/VIM
31	KF-K48	79	M	SA	ETT	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
32	KF-K50	74	M	SA	Urine	ICU	KF	I (2)	R (8)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
33	AR-K51	82	M	SA	Blood	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP	OXA-48
34	PM-K52	59	F	SA	Urine	FMW	PM	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
35	PM-K53	73	M	SA	Sputum	ICU	PM	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
36	PM-K54	67	M	SA	Sputum	ICU	PM	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
37	KF-K55	88	M	SA	Sputum	ICU	KF	I (2)	R (8)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
38	KF-K56	32	F	SA	Urine	FMW	KF	R (≥ 16)	R (≥ 16)	N/T	AMC, CAZ, CFE, TZP	NDM
39	KF-K57	79	M	SA	Blood	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
40	PM-K59	75	F	SA	Wound Swab	ICU	PM	R (> 8)	R (> 8)	R (> 4)	AMC, CAZ, CFE, TZP	NDM
41	AR-K62	59	F	SA	Urine	FMW	AR	R (> 8)	R (> 8)	R (> 4)	AMC, CAZ, CFE, CIP, TZP, TGC, SXT	NDM/VIM
42	AR-K63	69	M	SA	Wound Swab	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP	OXA-48
43	PM-K64	53	F	SA	Wound Swab	ICU	PM	S (≤ 4)	R (8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP	OXA-48
44	PM-K65	64	M	SA	Wound Swab	MMW	PM	S (≤ 4)	R (4)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
45	AR-K67	53	F	SA	Blood	FMW	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
46	AR-K71	63	F	SA	Blood	ICU	AR	R (> 8)	R (8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
47	AR-K75	68	M	SA	Sputum	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
48	PM-K76	90	F	SA	Sputum	ICU	PM	S (≤ 4)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	OXA-48
49	AR-K78	57	M	SA	Sputum	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
50	AR-K80	58	M	SA	Sputum	ICU	AR	R (> 4)	R (> 4)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
51	KF-K83	30	F	NS	HVS	FMW	KF	R (≥ 16)	R (≥ 16)	N/T	AMC, CAZ, CFE, CIP, TZP, SXT	NDM/VIM
52	PM-K85	37	M	SA	Tip Culture	CCU	PM	I (2)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
53	PM-K89	59	M	SA	Sputum	ICU	PM	I (2)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
54	PM-K91	56	F	SA	Urine	FMW	PM	S (≤ 4)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
55	PM-K94	54	F	SA	Urine	CCU	PM	I (2)	R (8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
56	KF-K99	64	M	SA	Sputum	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
57	KF-K100	59	M	SA	Urine	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
58	KF-K101	54	F	SA	Blood	ICU	KF	I (2)	R (≥ 16)	N/T	AMC, CAZ, CFE, TZP	OXA-48
59	PM-K102	67	M	SA	Wound Swab	CCU	PM	R (4)	R (> 8)	R (> 1)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
60	AR-K103	59	F	SA	Urine	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
61	AR-K104	73	M	SA	ETA	ICU	AR	R (> 8)	R (> 8)	R (> 4)	AMC, CAZ, CFE, CIP, TZP, SXT	OXA-48
62	KF-K105	69	F	SA	Blood	ICU	KF	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, TGC, SXT	KPC/NDM/VIM
63	KF-K108	49	M	NS	PF	ICU	KF	R (> 8)	R (> 8)	R (> 4)	AMC, AMK, CAZ, CFE, CIP, GEN, TZP, SXT	OXA-48
64	KF-K109	53	M	NS	ETA	ICU	KF	R (> 8)	R (> 8)	R (> 4)	AMC, CAZ, CFE, CIP, TZP	NDM

M: Male F: Female PN: Patient nationality; SA: Saudi Arabia; NS: Non-Saudi; PM: Prince Mohammed; KF: King Fahad; AR: Armed Forces; ICU: Intensive care unit; CCU: Cardiac care unit; MMW: Male medical ward; MSW: Male surgical ward; FMW: Female medical ward; AST: Antibiotic susceptibility test; MIC: Minimum inhibitory concentrations; S: Sensitive; R: Resistance; I: Intermediate; AMC: Amoxicillin-Clavulanic acid; AMK: Amikacin; CFE: Cefepime; CAZ: Ceftazidime; CIP: Ciprofloxacin; ETP: Ertapenem; GEN: Gentamycin; IPM: Imipenem; MEM: Meropenem; TZP: Piperacillin/Tazobactam; TGC: Tigecycline; SXT: Trimethoprim/Sulfamethoxazole; N/T: Not tested; ETT: Endotracheal Tube Tip; HVS: High Vaginal Swab; ETA: Endotracheal Aspirate; PF: Peritoneal Fluid.

carbapenemases, *bla*$_{OXA}$ types, *bla*$_{VIM}$ and *bla*$_{NDM}$ was 52.7%, 27.7%, and 26.3%, respectively [25]. In Saudi Arabia, different regions face a remarkable threat of clinical CPKP isolates. The 2017-2018 report of the Global Antimicrobial Resistance Surveillance System (GLASS), released by the WHO, revealed that the prevalence rate of carbapenemase-producing *Enterobacteriaceae* in Saudi Arabia ranged from 10% to 30% [2]. Here we reported for the first time the prevalence and genetic determinants of clinical CPKP isolates from the Jazan region.

The detection of carbapenemase-producing isolates is considered a challenge for many clinical laboratories and the automated susceptibility testing systems have shown variable results in detecting these isolates [26,27]. The current study showed complete resistance to meropenem (100%) compared to imipenem, and this result may support other studies that reported that meropenem is considered the most sensitive indicator for the activity of carbapenemases [28,29].

According to AST findings, the resistance rate of our isolates to different antimicrobial agents was high, which may be attributed to the acquisition of various resistance mechanisms. This resistance may result from the excessive use of different antimicrobial groups to treat an invasive infection caused by Gram-negative bacilli (GNB) isolates. In general, this result proved that awareness and proper usage of antimicrobial agents in healthcare facilities are still essential to reduce the rate of antimicrobial resistance. Molecular-based techniques, including multiplex PCR, have become the favorite choice for detecting of carbapenemase-producing isolates. The results of this technique revealed that the proportion of CPKP among CRKP isolates in our hospitals was 74.4%. This prevalence rate was lower than local reports from the southern region (90.8%) and central region (80.3%) [21,30].

From our CPKP isolates, 39.1% were obtained from elderly male patients, and 53.1% were isolated from urine and sputum together (Table 1). These findings are consistent with global data obtained from Spain [31] and South Korea [32]. Many reports of hospital surveillance for CRKP infections have commonly been associated with ICU patients [21,33,34]. Indeed, the variation in the proportion of CPKP isolates between ICU (75%) and other in-patient wards (25%) was observed in this study. In addition, several studies have shown that ICU hospitalization has been commonly associated with an increasing rate of CRKP infection or colonization due to prolonged hospitalization, poor functional status of patients, and uncontrolled treatment with antimicrobials [23,35,36].

Currently, *bla*$_{OXA-48}$ is considered the major carbapenemase-encoding gene causing carbapenem-resistance among *Enterobacteriaceae*, which is widely disseminated in the Middle East, including Arabian Gulf countries [17,37,38]. In the present study, *bla*$_{OXA-48}$ was expected to be a predominantly detected gene (81.2%) among CPKP isolates. This result is similar to the local report from the central region (67.6%) [30]. Additionally, this high prevalence of *bla*$_{OXA-48}$ further supports the previous study which reported that the *bla*$_{OXA-48}$-harboring *K. pneumoniae* was epidemic in the southern regions of Saudi Arabia [21,39]. Moreover, the rapid spreading of NDM-producing isolates has been reported worldwide, particularly in countries of the Indian subcontinent, which is currently considered the major endemic area for the *bla*$_{NDM}$ gene [12]. Recently, the epidemiology of NDM-producing *Enterobacteriaceae* was reported as a regional spread in the neighboring countries of Saudi Arabia, such as Sudan [36], Egypt [40], and Yemen [41]. Among our CPKP isolates, the presence of the *bla*$_{NDM}$ gene was 11.6%, which is in accordance with multicenter study [39], other local reports in Riyadh [30], southern region [21], and the western region [20]. The presence of the *bla*$_{VIM}$ gene in Saudi hospitals is less prevalent and mainly detected among non-*Enterobacteriaceae* isolates [42,43]. Therefore, the rate of the *bla*$_{VIM}$ gene in our study was only 5.8%, which supports their sporadic incidence in our countries.

In the last decade, many reports from the USA, Europe, China, and Australia have revealed the rapid dissemination of the KPC-producing isolates in healthcare facilities [16]. Notably, CRE in the Arabian Gulf region has been rarely associated with *bla*$_{KPC}$. However, the first *bla*$_{KPC}$-harboring *K. pneumoniae* in the Arabian Peninsula was reported in the United Arab Emirates [44]. Moreover, the neighboring countries of Saudi Arabia, such as Sudan and Egypt, have also reported the frequency of *bla*$_{KPC}$-harboring *K. pneumoniae* among hospitalized patients [45,46]. After the initial emergence of *bla*$_{KPC}$ among clinical *K. pneumoniae* isolates in Riyadh [19] and the western region [20], the current study also revealed the presence of *bla*$_{KPC}$ in only one isolate, which can be considered the third emergence in Saudi Arabia. Although there was no clinical information available related to patients with *bla*$_{KPC}$, this case may be justified by the fact that the traveling of Saudi people to *bla*$_{KPC}$-epidemic countries for study, tourism, or medication may lead to the dissemination of KPC-producing *K. pneumoniae* isolates in Saudi Arabia hospitals.

Although many previous studies [47-49] have reported that *bla*$_{NDM}$ is the main mechanism of carbapenem resistance in Gram-negative bacteria including *K. pneumoniae*, this study showed a clear correlation between carrying the *bla*$_{OXA-48}$ gene and imipenem resistance (with p values of < 0.014). Consequently, the *bla*$_{OXA-48}$ gene may play a significant role in carbapenem resistance, but other factors such as deficiency or decreased expression of porins and increased activity of bacterial efflux pump need to be considered.

Harboring multiple carbapenemase genes in a single isolate has become common in recent nosocomial isolates, particularly in GNB, due to their mobile genetic elements [50,51]. Remarkably, in this study, three isolates had co-harboring of double genes (*bla*$_{NDM}$ and *bla*$_{VIM}$), while one isolate had co-harboring of triple resistant genes (*bla*$_{NDM}$, *bla*$_{VIM,}$ and *bla*$_{KPC}$). In contrast, several local studies frequently reported the co-acquisition of *bla*$_{OXA-48}$ and *bla*$_{NDM-1}$ among *K. pneumoniae* isolates [17,30,39]. The emergence of such isolates in our region could be explained by the fact that traveling and/or migration of foreigners from endemic areas, such as the Indian Subcontinent and East Africa, to the southern region of Saudi Arabia account for the spread of these resistance mechanisms.

The COVID-19 pandemic represents a serious public health challenge worldwide. The impacts of this pandemic on global health are still unknown, including the potential effect of the COVID-19 pandemic on the spread and emergence of global antimicrobial resistance. Recently, the incidence of secondary bacterial infections due to CRKP was reported globally during the COVID-19 pandemic, particularly among ICU patients [45,52-55]. Here, we identified a clinical CPKP isolate that carried triple carbapenemase genes (*bla*$_{NDM}$, *bla*$_{VIM}$ and *bla*$_{KPC}$) in an ICU- patient hospitalized with COVID-19 who died. This unusual isolate is in addition to previous isolates reported from the US [56] and Saudi Arabia [20].

Conclusions

We introduced the first report about the prevalence of CPKP isolates in the Jazan region. A high-frequency isolation of OXA-48-producing *K. pneumoniae* was observed in Jazan hospitals, which confirms the continued dissemination of carbapenem resistance in Saudi hospitals. Although *K. pneumoniae* co-harboring *bla*$_{NDM}$ and *bla*$_{VIM}$ genes remained less spread in Saudi hospitals, regular surveillance for CPKP is required. A *K. pneumoniae* strain with triple carbapenemase genes was reported for the first time in southern Saudi Arabia. The emergence of such isolate in Saudi hospitals could threaten patients and healthcare workers and require great attention with rapid interventions to avoid further dissemination, particularly during the pandemic of COVID-19. The major limitation of this study is that the study did not include all carbapenem-susceptible and resistant isolates to accurately calculate the proportion of *K. pneumoniae* that are carbapenemase producers. The lack of sufficient funding also prevented the performing of whole-genome sequencing typing (WGST) to identify the common clones of CPKP. Therefore, further work should focus on determining the dissemination of common clones of CRKP in our hospitals.

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Authors' contributions

TB, MY, IA designed, performed the experiments, and edited the manuscript. TA, AKA, EA, MH collected the isolates, analyzed data, and helped in drafting the manuscript. All authors revised and approved the final manuscript.

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