EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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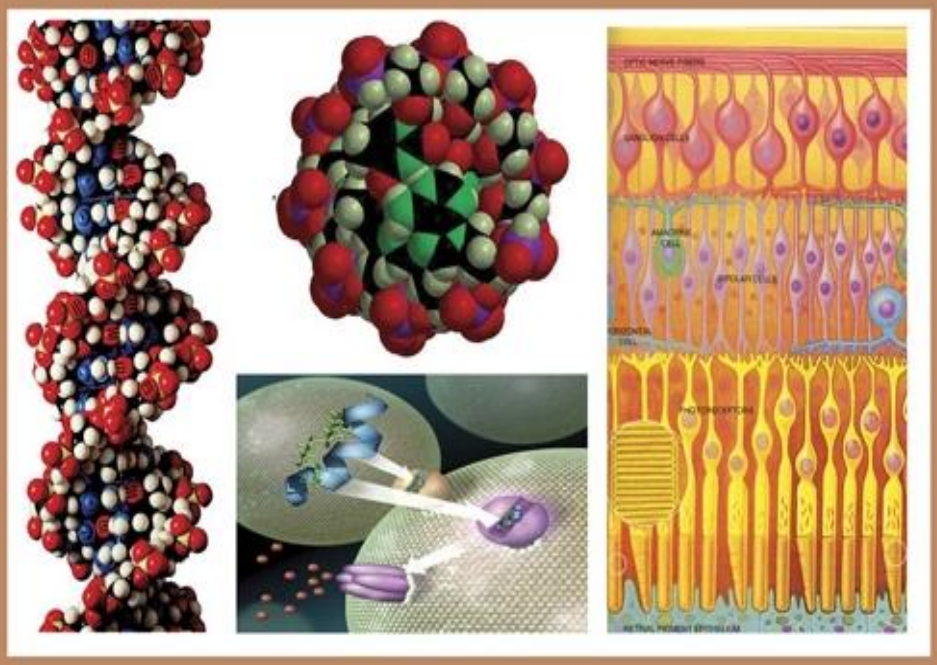

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)

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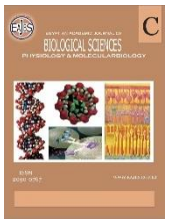
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(2) pp389-401 (2022)*
DOI: 10.21608/EAJBSC.2022.277002

Egypt. Acad. J. Biolog. Sci., 14(2):389-401 (2022)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Molecular Characterization and Biofilm Formation of Carbapenem-Resistant Gram-Negative Bacteria in King Faisal Medical Complex Hospital in Taif, Saudi Arabia

Reda Amashah[1], Rawan Abou-assy[1], Moufag H. Fida[2], Samyah Jastaniah[1], Sharifah Fahmi[3], Magda Aly[1,4*]

1-Department of Biology, King Abdulaziz University, Jeddah, Saudi Arabia.
2-Faculty of Medicine, King Abdulaziz University, Saudi Arabia.
3-Department of Microbiology, King Faisal Medical Complex, Taif, Makkah, Saudi Arabia.
4-Department of Botany and Microbiology, Kafrelsheikh University, Kafrelsheikh, Egypt.
*E. Mail: mmmohammad@kau.edu.sa

ARTICLE INFO
Article History
Received:13/10/2022
Accepted:30/11/2022
Available:5/12/2022

Keywords:
Carbapenemases, Enterobacterales, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA}$, *bla*$_{VIM}$, *bla*$_{IMP}$, lactamase, biofilm.

ABSTRACT

The aim of this study was to identify carbapenemase genes distributed among carbapenem-resistant Gram-negative bacteria (CR-GNB), previously collected from medical samples from different sites in King Faisal Medical Complex Hospital (KFMC), Taif, Saudi Arabia, and to determine the ability of these CR-GNB isolates to biofilm formation. The bacterial samples, collected from patients at FMC from November 2021 to April 2022 (6 months), were screened for carbapenem resistance (CR) by Phoenix System. Further, the detection of carbapenemase producer (CP) genes was performed using the XpertCarba-R molecular method. Overall, 236 clinical samples were determined as CR-GNB from 167 CR patients. The real-time PCR results show that 40.7% (68/167) of the CR isolates were positive for *bla*$_{NDM}$ and 33.5% (56/167) were positive for *bla*$_{OXA}$ while no isolates were positive for *bla*$_{KPC}$, *bla*$_{VIM}$ and *bla*$_{IMP}$ which mean41.3% (69/167) of the CR isolates didn't have any carbapenemase genes. There was a significant association between the type of carbapenem resistance genes within various CRorganisms, antibiotypes categories, and hospital service wards. There were three distinct groups of CP isolates circulating in KFMC. The first group of strains was positive for *bla*$_{OXA}$ (30, 18.6%). The second group of strains was positive for *bla*$_{NDM}$ 42 (25.1 %). The third group of isolates was positive for both *bla*$_{NDM}$ and *bla*$_{OXA}$ (26, 15.6%). Most CR *Klebsiella* spp. isolates were strong biofilm former (74/99, 74.7%) while lower numbers of CR *Klebsiella* spp. formmoderate biofilm (25/99, 25.3%). Also, the strong biofilms were the most common among CR *Pseudomonas* spp. (18/23, 78.3%), *Acinetobacter baumannii* (25/32, 78.1%), and other organisms (13/13, 100%). Weak biofilm just appeared in two isolates (8.7%) of *Pseudomonas* spp.It is clear that biofilm formation ability didn't distribute randomly in the various CR organisms and antibiotype categories. Carbapenemase-producing ability and biofilm formation ability are the most virulent factors of CR-GNB, the increase of carbapenemase-producing organisms in biofilm-producing isolates of CR-GNB is considered a serious alert.

INTRODUCTION

Carbapenem-Resistant Gram-Negative Bacteria (CR-GNB) is a worldwide urgent public health problem.

Similar to other countries, Saudi Arabia is facing the challenge of increasingly reported cases of CR-GNB(Abou-assy *et al.*, 2022; Alotaibi, 2019), while Europe and the US have established monitoring programs for surveillance of antibiotic resistance, the rest of the world is still behind. The reports of the Global Antimicrobial Resistance Surveillance System (GLASS) 2017 and 2018 showed that the prevalence rate of carbapenemase producers among Enterobacterales in Saudi Arabia ranged from 10-30% (WHO, 2017, 2018, Mendelson and Matsoso, 2015). It occurs mainly among *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Nordmann and Poirel, 2019), and may be intrinsic or mediated by transferable carbapenemase-encoding genes, the most effective carbapenemases reported worldwide among Enterobacteriaceae are the Ambler class A *K. pneumoniae* carbapenemase (*bla*$_{KPC}$), class B Metallo-β-lactamases (*bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM}$), and class D (*bla*$_{OXA-48}$) types (Meletis et al., 2012; Han et al., 2020; Zarakolu *et al.*, 2022)**.** In Saudi Arabia, recent studies have shown the predominance of *bla*$_{OXA-48}$ and *bla*$_{NDM}$ genes of carbapenemases (Al-Abdely *et al.*, 2021; Al Mutair *et al.*, 2021)**.** Virulence factors of CR-GNB include different adhesions, carbapenemase production, serum resistance, hemolysin production, and biofilm formation (Codjoe and Donkor, 2017). A biofilm is a slimy layer of bacterial cells that stick to wet surfaces that are enclosed in a self-produced matrix mainly composed of polysaccharides, secreted proteins, and extracellular DNAs, and adherent to an inert or living surface (Muhammad *et al.*, 2020). Bacterial biofilms are recognized as an important cause of many infections as 65-80% of all bacterial infections are related to biofilm formation (Costerton *et al.*, 1999). The association between antibiotic resistance and biofilm formation was reported many times (Al-Bayati and Samarasinghe, 2022; Yaita *et al.*, 2019).

Makkah region of Saudi Arabia includes the two largest cities (Makkah and Jeddah) with the highest population outside the central region. Millions of Muslims from across the globe arrive annually in Makkah to perform the Pilgrimage and Umrah. These mass gatherings could be a good environment for spreading multi-drug resistant organisms in this region, then around the world (Ibrahim, 2019; Leangapichart *et al.*, 2016). There were nine studies about carbapenem resistance published in the last five years from Makkah region, five of them from Makkah city (Al-Sultan, 2021; Al-Zahrani and Al-Ahmadi, 2021; Alraddadi *et al.*, 2022; Faidah *et al.*, 2017; Khan *et al.*, 2019), three studies from Jeddah city(Alhazmi *et al.*, 2022; Hala *et al.*, 2019; Shah *et al.*, 2019), and just one study reported from Taif (El-Badawy *et al.*, 2019), in addition, this study unincluded all Gram-negative bacteria. Seven of the previous studies detect the carbapenemase genes dissemination ratios. This study aims to identify carbapenemase genes distribution among all carbapenem resistance Gram-negative bacteria (CR-GNB) infections from King Faisal Medical Complex (KFMC), at Taif, Saudi Arabia, and to determine the ability to form biofilm of these CR-GNB during a study period and the correlation between the strong biofilm and CR genes.

MATERIALS AND METHODS

1. Source of Isolates and Study Design:

Isolates of CR-GNB were previously collected during six months, from November 2021 to April 2022 from KFMC in Taif, Saudi Arabia, characterized, and identified by Abou-assy *et al.*, (2022). The approval number from King Abdulaziz City for Science and Technology (KACST) was HAP-02-T-067.

2. Identification and Susceptibility Test of CR-GNB:

All clinical specimens from all units of KFMC were previously collected, transferred to the Clinical Microbiology Laboratory and grown on blood agar and MacConkey agar, identified using BD

Phoenix System 100 (Sparks, MD, USA) were tested for their sensitivity of carbapenem using BD Phoenix System by Abou-assy *et al.,* (2022). The sensitivity of carbapenem was confirmed by the disc diffusion method (Jin *et al.,* 2020, Yen *et al.,* 2022) and all the CR isolates were maintained on Glycerol Nutrient Broth medium (20% glycerol) at -70 to-80° C for a long period of storage until used (Bahramian *et al.,* 2019).

3. Molecular Characterization by XpertCarba-R assay:

XpertCarba-R (GeneXpert Cepheid, USA) was performed on significant isolates that indicated CR on automated drug susceptibility testing (Phoenix). This assay was performed using the GeneXpert platform (Cepheid, USA). The Xpert Carba-R assay is a qualitative *in vitro* real-time PCR assay designed to detect five carbapenemase gene families, including *bla*$_{IMP}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$, and *bla*$_{VIM}$. In more detail, the pure colony of the organism was transferred into the elution reagent tube and vortexed at high speed for 10 sec. Following the manufacturer's instructions, the contents of the elution reagent tube were transferred using the transfer pipette provided (approximately 1.7 ml) into the specimen chamber of the XpertCarba-R cartridge, and the run time was 48 min. The results were interpreted by the GeneXpert System (Sheth *et al.,* 2022).

4. Biofilm Formation Ability of CR-GNB:

A microtiter biofilm assay was performed to assess biofilm formation (Christensen *et al.,* 1985; Cusumano *et al.,* 2019). This assay is considered the standard for the evaluation of bacterial attachment and biofilm formation *in vitro*. Isolates were obtained from culture stocks, and stored at -80°C. After streaking on tryptic soy agar and incubating for 18 to 24 h, an inoculum of 10^6 CFU/mL in tryptic soy broth was prepared, and 10 µl activated of each CR-GNB isolate was added. All isolates were implemented in triplicate and the results were averaged. The total volume in each well was adjusted to 250 µl using tryptic soy broth (TSB). Plates were incubated at 37^{o} C for 24 h. After the incubation period, the contents of the microtiter plates were emptied, and the wells were washed three times with 300 µl of phosphate-buffered saline (PBS, pH 7.2). The remaining adhered bacteria were fixed with 250 µl of methanol per well. After 15 min, microtiter plates were poured off and air-dried. Wells were stained with 0.1% crystal violet (CV), for 5 min. The surplus of stain was rinsed off by placing the microtiter plates under slow-running tap water. After drying the microtiter plates, the dye bound to the adherent cells was resolubilized with 33% glacial acetic acid (El-Deeb *et al.,* 2018).

The absorbance of each well was measured at 595 nm using an ELISA reader (HumaReader HS, Human, Germany). *K. pneumoniae* (ATCC 700603) served as the biofilm-positive control and media alone was the negative control. The cut-off absorbance (Ac) was the mean absorbance of wells containing TSB only, without bacterial cells (negative control). Based on optical density OD_{595} obtained after 24 h by bacterial biofilms, strains were classified into four categories. Briefly, Strains were classified as follows: A = Ac = no biofilm producer (-); Ac < A ≤ (2Ac) = weak biofilm producer (+); (2 Ac) < A ≤ (4 Ac) = moderate biofilm producer (++); (4 Ac) < A = strong biofilm producer (+++) (Behzadi et al., 2022).

5. Statistical Analysis:

All statistical analyses were conducted with SPSS software (IBM Corp. released 2017, IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Categorical variables were compared by the Chi-square test or Fisher exact test, and *P* values ≤0.05 were considered significant (Al-Abdely et al., 2021).

RESULTS

A total, 236 of CR-GNB from the clinical samples sent from different KFMC wards from various clinical samples during the study period from November 2021 to April 2022 were molecularly characterized and these isolates referred to 167 patients. All the bacterial isolates from various

samples (Ex., urine, blood, sputum, and wound) to the same patient and have the same sensitivity profile was considered the same biotype.

1. Molecular Typing Of CR-GNB in KFMC for 6 Months:

Clinical specimens of CR-GNB isolates for 167 patients for 6 months were cultured and the Cepheid XpertCarba-R test was performed to screen for the presence of the carbapenemase-group genes (*bla*$_{OXA}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$ and *bla*$_{IMP}$), The prevalence of carbapenemase in CR-GNB in KFMC which responsible of high transmission of CR infection and cause an outbreak was recorded (98/167, 58.7%). Cepheid Gene Xpert real-time PCR showed that 40.7% (68/167) of the CR isolates were positive for *bla*$_{NDM}$, 33.5% (56/167) were positive for *bla*$_{OXA}$, and no isolates were positive for *bla*$_{KPC}$, *bla*$_{VIM}$ and *bla*$_{IMP}$ (Table 1). The real-time PCR results were positive for *bla*$_{OXA}$ of CR *Klebsiella* spp. (54/99, 54.5%), and *Pseudomonas* spp. (2/23, 8.7%) but the results of *bla*$_{OXA}$ were negative for *A. baumannii* (0/32, 0%) and other organisms (0/13, 0%). In another hand, CR *Klebsiella* spp. (51/99, 51.5%), *Pseudomonas* spp. (3/23, 13%), *A. baumannii* (10/32, 31.3%) and other organisms (4/13, 30.8%) were positive for *bla*$_{NDM}$. There 41.3% (69/167) of the CR isolates didn't have any carbapenemase genes. In detail, 20.2% (20/99) of CR *Klebsiella* spp., 78.3% (18/23) of CR *Pseudomonas* spp., 68.8% (22/32) of CR *A. baumannii* and 69.2% (9/13) of other CR organisms showed negative results for all five carbapenem resistance genes. There was a significant association between the types of carbapenem resistance genes within various CR organisms ($P < 0.05$, Chi-square test) which means that the distribution of CR genes was related to types of CR organisms.

Table 1 Distribution of the CR genes (*bla*$_{OXA}$, *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$ and *bla*$_{IMP}$) to 167 CR-GNB bacterial isolates.

		Positive results					Negative results (%)
		bla$_{OXA}$ (%)	*bla*$_{NDM}$ (%)	*bla*$_{KPC}$ (%)	*bla*$_{VIM}$ (%)	*bla*$_{IMP}$(%)	
***Klebsiella* spp.**	**(%)**	54.5	51.5	0	0	0	20.2
(n=99)	**No.**	(54)	(51)	0	0	0	(20)
***Pseudomonas* spp.**	**(%)**	8.7	13	0	0	0	78.3
(n=23)	**No.**	(2)	(3)	0	0	0	(18)
A. baumannii	**(%)**	0	31.3	0	0	0	68.8
(n=32)	**No.**	0	(10)	0	0	0	(22)
Others	**(%)**	0	30.8	0	0	0	69.2
(n=13)	**No.**	0	(4)	0	0	0	(9)
Total	**(%)**	33.5	40.7	0	0	0	41.3
(n=167)	**No.**	(56)	(68)	0	0	0	(69)
P-value		0.000					

The real-time PCR results showed that there were four distinct groups of CR isolates circulating in KFMC. The first group of isolates was positive for *bla*$_{OXA}$ (30/167, 18.6%). The second group of strains was positive for *bla*$_{NDM}$ (42/167, 25.1 %). The third group of strains was positive for both *bla*$_{NDM}$ and *bla*$_{OXA}$ (26/167, 15.6%). The fourth group of strains was negative for all carbapenem resistance genes (69/167, 41.3%) as shown in Table 2.

Table 2. CP gene patterns prevalence by real-time PCR in the 167 resistant isolates collected for 6 months.

CRE Positive Prevalence (%)		*bla*$_{OXA}$	*bla*$_{NDM}$	*bla*$_{KPC}$	*bla*$_{VIM}$	*bla*$_{IMP}$
No.	30	+	-	-	-	-
(%)	18.6					
No.	42	-	+	-	-	-
(%)	25.1					
No.	26	+	+	-	-	-
(%)	15.6					
Total	98	**56**	**68**	**0**	**0**	**0**
(%)	58.7	**33.5**	**40.7**	**0**	**0**	**0**

2. Distribution of CR Genes Patterns Among Biotypes, Antibiotypes Categories, Hospital Wards and Bodies Sites:

Out of 99 CR *Klebsiella* spp., 25 contained only *bla*$_{NDM}$, 28 contained *bla*$_{OXA}$, 26 contained *bla*$_{NDM}$ + *bla*$_{OXA}$ and CR genes disappeared in the other 20 isolates.Out of 32 CR *A. baumannii*, 10 *A. baumannii* contained only *bla*$_{NDM}$, in 22 isolates CR genes disappeared. Out of 23 CR *Pseudomonas* spp., 3 contained only *bla*$_{NDM}$, 2 contained the *bla*$_{OXA}$ gene and in the other 18 *Pseudomonas* spp. isolates CR genes disappeared. Statistically, there are significant differences between the CR genes pattern and biotype ($P < 0.05$ by Chi-Square test) which means that each kind of biotype has a special CR genes pattern shown in (Table 3). The antibiotic susceptibility profiles of CR-GNB isolates were studied to detect antibiotype categories (MDR, XDR, and PDR) percentages among several CR gene patterns. Multiple drug resistance (MDR) is antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories, extensively drug-resistant (XDR) is the resistance of one bacteria species to all antimicrobial agents except in two or fewer antimicrobial categories but pan-drug resistant (PDR) is the non-susceptibility of bacteria to all antimicrobial agents in all antimicrobial categories. According to the broth microdilution test, out of 167 carbapenem resistance isolates, 39 strains were MDR to antimicrobials, 12 strains contained *bla*$_{NDM}$, 5 strains contained *bla*$_{NDM}$ + *bla*$_{OXA}$ genes, and 22 strains had no CR genes. In addition, 100 isolates showed XDR, 22 isolates contained *bla*$_{NDM}$, 18 isolates contained *bla*$_{OXA}$ and 17 isolates contained *bla*$_{NDM}$ and *bla*$_{OXA}$. Twenty-eight strains were pan-drug resistant, 8 strains of them contained *bla*$_{NDM}$, 12 strains contained *bla*$_{OXA}$, 4 strains contained *bla*$_{NDM}$+*bla*$_{OXA}$ genes, and 4 strains have no CR genes. There was a significant difference between CR gene patterns among antibiotypes categories of resistance ($P < 0.05$ by Chi-Square test).

According to the distribution of CR genes pattern among KFMC service wards, the *bla*$_{OXA}$ gene most commonly appeared in the ICU-CCU units, while the *bla*$_{NDM}$ gene most commonly appeared in Burn Unit, HDU-BED, MMW, FMW, MSWand OPD units. In addition, *bla*$_{NDM}$ + *bla*$_{OXA}$ were the most common CR genes pattern in ISO, Maternity, ER and LTCU units. There is a significant association between CR gene patterns and Service Units ($P < 0.05$, Chi-Square test) which means that the CR pattern of the biotype wasn't distributed randomly in the hospital wards.

About the site of infection, the *bla*$_{OXA}$ gene most appeared in blood, urine, and sputum samples, while the *bla*$_{NDM}$ gene most appeared in wound samples, but there was an insignificant association between the site of infection and CR gene patterns ($P > 0.05$ by Chi-Square test).

Table 3: Significant parameter of 167 CR-GNB biotypes and CR genes using Chi-Square test.

Prognostic factors		*bla*NDM	*bla*OXA	*bla*NDM+*bla*OXA	No gene	P value
Biotype	*Klebsiella* spp.	25	28	26	20	0.000*
	Pseudomonas spp.	3	2	0	18	
	A. baumannii	10	0	0	22	
	Others	4	0	0	9	
	Total	(n=42)	(n=30)	(n=26)	(n=69)	
Antibiotype	MDR	12	0	5	22	0.000*
	XDR	22	18	17	43	
	PDR	8	12	4	4	
	Total	(n=42)	(n=30)	(n=26)	(n=69)	
Hospital Service	ICU-CCU	12	16	6	12	0.003*
	Burn Unit	2	0	0	0	
	HDU-BED	8	2	4	12	
	MMW	6	2	2	8	
	FMW	5	3	2	4	
	MSW	8	6	4	12	
	FSW	2	0	2	8	
	ISO	2	0	4	0	
	Maternity and Delivery	0	0	1	0	
	ER	0	0	4	4	
	LTCU	2	2	6	2	
	NICU	0	0	0	2	
	OPD	2	0	0	0	
	INPS	0	0	0	2	
	ANT5	0	2	2	2	
		49	33	37	68	
Source	Sputum	11	17	12	23	0.206
	Blood	16	17	14	24	
	Urine	14	15	12	24	
	Wound	11	4	5	2	
	Others	4	2	5	3	
		56	55	48	76	

ICU: intensive care unit; CCU: cardiac care unit; HDU-BED: high dependent unit; MMW: male medical ward; FMW: female medical ward; MSW: male surgical ward; FSW: female surgical ward; ISO: isolation; ER: emergencyroom; LTCU: long term care unit; NICU: nursery intensive care unit; OPD: outpatient department; INPS: infants and pediatrics isolation; ANT5: antenatal care.* Significant association

3. Ability of CR-GNB to Biofilm Formation:

Optical density (OD_{595}) for all 167 isolates to evaluate the ability of biofilm formation was measured and divided into three categories as follows: weak (n = 2; $OD_{595} \leq 0.13$), moderate (n = 35; $0.13 < OD_{595} > 0.26$), and strong biofilm formers (n = 130; $OD_{595} \geq 0.26$). The most common category of biofilm formation among CR-GNB was strong (130/167, 77.8%), then was moderate biofilm former (35/167, 21%), and slightly weak biofilm former (2/167, 1.2%). Out of 99 CR *Klebsiella* spp. isolates, seventy-four (74.7%) isolates were strong biofilm former, and twenty-five (25.3%) isolates were moderate biofilm former. Also, the strong biofilms were the most common among CR *Pseudomonas* spp. (18/23, 78.3%), *A. baumannii* (25/32, 78.1%), and other organisms (13/13, 100%). Weak biofilm just appeared in two isolates (8.7%) of *Pseudomonas* spp. as appeared in (Table 4). There is a significant association between CR organisms and the ability of biofilm formation ($P < 0.05$, Chi-Square test) which means that biofilm formation ability wasn't distributed randomly in the various CR organisms, but there is no impact of CR gene's various patterns on the ability of biofilm formation ($P>0.05$, Chi-square test).

Strong biofilms were more common and similarly established among XDR (n = 81, 81%), MDR (n = 31, 79.5%) and PDR (n = 18, 64.3%). PDR isolates (n = 28) were more common among moderate biofilm formers (n = 8, 28.6%) versus weak biofilm formers (n = 2, 7.1%; P = 0.02). The mean OD_{595} values of 103 CR-GNB strains were more than 0.6 in the microtiter biofilm assay, which indicated a very high activity of biofilm formation. There is a significant difference in biofilm formation among MDR, XDR and PDR ($P < 0.05$, Chi-Square test).

Table 4. The ability of CR-GN biofilm formation among different biotypes, CR-GNB genes and antibiotypes.

		Biofilm formation			Total (%)	P-value
		Weak (%)	Moderate (%)	Strong (%)		
Biotype	*Klebsiella* spp.	0	25	74	99	0.007*
		0.0	25.3	74.7	100.0	
	Pseudomonas spp.	2	3	18	23	
		8.7	13.0	78.3	100.0	
	A.baumannii	0	7	25	32	
		0.0	21.9	78.1	100.0	
	Others	0	0	13	13	
		0	0	100	100	
CR-GN genes	bla_{NDM}	0	4	38	42	0.115
		0.0	9.5	90.5	100.0	
	bla_{OXA}	0	10	20	30	
		0.0	33.3	66.7	100.0	
	bla_{NDM}+bla_{OXA}	0	4	22	26	
		0.0	15.4	84.6	100.0	
	No genes	2	17	50	69	
		2.9	24.6	72.5	100.0	
Antibiotype	MDR	0	8	31	39	0.02*
		0.0	20.5	79.5	100.0	
	XDR	0	19	81	100	
		0	19	81	100	
	PDR	2	8	18	28	
		7.1	28.6	64.3	100.0	
Total		**2**	**35**	**130**	**167**	

*** Significant association**

DISCUSSION

Detecting infection and colonization with Carbapenemase is important information for antimicrobial stewardship programs and infection control, for undertaking such surveys (Balkhy *et al.*, 2018). This is consistent with guidance from the Centers for Disease Control and Prevention (US CDC and the eCDC) that carbapenemase producers have the highest impact on infection control activities (Lyman *et al.*, 2015; Prevention and Control, 2014). However, the rate of clinical CR-GNB detection in KFMC at Taif was 32% whereas the CR percentage among all clinical specimens was 1.8% (Abou-assy *et al.*, 2022). In this study, several methods to detect and confirm the carbapenem resistance infection were used including culture (Phoenics automated system, and Kirby Bauer) and molecular method (XpertCarba-R Assay). However, culture methods capture all mechanisms of carbapenem resistance, including efflux and porin-mediated resistance, but molecular method detect carbapenemase genes which are known as transmissible mechanisms of resistance. Without culture results, it is not clear whether the detection of multiple CR genes represents a single organism with multiple CR genes, or separate organisms each with a single resistance gene (Kralik and Ricchi, 2017). In addition to the culture method, we focus on carbapenemase producer organisms, whereas the prevalence of CP in our study was 16.3% among clinical infection (124/763 bacterial isolates) and 52.5% of CR isolates (124/236 CR-GNB), lower than the CP percentage in recent U.S. study(Wilson *et al.*, 2021), one-third of clinical cultures were CP and an apparent increase in the number of CP in CR cultures from %23.3in 2013 to 46.3% in 2018 was reported(Wilson *et al.*, 2021). Compared with the recent study from Gulf Cooperation Council hospitals which screened 529 specimens, 138 (26.1%) were positive for one or more CR genes. The positivity rates

of CP among the hospitals ranged from 8.0% to 67.3%; 20% of the positive specimens harbored 2 CRGs. The most common CR gene detected was *bla*$_{\text{OXA-48}}$ (15.5%) (Alqahtani *et al.,* 2021). The most common CP genes in this study were *bla*$_{\text{NDM}}$ (68/167, 40.7%), and *bla*$_{\text{OXA}}$ (56/167, 33.5%), and no isolates were positive for *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$. This is in contrast with other countries such as the USA, Israel, Greece, and some countries in Latin America where *bla*$_{\text{KPC}}$ continues to be the predominant CP gene (Karlowsky *et al.,* 2017).

In this study, there were 41.3% (69/167) of the CR isolates in KFMC at Taif didn't have any carbapenemase genes and these isolates might harbor other carbapenemases enzymes not included in XpertCarba-R Assay like *bla*$_{\text{OXA-48-like}}$ carbapenemase or they might contain other mechanisms for carbapenems resistance including the diminishment of outer membrane permeability, or *bla*$_{\text{Amp-C}}$ β-lactamases which consists with another study (Al-Abdely *et al.,* 2021) which reported15.4% of CR isolates weren't CP organisms by Xpert Carba-R Assay. The mean percentages of genes encoding for carbapenemase-producing *K. pneumoniae* were 28/99 (28.3%) for *bla*$_{\text{OXA}}$, 26/99 (26.3%) for *bla*$_{\text{NDM}}$ + *bla*$_{\text{OXA}}$ and 25/99 (25.3%) for *bla*$_{\text{NDM}}$. Nearly was similar to previous studies whereas the single *bla*$_{\text{OXA-48}}$ type was the most common (71.2%) followed by *bla*$_{\text{NDM-1}}$ (20.5%), and the least common isolates were those harboring both *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM-1}}$, across all study centers (Al-Abdely *et al.,* 2021). Another more recent study from the central region performed on 31 carbapenem-resistant Enterobacterales showed that *bla*$_{\text{OXA-48}}$ and *bla*$_{\text{NDM-1}}$ accounted for 58% and 42%, respectively (Al-Agamy *et al.,* 2018). Another study from the two largest hospitals in the southern region detected 81.5% *bla*$_{\text{OXA-48}}$ and 7.4% *bla*$_{\text{NDM-1}}$, respectively (Al-Zahrani and Alasiri, 2018).

In this study, all carbapenemase-producing *A. baumannii* expressed from the *bla*$_{\text{NDM}}$ gene (10/32, 31.3%), and 68.8% (22/32) of CR *A. baumannii* showed negative results for all five carbapenem resistance genes in Carba-R assay. In contrast, CR *A. baumannii* isolates in central rejoin showed a prevalence of the *bla*$_{\text{OXA-23}}$ gene with an occurrence of 85.7%, and the ISAba-1 insertion sequence was found in 27 isolates (Jawhar et al., 2020). The mean percentages of genes encoding for carbapenemase-producing *Pseudomonas* spp. were (2/23, 8.7%) for *bla*$_{\text{OXA}}$, and (3/23, 13%) for *bla*$_{\text{NDM}}$,78.3% (18/23) of CR *Pseudomonas* spp. didn't have any carbapenemase genes. In contrast to the previous study from Riyadh, any traces of *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$, *bla*$_{\text{NDM}}$ and *bla*$_{\text{KPC}}$ were not detected in any of CR *P. aeruginosa* isolates while, the most prevalent genes were *bla*$_{\text{OXA-23}}$ (55%) followed by *bla*$_{\text{VIM}}$ (46%), *bla*$_{\text{OXA-1}}$ (22%) and *bla*$_{\text{GIM}}$ gene (15%) of the isolates(Jawhar *et al.,* 2020). Also, Al gamy *et al.* (2016) reported that *bla*$_{\text{VIM-like}}$ genes were the predominant MβLs among *P. aeruginosa.* isolates in Riyadh, Saudi Arabia (Al-Agamy *et al.,* 2018). But, the most common carbapenemase-encoding gene of CR*P. aeruginosa* isolates (n=35) were collected from a tertiary and quaternary hospital in Makkah was *bla*$_{\text{VIM}}$ (n=11), while *bla*$_{\text{GES}}$ was reported in only three isolates, whereas 56% were negative for common carbapenemase genes and approximately one-third (31.4%) of the isolates belonged to two the high-risk clones (ST235 and ST654) (Al-Zahrani and Al-Ahmadi, 2021). This finding probably reflects the fact that in the absence of carbapenem hydrolyzing enzymes, carbapenem resistance in *P. aeruginosa* frequently originates from the loss of the porin and the overexpression of efflux pumps (McCracken *et al.,* 2019).

The results of a previously published article from Makkah city were as that, 120 confirmed Enterobacteriaceae isolates were investigated, 21.7% of isolates showed resistance to carbapenems, and the majority were *K. pneumoniae*. Remarkably, 65% of isolates carried triple resistant genes (*bla*$_{\text{KPC}}$, *bla*$_{\text{NDM-1}}$, and*bla*$_{\text{OXA-48}}$) while 15% carried double resistant genes (*bla*$_{\text{KPC}}$ and

bla$_{OXA-48}$) or *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ (Khan *et al.*, 2019). Another earlier study from Makkah indicated that most of the cases of *A. baumannii* infection were acquired in the hospital, 55.6% of *A. baumannii* isolates were CR isolates. The *bla*$_{OXA-23}$ gene and ISAba1 upstream of *bla*$_{OXA-23}$ were detected in 92% of the CR isolates, while all CR isolates were found to carry *bla*$_{OXA-51}$, and *bla*$_{ADC-type}$ (*Acinetobacter*-derived cephalosporins, ADC) gene. The *bla*$_{IMP}$ gene was detected in 84% of isolates, and two isolates carried the *bla*$_{NDM-1}$ gene (Shah *et al.*, 2019), while El-Badawy *et al.* (2019) study was the only study from Al-Taif city and examined ten common carbapenemase genes among 32 clinical CR *A. baumannii* isolates, *bla*$_{OXA-51}$, *bla*$_{IMP}$, *bla*$_{NDM}$ and *bla*$_{OXA-23}$ were detected in 100%, 87.5%, 62.5% and 59.4% of isolates, respectively. Also, *bla*$_{VIM}$ and *bla*$_{OXA-40}$ were less prevalent and were detected in 9.3% and 3.1% of the isolates, respectively. In addition, *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{OXA-58}$, and *bla*$_{OXA-181}$ were not detected in any isolate, and all the CR isolates were sensitive to polymyxin B and colistin (El-Badawy *et al.*, 2019).

Most CR-GNB was strong biofilm formers (77.8%), while the weak biofilm just appeared in two isolates (8.7%) of *Pseudomonas* spp. in KFMC at Taif. Similar to another study, 77.9% and 22% of isolates were reported as strong and moderate biofilm producers, the significant correlation also was seen between biofilm formation ability and carbapenem-resistant isolates but a weak biofilm producer hasn't been seen (Rahdar *et al.*, 2019; Jamal *et al.*, 2022). But in contrast, a previous study showed that CR *K. pneumoniae* isolates were 91% less likely to be strong biofilm formers (Cusumano *et al.*, 2019). In this study, there was an insignificant association between CR genes and the biofilm formation category, in contrast, to earlier two studies, a significant correlation between strong biofilm formation and the prevalence of some CR genes was reported among*bla*$_{VIM1}$ and *bla*$_{IMP1}$ genes(Khodadadian *et al.*, 2018) and *bla*$_{IMP}$ of *E. coli* and *bla*$_{NDM-1}$ of *K. pneumoniae*(Al-Bayati and Samarasinghe, 2022).

Conclusion

Carbapenem resistance Gram-negative bacteria have survived in harsh conditions using biofilm formation in hospital equipment and cause nosocomial infections, the emergence and extension of carbapenem resistance burden among CR-GNB producing biofilm is the current concern of public health services. Carbapenemase-producing organisms have a high transmission ability and cause severe infection in KFMC at Taif, our study provides valuable epidemiological data for use of newβ-lactamase inhibitor combinations, which have variable antibacterial activity depending on the molecular type of CR-GNB; for example, ceftazidime/avibactam has higher activity against *bla*$_{OXA-48}$CR-GNB type and the addition of aztreonam is required to treat *bla*$_{NDM-1}$CR-GNB types.

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