original article

Molecular characterization of carbapenem-resistant *Enterobacterales* in thirteen tertiary care hospitals in Saudi Arabia

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BACKGROUND: Carbapenems are the antibiotics of last-resort for the treatment of bacterial infections caused by multidrug-resistant organisms. The emergence of resistance is a critical and worrisome problem for clinicians and patients. Carbapenem-resistant *Enterobacterales* (CRE) are spreading globally, are associated with an increased frequency of reported outbreaks in many regions, and are becoming endemic in many others.

OBJECTIVES: Determine the molecular epidemiology of CRE isolates from various regions of Saudi Arabia to identify the genes encoding resistance and their clones for a better understanding of the epidemiological origin and national spread.

DESIGN: Multicenter, cross-sectional, laboratory-based study.

SETTING: Samples were collected from 13 Ministry of Health tertiary-care hospitals from five different regions of Saudi Arabia.

METHODS: Isolates were tested using the GeneXpert molecular platform to classify CRE.

MAIN OUTCOME MEASURES: Prevalence of various types of CRE in Saudi Arabia.

SAMPLE SIZE: 519 carbapenem-resistant isolates.

RESULT: Of 519 isolates, 440 (84.7%) were positive for CRE, with *Klebsiella pneumoniae* (410/456, 90%) being the most commonly isolated pathogen. The distribution of the CRE-positive *K pneumoniae* resistance genes was as follows: OXA-48 (n=292, 71.2%), NDM-1 (n=85, 20.7%), and NDM+OXA-48 (n=33, 8%). The highest percentage of a single blaOXA-48 gene was detected in the central and eastern regions (77%), while the bla_{NDM}-gene was the predominant type in the northern region (27%). The southern regions showed the lowest percentages for harboring both bla_{OXA-48} and bla_{NDM} genes (4%), while the

western region isolates showed the highest percentage of harboring both genes (14%).

CONCLUSION: The results illustrate the importance of molecular characterization of CRE isolates for patient care and infection prevention and control. Larger multicenter studies are needed to critically evaluate the risk factors and trends over time to understand the dynamics of spread and effective methods of control.

LIMITATIONS: Lack of phenotypic susceptibility and clinical data.

CONFLICT OF INTEREST: None.

Antimicrobial resistance (AMR) is a major concern nationally and internationally. Major international efforts are ongoing by the World Health Organization to combat AMR through the initiation of a global action plan for antimicrobial resistance, the Global Antimicrobial Resistance Surveillance System (GLASS). The most recent reports of GLASS 2017 and 2018 showed that the prevalence rate of carbapenemase producers among *Enterobacterales* in Saudi Arabia range from 10-30%.[1-3] Saudi Arabia became a leading member of these two programs in the Middle East. Continuous monitoring, molecular characterization, and identification of the source of these mechanisms of resistance are required to limit the spread of AMR.[4,5] Globally, infections caused by gram-negative bacteria are associated with high rates of morbidity and mortality and cause many types of infections including bloodstream, urinary tract, respiratory tract and gastrointestinal tract infections.[6] The emergence and dissemination of antibiotic resistance in gram-negative bacteria is complicating the treatment of serious nosocomial infections and is already creating bacterial species resistant to all currently available agents.[7,8]

Increasing degrees of resistance to major antimicrobials were first caused by extended-spectrum-β-lactamase (ESBL) producing gram-negative bacteria in the mid-2000's, and more recently by carbapenemase-producers, which are a huge clinical and public health concern worldwide; countries of the Gulf Cooperation Council (GCC) are no exception.[9] Carbapenems are the last-resort antibiotics in the treatment of bacterial infections caused by multidrug-resistant organisms, and the emergence of resistance is an extremely critical and worrisome problem for clinicians and patients. The WHO considers carbapenem-resistant *Enterobacterales* to be a critical public health issue of global concern that requires immediate action.[10] Resistance to carbapenems develops by several mechanisms; in *Enterobacterales,* this is mainly achieved by the acquisition of carbapenemases, resulting in carbapenem-resistant *Enterobacterales* (CRE). CRE are now spreading globally and are associated with an increased frequency of reported outbreaks in many regions; CRE are becoming endemic in some parts of the world.[11-13] Other mechanisms of carbapenem resistance in *Enterobacterales* include permeability changes, AmpC- and ESBL acquisition/overexpression, and an efflux mechanism.[14]

There are five classes of carbapenemases found globally, which include three Ambler classes: KPC (*Klebsiella pneumoniae* carbapenemase) (class A); IMP (imipenem), NDM-1 (New Delhi metallo-β-lactamase), and VIM (Verona integron-encoded metallo-β-lactamase) (class B); and OXA-48-like (OXA: oxacillinase) carbapenemase (class D).[12] Carbapenemase genes have very effective transmission abilities because they are encoded on mobile genetic elements, and their spread has been associated with both successful high-risk clones (e.g., KPC in *Klebsiella pneumoniae* clonal group ST258)[15] and inter- and intra-species plasmid spread (e.g., bla_{KPC} in pKpQIL and $bla_{OXA-48-like}$ in pOXA-48a).[16] Additionally, these carbapenemase plasmids can harbor genes that encode resistance to other antibiotic classes like quinolones, polymyxin, and aminoglycosides that might be encoded on other plasmids or the chromosome, resulting in difficulty in finding other options to treat a patient infected with these organisms. These infections are associated with prolonged hospital stays and increased mortality.[17-20] In this study, we aimed to determine the molecular characteristics of CREs isolated from various regions of Saudi Arabia to identify the genes and clones encoding resistance to better understand their epidemiological origin and spread.

METHODS

This multicenter, cross-sectional, laboratory-based study included Ministry of Health tertiary-care hospitals and laboratories selected from five different regions Saudi Arabia, which makes the data representative of

the country. These hospital selections were based on special consideration, i.e., the number of beds, scope of services provided, and number of isolates obtained over one year starting from January to December, 2018. We included the first non-duplicate CRE isolated from each patient received from these hospitals, along with the demographic information on patients per the study protocol. All participating laboratories used the Clinical and Laboratory Standards Institute (CLSI) definition of CRE, screening by an automated system, and confirmation by E-test if the MIC was ≥1 for imipenem or meropenem. Molecular testing was performed using the Xpert Carba-R Assay (Cepheid, Sunnyvale, California, United States), a real-time polymerase chain reaction assay for the detection of bla_{KPC}, blaNDM-1, bla_{VIM}, bla_{IMP}, and bla_{OXA-48} carbapenem resistance genes from bacterial isolates grown on blood agar or MacConkey agar.[21] All data were analyzed using SPSS software (IBM Corp. released 2017, IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) to calculate percentages, chi-square values and *P* values. The respective institutional ethics and review boards of the included centers approved this study (Research Project No. E-20-4613).

RESULTS

Of the 519 carbapenems-resistant isolates tested, 440 (84.7%) tested positive for CRE (**Table 1**). The rate of CRE was highest among *K pneumoniae*, with relatively few numbers of other gram-negative bacteria. All the three CRE-positive *K oxytoca* isolates were NDM-1 producers. Similarly, NDM-1 production was detected in 5/8 CRE-positive E cloacae, followed by 3 OXA-48 (n=3, 38%). IMP1, VIM, and KPC were not detected in any of these isolates. The anatomical source and classification of the 519 carbapenem-resistant *Enterobacterales* isolates are shown in **Table 2**. The distribution by region is shown in **Figure 1**.

Tables 3 and 4 show the molecular classification of CRE-positive *K pneumoniae* across the different Saudi regions and hospitals participating in the study. The highest percentage of a single bla_{OXA-48} gene was detected in the central and eastern regions (77%), and the lowest percentage was detected in the northern region (61%). For the single bla_{NDM-1} gene the highest percentage was in the northern region (27%) and lowest in the central and eastern regions (15%). The southern region isolates showed the lowest percentage (4%) of both bla_{OXA-48} and bla_{NDM-1} genes present together whereas the western region isolates showed the highest percentage harboring both genes (14%). This slight difference in the prevalence of carbapenemase types among regions was not statistically significant (chi-square=9.2854, df=6, *P*=.1582).

DISCUSSION

Over the past ten years, carbapenemase-producing *Enterobacterales* (CRE) have been increasingly report-

Table 1. Molecular characterization of the 519 carbapenem-resistant *Enterobacterales* isolates.

	Positive result	None Detected	Molecular classification			
			NDM-1	OXA-48	NDM-1 + OXA-48	VIM
Number of isolates (n=519)	440/519 (85)	79/519 (15)	103/519 (20)	298/519 (57)	37/519 (7)	2/519 (0.4)
K pneumoniae (n=456)	410/456 (89.9)	46/456 (10.1)	85/410 (20.7)	292/410 (71.2)	33/410 (8)	-
K oxytoca (n=3)	3/3 (100)	-	3/3 (100)	-	-	-
E coli (n=21)	13/21 (61.9)	8/X (30)	9/13 (69)	1/13 (7.7)	3/13 (23)	-
E cloacae (n=13)	8/13 (62)	5/13 (38)	5/8 (63)	3/8 (38)	-	-
Other gram-negative organisms[a] (n=26)	26 (100)	20/26 (76.9)	1 (3.8)	2 (7.7)	1 (3.8)	2 (7.7)

Data are number (%). [a]*Proteus mirabilis* VIM and OXA-48 (n=2/8), *Pseudomonas fluorescens* VIM (n=1/1), *Acinetobacter baumannii* NDM (n=1/8), *K ozaenae* OXA-48 (n=1/1), *Prevotella denticola* NDM (n=1) and 1 OXA-48 (n=1), and *Serratia marcescens* (n=3) *Morganella morganii* (n=2) *E aerogenes* (n=1), *Pseudomonas* (n=1) negative for all genes.

ed worldwide,[22-24] and their dissemination has been highlighted as a significant global public health threat. *Enterobacterales* that produce carbapenemases commonly have so few treatment options that their spread threatens our system of modern healthcare. Recent GLASS data have shown an increase in the rate of carbapenem-resistant *K pneumoniae* isolated in 2017 and 2018 from different infection sites including urine isolates (increased from 15% to 22%) and blood isolates (increased from 30% to 35%).[25]

Table 2. The anatomical source of 519 carbapenem-resistant *Enterobacterales* isolates (n=519).

Respiratory system	136 (26.2)
Urine	115 (22.2)
Tissue and wound	103 (19.8)
Blood	62 (11.9)
Swab	32 (6.17)
Fluid	17 (3.28)
Unknown	54 (10.4)

Data are number (%).

Since the discovery and early isolation of the first metallo-β-lactamase from a urinary tract infection in a Swedish patient who had traveled to New Delhi in 2008,[26] there has been a dramatic spread and global dissemination of this carbapenemase-producing gene (named the New Delhi metallo-β-lactamase) worldwide, with several reports showing variability in the prevalence of NDM-1 and other types of carbapenemases in *Enterobacterales* across different countries. Before long, CRE became endemic to Asia, northern European and Pacific regions, the UK, and the Middle East.[27-30] The other types of carbapenemases in *Enterobacterales* common to the Middle East and Arabian countries are OXA-48 and OXA-48-like (i.e., OXA-163 and OXA-181) carbapenemases with a prevalence range of 2–10%.[30-33]

In the current study, the mean percentages of genes encoding for carbapenemase-producing *K pneumoniae* were consistent with previous studies: the single OXA-48 type was the most common (71.2%) followed by NDM-1 (20.5%), and the least common isolates were those harboring both OXA-48 and NDM-1, across all study centers. Two earlier studies from central Saudi Arabia reported the following: the first one performed

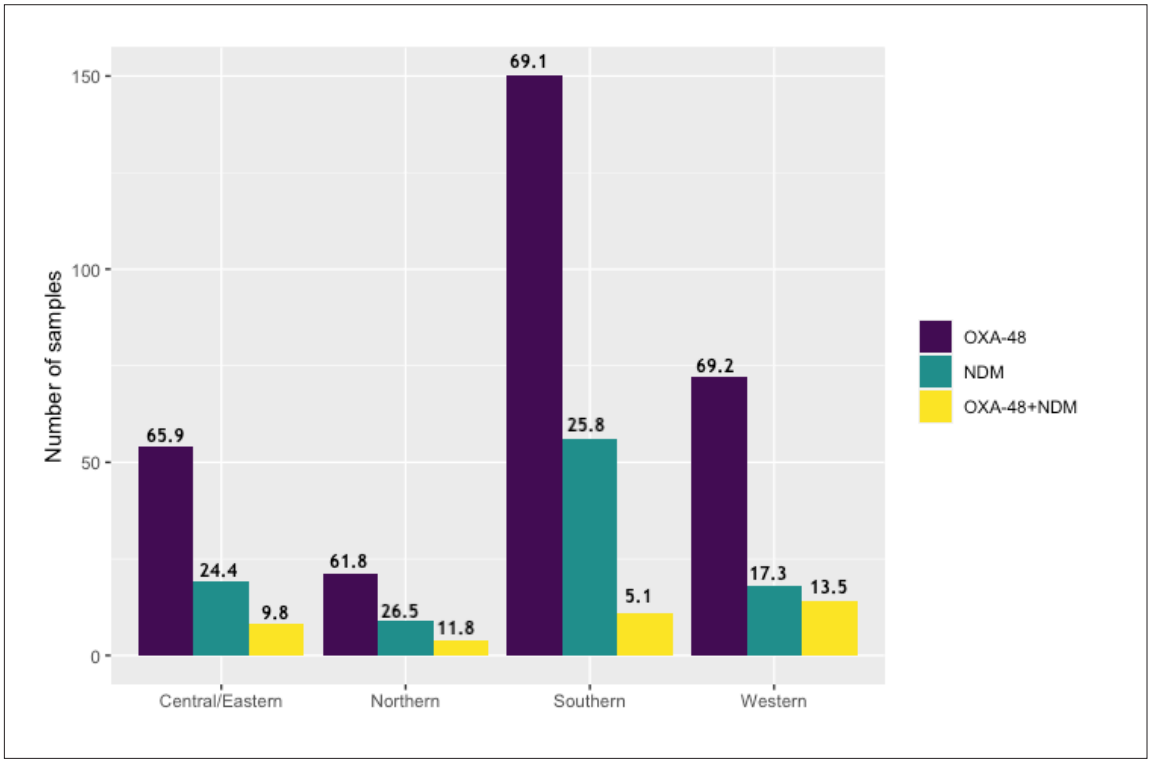

Figure 1. Participating regions and molecular classification of the 436 carbapenem-resistant *Enterobacterales* isolates by region (percentage for region shown above bars).

Table 3. Molecular classification of 456 isolates of *K pneumoniae* submitted for molecular testing by region.

Regions	*K pneumoniae* received (n=456)	*K pneumoniae* positive (n=410)	Single NDM-1 (n=85)	Single OXA-48 (n=292)	NDM-1+OXA-48 (n=33)
Northern	35	33	9 (27)	20 (61)	4 (12)
Central and Eastern	90	78	12 (15)	60 (77)	6 (7)
Western	111	94	15 (16)	65 (69)	14 (14)
Southern	220	205	49 (24)	147 (72)	9 (4)
Mean number (%) for all regions	114	102.5	21.1 (20.5)	73 (71.2)	8.25 (8.1)

Data are number (%). Chi-square=9.2854, df=6, *P*=.1582 for comparison of genetic types (shaded light blue).

Table 4. Participating hospitals and molecular classification of 456 isolates of *K pneumoniae*.

Region	Participating hospitals	*K pneumoniae* received (n=456)	*K pneumoniae* positive (n=410)	NDM-1 detected (n=85)	OXA48 detected (n=292)	NDM-1 +OXA48 detected (n=33)
Central	King Salman Bin Abdulaziz Hospital, Riyadh (n=54)	47	45 (96)	5 (11)	40 (89)	0 (0)
Central	King Khalid Hospital and Prince Sultan Center for Health Service, Al Kharj (n=24)	17	9 (53)	3 (33)	6 (67)	0 (0)
Central	King Fahd Specialist Hospital, Buraydah (n=22)	20	18 (90)	4 (22)	8 (44)	6 (33)
East	Regional Laboratory and Blood Bank, Microbiology Department, Dammam (n=7)	6	6 (100)	0 (0)	6 (100)	0 (0)
North	Gurayat General Hospital (n=23)	23	22 (96)	7 (32)	11 (50)	4 (18)
North	Hael General Hospital (n=20)	6	5 (83)	0 (0)	5 (100)	0 (0)
North	Arar Central Hospital (n=6)	6	6 (100)	2 (33)	4 (67)	0 (0)
South	Asir Hospital (n=222)	208	193 (93)	49 (25)	137 (71)	7 (4)
South	King Fahd Hospital, Al Baha, (n=12)	12	12 (100)	0 (0)	10 (83)	2 (17)
West	King Faisal Medical Complex, Taif (n=15)	15	12 (80)	2 (17)	8 (67)	2 (17)
West	King Abdulaziz Specialist Hospital, Taif (n=8)	8	8 (100)	1 (13)	7 (88)	0 (0)
West	King Fahad Hospital, Madina Munawara (n=95)	83	70 (84)	11 (16)	49 (70)	10 (!4)
West	Hera General Hospital (n=11)	5	4 (80)	1 (25)	1 (25)	2 (50)

Data are number (%).

on 71 CRE isolates showed that OXA-48 was the most common type (67.6%), followed by NDM-1 (12.7%) and both (8.5%),[34] The second study performed on 60 CRE isolates which showed that OXA-48 was detected in 78.3% and NDM-1 in 20%.[35] Another more recent study from the same region performed on 31 CREs isolated from three hospitals showed that OXA-48 and NDM-1 accounted for 58% and 42%, respectively.[36] In 2015, another study on a smaller number of isolates from the two largest hospitals in the southern region detected 81.5% OXA-48 and 7.4% NDM-1, respectively.[37] Recently several studies reported the appearance of the KPC type in the Central and Western regions of Saudi Arabia, the GCC and other Middle Eastern countries.[30,38-41]

In this study, some of the isolates tested negative for carbapenemases (n=79 [15.4%]) and these isolates might harbor other carbapenemases enzymes not included in Xpert Carba-R Assay like OXA-48-like carbapenemase or they might contain other mechanisms for carbapenems resistance including the diminishment of outer membrane permeability due to decreased expression or loss of *OprD* porin expression, efflux pumps, or AmpC β-lactamases.[42] There was a slight variability in the percentage of CRE encoding genes between regions, which could be explained by the circulating strain causing outbreaks in that specific region during the course of the study. The variability among studies can also be explained by the inclusion criteria, types and number of patients, and the geographic location of the study.

In the GCC, a molecular classification of 45 CRE isolates found that the bla_{OXA-48} gene was detected in 56.4%, bla_{NDM-1} gene in 25.8%, and both bla_{OXA-48} and bla_{NDM-1} genes were detected in 9.7% of the isolates.[30] In the Gulf region, the most prevalent carbapenemase classes are NDM-1 and OXA-48, and these genes are carried on plasmid replicon type Inc L/M, Tn1999, IS1999 and rarely IncA/C in OXA-48-like. A mix of IncR, IncFII, were responsible for a spread of NDM-1.[43] These plasmids are mobile elements and facilitate the horizontal transmission of these resistance mechanisms among patients in hospitals, especially those with a risk factor for colonization and infection with CRE.[44]

In the current study, nearly 10% of carbapenem-resistant *K pneumoniae* isolates were negative for all genes encoding the CRE type on the Xpert Carba-R Assay, which is most likely indicative of other mechanisms of resistance like AmpC hyper-production with efflux, loss of porins, or outer membrane protein mutations.[45]

Recently, the rate of colonization in hospitalized patients with CRE has been confirmed to be in the range of 2–13.5%, especially among high-risk groups such as patients in ICUs and patients on multiple antibiotics, which can lead to the spread of this organism in healthcare facilities, longer hospital stays, and a higher rate of morbidity and mortality.[46-49] Asymptomatic carriage of CREs has been documented in patients admitted to the hospital; in a survey of 1806 hospitals, 299 (16.5%) patients showed colonization with CRE, which progressed to clinical infection.[50] This might be due to widespread CRE contamination of the environment, especially drinking and sewage water, which was shown earlier in a study in India.[51]

Various laboratory methods including both phenotypic and genotypic methods, have been used to screen patients for the carriage of CRE. The implementation varies from one clinical microbiologist to another and depends on the available resources; however, the molecular methods are rapid, sensitive, and specific, and have been made more affordable in recent years.[52] To prove transmission and document an outbreak of CRE, molecular typing using whole-genome sequencing can be performed to determine if the strains of the outbreak are related to the same clone or sequence type or not. The majority of OXA-48 are related to ST147, ST11, ST101, ST405, and ST395 while NDM-1 are related to ST101, ST11.[53]

This study is an initial surveillance study to determine the most common CRE types nationwide. Our future plan is to perform molecular sequencing to identify the ST and plasmids associated with the spread and identify whether differences exist between different geographic regions. We have not included the phenotypic susceptibility testing and minimal inhibitory concentration of these isolates to carbapenems because of inconsistency in the methods of testing and reporting in these hospitals. Despite that, our study provides valuable epidemiological data and likely, good guidance for future national antimicrobial guidelines and use of new β-lactamase inhibitor combinations, which have variable antibacterial activity depending on the molecular type of CRE; for example ceftazidime/avibactam has higher activity against OXA-48-like CRE type and the addition of aztreonam is required to treat NDM-1 CRE types.[54-55]

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