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Phenotypic and Genotypic Characterization of Pan-Drug Resistant *Klebsiella pneumoniae* Isolated in Qatar

Mazen A. Sid Ahmed [1], Jemal M. Hamid [2], Ahmed M. M. Hassan [2], Sulieman Abu Jarir [3], Emad Bashir Ibrahim [2,4] and Hamad Abdel Hadi [3,*]

[1] Department of Public Health, Laboratory Services, Philadelphia, USA
[2] Division of Microbiology, Department of Pathology and Laboratory Medicine, Hamad Medical Corporation, Doha, Qatar
[3] Division of Infectious Diseases, Communicable Diseases Centre, Hamad Medical Corporation, Doha, Qatar
[4] Biomedical Research Centre, Qatar University, Doha, Qatar
* Correspondence: habdelhadi@hamad.qa

Abstract: At secondary healthcare, Carbapenem-Resistant Enterobacterales (CREs) such as observed in *Klebsiella pneumoniae* are a global public health priority with significant clinical outcomes. In this study, we describe clinical, phenotypic, and genotypic characteristics of three pan-drug resistant (PDR) isolates that demonstrated extended resistance to conventional and novel antimicrobials. All patients had risk factors for acquisition of multidrug resistant organisms while microbiological susceptibility testing showed resistance to all conventional antimicrobials. Advanced susceptibility testing demonstrated resistance to broad agents such as ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem-vaborbactam. Nevertheless, all isolates were susceptible to cefiderocol, one of the novel antimicrobials that demonstrated potent *in vitro activity* against resistant Gram-negative bacteria including CREs pointing towards potential therapeutic role for PDR pathogens. Expanded genomic studies revealed multiple antimicrobial resistant genes (ARGs), including *bla*NMD-5 and *bla*OXA derivative types as well as a mutated outer membrane porin protein (OmpK37).

Keywords : antimicrobial resistance ; AMR; gram negative bacteria (GNB) ; *Klebsiella pneumoniae*

1. Introduction

Carbapenem-resistant Enterobacterales (CREs) are resistant gram-negative bacteria of recognized public health priority with significant clinical and economic outcomes (1, 2). At its forefront, *Klebsiella pneumonia* is responsible for a wide spectrum of presentations including intraabdominal, urinary, and respiratory tracts infections frequently presenting as community as well as hospital-associated infections with significant morbidity and mortality (3, 4). The pathogen frequently affects vulnerable patients such as those with chronic comorbidities, immunocompromised or with critical diseases particularly following invasive surgery or procedures at critical care units (3, 5). Globally, the past decades witnessed alarming challenges of *K.pneumoniae* due to its recognized capabilities as a prime community and nosocomial infection , ability to accumulate extensive resistance phenotypes with special attention directed to its hypervirulent , spreading multidrug-resistant (MDR) and extensively drug-resistant (XDR) clones (6).

To evade antimicrobials, isolates of *K. pneumoniae* exhibit various complex resistance mechanisms which are acquired through random genetic mutations or horizontally through mobile genetic elements which can be subsequently amplified through upregulation of resistance genes or continuously disseminated (7) . Studies conducted in this field, describe a large group of antimicrobial resistance genes (ARGs), that impede therapeutic function of broad spectrum antimicrobials such as carbapenems as well as novel βlactam-βlactamase Inhibitors (BLBLIs) which are considered as the ultimate management of serious infections secondary to Gram-negative bacteria (GNB) (8, 9).

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Worryingly, recent reports highlighted the emergence of extensively and Pan-drug resistant (PDR) strains of *K. pneumoniae* from different countries limiting further treatment options (10-13). Due to regional variations, in the Gulf countries including Qatar, there is lack of data to correspond pathogens phenotypic with genotypic characteristics to obtain a reliable molecular epidemiology of AMR in the region. In the current study, we outline clinical, phenotypic, and genotypic characteristics of three PDR *K. pneumoniae* isolates aiming to describe the underlying resistance mechanisms using observations obtained from antimicrobial susceptibility tests (AST) as well as whole-genome sequencing (WGS).

2. Results

All three cases were hospital-acquired isolated from patients admitted to critical care units who had multiple risk factors for MDR infections, including recent systemic antimicrobial therapy (3/3), hospitalization or outpatient hospital attendance within the previous 90 days (2/3), invasive medical devices (2/3), and history of MDR infection or colonization within the past 90 days prior to hospitalization (2/3) as shown in patients' demographics in **Table 1**. The most frequent underlying comorbidities were diabetes mellitus (2/3), and immune suppression following transplant (1/3). Meropenem, tigecycline, and colistin were used to treat the patient with KP1 isolate with new signs of infection, while the two colonized patients (isolates KP2 and KP3) did not receive specific therapy as evaluated as colonization.

Table 1. Demographic, clinical characteristics and outcomes of three patients colonized or infected with PDR *K. pneumoniae.*

Characteristics	PA1 (ST383)	PA2 (ST231)	PA3 (ST231)
Age	66	51	72
Gender	Male	Female	Male
Location	Critical Care Unit	Critical Care Unit	Critical Care Unit
Isolation site	Urine tract	Lower respiratory tract	Lower respiratory tract
Common associated underlying conditions			
Extensive health care contact [a]	No	Yes	Yes
History of antibiotic exposure within 90 days prior to hospital admission	Yes	Yes	Yes
Invasive devices [b]	Yes	Yes	No
Diabetes mellitus	Yes	Yes	No
History of MDR infection or colonization within prior 90 days	No	Yes	Yes
Co-infection with other microorganisms [c]	Yes	Yes	No
Heart failure	Yes	No	No
Chronic lung disease	No	No	Yes
Post-transplantation	No	Yes	Yes
Chronic liver disease	No	Yes	No
Acquisition	Hospital	Hospital	Hospital
Disease evaluation	Colonization	Colonization	Sepsis
Antibiotic treatment			
Meropenem [d]	No	No	Yes
Tigecycline[e]	No	No	Yes

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Colistin nebulizer	No	No	Yes

Patients and pathogens identification: PA refer to patients 1,2 and 3 while ST refer to pathogens sequence types. [a] Extensive health care contact involves regular visits to outpatient medical facilities, a regular home visit by home care teams, hospitalization within the preceding 90 days, or residency in a long-term care facility. [b] Invasive devices involve central line, Foley's catheter, and tracheostomy. [c] Co-infection is associated with the following organisms: other strains of MDR K. pneumonia and MDR *Acinetobacter baumannii*. [d] High dose meropenem 2000 mg/ml IV 8 hourly. [e] High dose tigecycline 200 mg loading followed by 100 mg IV 12 hourly.

The ASTs for the isolates are outlined at **Table 2** demonstrating resistant to all conventional anti-GNB agents from different antimicrobial classes. Additional ASTs for novel antimicrobials revealed susceptibility to cefiderocol 3/3, while 2/3 isolates were susceptible to eravacycline, omadacycline and ceftazidime/avibactam, and none to plazomicin, ceftolozane/tazobactam, imipenem/relebactam and meropenem/vabrobactam.

Table 2. Antimicrobial susceptibility profile of three pan-drug resistant *K. pneumonia* isolates against conventional and novel antimicrobials.

Antimicrobial class	**Antimicrobial drug**	**Isolate number**					
		KP1		**KP2**		**KP3**	
Tested by BD Phoenix							
Penicillins	Ampicillin	>16	R	>16	R	>16	R
Cephalosporins	Cefazolin	–	R	–	R	–	R
	Cefepime	>16	R	>16	R	>16	R
	Cefoxitin	>16	R	>16	R	>16	R
	Ceftazidime	>16	R	>16	R	>16	R
	Ceftriaxone	>32	R	>32	R	>32	R
	Cefuroxime	>16	R	>16	R	>16	R
	Cephalothin	>16	R	>16	R	>16	R
Monobactam	Aztreonam	>16	R	>16	R	>16	R
Carbapenems	Ertapenem	>4	R	>4	R	>4	R
	Imipenem	>8	R	>8	R	>8	R
	Meropenem	>8	R	>8	R	>8	R
β-lactam-β-lactamase inhibitors	Amoxicillin/clavulanate	>16/8	R	>16/8	R	>16/8	R
	Piperacillin/tazobactam	>64/4	R	>64/4	R	>64/4	R
Aminoglycosides	Amikacin	>32	R	>32	R	>32	R
	Gentamicin	>8	R	>8	R	>8	R
Fluoroquinolones	Ciprofloxacin	>2	R	>2	R	>2	R
	Levofloxacin	>4	R	>4	R	>4	R
	Nitrofurantoin	>64	R	>64	R	>64	R
Folate-pathway inhibitors	Trimethoprim/sulfamethoxazole	>4/76	R	>4/76	R	>4/76	R
Glycylcyclines	Tigecycline	4	R	2	I	2	I
Additional tested antimicrobials using MIC Test Strip							
Fosfomycin	Fosfomycin	48	R	48	R	256	R
Cephalosporins	Cefiderocol	0.38	S	0.38	S	0.094	S
Aminoglycosides	Plazomicin	256	R	256	R	256	R
Tetracycline	Omadacycline	32	R	3	S	3	S

 doi:10.20944/preprints202401.1775.v1

	Eravacycline	32	R	0.75	S	1.5	S
	Doxycycline	32	R	2	S	32	R
New β-lactam-β-lactamase inhibitors	Ceftazidime/avibactam	256	R	0.75	S	1	S
	Imipenem/relebactam	32	R	2	I	2	I
	Ceftolozane/tazobactam	256	R	256	R	16	R
	Meropenem/vaborbactam	32	R	12	I	8	I
Tested by using Broth Microdilution Method							
Polymyxin	Colistin*	16	R	16	R	8	R

Novel antimicrobials agents include: Cefiderocol, plazomycin, omadacyline, eravacycline, ceftazidime-avibactam, ceftolozane-tazobactam imipenem/relebactam and meropenem/vaborbactam. *Colistin: antimicrobial susceptibility tested through broth microdilution methods.

The genomic size of the three PDR *K. pneumoniae* isolates KP1, KP2, KP3 were 5,617,763 base pair (bp), 5,617,835 bp, and 5,677,828 bp respectively . Two of the three PDR *K. pneumoniae* isolates belonged to sequence type ST231 while the last to ST383. Genomic data of the three PDR *K. pneumoniae* isolates revealed that they possessed 7 different β-lactamase genes from all classes; Class A ESBL (CTX-M-15 and TEM-1) in all isolates (3/3), while class A β-lactamase (CTX-M-14 and SHV-1), class B β-lactamase (NDM-5) and Class D β-lactamase (OXA-232) were present in (2/3) (**Table 3**). Different enzyme encoding genes highlighting antibiotics target alteration such as mutant *gyrA*, penicillin-binding protein (PBP3), and16S rRNA methyltransferase (rmtF) (G1405) and *K. pneumoniae* mutated outer membrane porin with reduced permeability (*OmpK37*) were detected in all isolates in addition to active efflux pump complexes such as emrB, baeR as depicted in **Tables 4 and 5.**

Table 3. Sequence types and enzymatic genotypic profiles of *K. pneumoniae* isolates.

Isolate number (sequence type)		**KP1 (ST383)**	**KP2 (ST231)**	**KP3 (ST231)**
Resistance gene	**Gene family**	**Gene presence (% identity)**		
AAC(6')-Ib	AAC(3), AAC(6')	Yes (100)	Yes (100)	Yes (100)
aadA	Amimonglycoside 3''-nucleotidyltransferases; ANT(3'')	VIM, Deletion b of E231 (99.23)	–	–
aadA2	ANT(3'')	–	Yes (100)	Yes (100)
APH(3')-Ia	Aminoglycoside 3'-phosphotransferases; APH(3')	L19M, R27K, N48D, A77E (98.52)	–	–
APH(3'')-Ib	APH(3'')	L116S (99.63)	–	–
APH (3')-VI	APH (3')	Yes (100)	–	–
APH (6)-Id	APH (6)	Q259E (99.64)	–	–
CTX-M-14	Class A β-lactamase	Yes (100)	–	–
CTX-M-15	Class A β-lactamase	Yes (100)	Yes (100)	Yes (100)
SHV-1	Class A β-lactamase	Yes (100)	–	Yes (100)
TEM-1	Class A β-lactamase	Yes (100)	Yes (100)	Yes (100)
NDM-5	Class B β-lactamase	Yes (100)	–	–
OXA-232	Class D β-lactamase	–	Yes (100)	Yes (100)
OXA-48	Class D β-lactamase	Yes (100)	–	–
arr-2	Rifampin ADP-ribosyl transferase (Arr)	Yes (100)	Yes (100)	Yes (100)
BRP(MBL)	Bleomycin resistant protein	–	–	–
catI	Chloramphenicol acetyltransferase (CAT)	–	Yes (100)	Yes (100)

 doi:10.20944/preprints202401.1775.v1

FosA6	Fosfomycin thiol transferase	Q130P, Q139E (98.56)	A86V, I91V, Q130P (97.84)	A86V, I91V, Q130P (97.84)
mphA	Macrolide phosphotransferase (MPH)	Yes (100)	Yes (100)	Yes (100)
mphE	Macrolide phosphotransferase (MPH)	Yes (100)	–	–
Disc diffusion test	ESBL	detected	detected	detected

Disk diffusion-based screening tests for extended-spectrum β-lactamases.

Table 4. Sequence types and genotypic profiles of encoding enzymes of *Klebsiella pneumoniae* isolates.

Isolate number (sequence type)		**KP1 (ST383)**	**KP2 (ST231)**	**KP3 (ST231)**
Resistance gene	**Drug class**	**Gene presence (% identity)**		
Antibiotic target alteration				
16S rRNA methyltransferase (armA), (G1405)	aminoglycoside	Yes (92.74)	–	–
Erm 23S ribosomal RNA methyltransferase (ErmB)	lincosamide, macrolide, streptogramin	Yes (97.96)	Yes (97.96)	Yes (97.96)
EF-Tu mutants	Pulvomycin	Yes (97.97)	Yes (98.06)	Yes (98.06)
gyrA	nybomycin, fluoroquinolone	Yes (95.67)	Yes (95.67)	Yes (92.23)
marR mutant	cephalosporin, fluoroquinolone, penam, phenicol, glycylcycline, tetracycline, rifamycin, triclosan	Yes (84.03)	Yes (84.03)	Yes (84.03)
parC	fluoroquinolone	Yes (94.41)	Yes (94.41)	Yes (94.41)
UhpT with mutation	fosfomycin	Yes (95.03)	Yes (95.25)	Yes (95.25)
PBP3	β-lactam	Yes (52.37)	Yes (52.37)	Yes (52.37)
16S rRNA methyltransferase (rmtF), (G1405)	aminoglycoside	Yes (98.36)	Yes (100)	Yes (100)
Antibiotic target protection				
ABC-F ATP-binding cassette ribosomal protection protein (msrE)	macrolide antibiotic, streptogramin	Yes (100)	–	–
QqnrS2	fluoroquinolone	Yes (100)	–	Yes (100)
ABC-F ATP-binding cassette ribosomal protection protein (vgaC)	streptogramin, pleuromutilin	Yes (100)	Yes (91.89)	Yes (91.78; 83.78) *
Antibiotic target replacement				
trimethoprim resistant dihydrofolate reductase (dfr); dfrA12	diaminopyrimidine	Yes (100)	Yes (100)	Yes (100)
dfrA5	diaminopyrimidine	Yes (100)	–	–
Sulfonamide resistant (sul1)	sulfonamide, sulfone	Yes (100)	Yes (100)	Yes (100)
Sulfonamide resistant (sul2)	sulfonamide, sulfone	Yes (100)	–	–
Reduced permeability to antibiotic				

 doi:10.20944/preprints202401.1775.v1

Klebsiella pneumoniae porin with reduced permeability (OmpK37)	β-lactams	Yes (99.47)	Yes (94.01)	Yes (94.01)
General Bacterial Porin with reduced permeability (marA)	β-lactam, fluoroquinolone, glycylcycline, triclosan, phenicol, tetracycline, rifamycin	Yes (92.74)	Yes (92.74)	Yes (92.74)

*Two different vgaC genes were detected.

Table 5. Occurrence of genotypic profile of efflux pump complexes and their regulators for *Klebsiella pneumoniae.*

Isolate number (sequence type)			**KP1 (ST383)**	**KP2 (ST231)**	**KP3 (ST231)**
	Gene family	**Drug class**	**Present or absent**		
Efflux pump complexes					
msbA	ABC[a]	nitroimidazole	+	+	+
emrB	MFS[b]	fluoroquinolone	+	+	+
QepA4	MFS	quinolone and fluoroquinolone antibiotics	+	+	–
tet(A)	MFS	tetracycline, glycylcycline	+	+	–
tet(C)	MFS	tetracycline	+	–	–
tetR	MFS	tetracycline	+	+	–
adeF	RND[c]	fluoroquinolone, tetracycline	+	+	+
baeR	RND	aminoglycoside	+	+	+
oqxA	RND	fluoroquinolone, nitrofuran, tetracycline, glycylcycline	+	+	+
Efflux pump regulators					
CRP	RND	macrolide, fluoroquinolone, penam	+	+	+
emrR	MFS	fluoroquinolone	+	+	+
H-NS	MFS, RND	cephamycin, cephalosporin, fluoroquinolone, tetracycline, penam	+	+	+

[a] ATP-binding cassette (ABC) antibiotic efflux pump. [b] Major facilitator superfamily (MFS) antibiotic efflux pump. [c] Resistance-nodulation-cell division (RND) antibiotic efflux pump.Discussion:

In order to understand the underlying mechanisms that derive antimicrobial resistance (AMR), a detailed evaluation of extensive drug resistant (XDR) and pan-drug resistant (PDR) organisms is of paramount importance (16). The problem of AMR is more evident in Gram negative bacteria (GNB) because of accumulated and diverse resistance mechanism that peaks at carbapenem resistance (CR) pathogens (17). The subset of CREs represented by *K. pneumoniae is* one of the leading challenges implicated in healthcare-associated infections (HAIs) with significant negative clinical and economic outcomes (3). Resistance in *K. pneumoniae* is increasingly reported particularly in patients with chronic comorbidities, immune-suppressions, previous antimicrobial exposure and prolonged

hospital or critical care length of stay (18). The described cases are from patients with all risk factors for AMR and HAIs, namely, prolonged hospital stay, prior antimicrobial therapy, invasive devices, and chronic comorbidities including immune suppression (19).

The outlined microbiological and genomic studies of the three cases of PDR *K. pneumoniae* demonstrate extensive resistance to conventional antimicrobials such as all third and fourth generations cephalosporins, monobactams, carbapenems, aminoglycosides, quinolones as well as polymyxins represented by colistin (Tables 1 and 2). Upon genomic characterization, two of the three isolates belong to the notorious epidemic clones: *K. pneumoniae* ST231 which was first described in South-East Asia and has been associated with healthcare outbreaks as well as demonstrated diverse ARGs spanning major classes, particularly *bla*OXA-48 (20). In a previous surveillance study from Qatar of 149 CREs, 8.6% of isolates were of the same epidemic clone which explains its endemicity (21). Additionally, Class A extended-spectrum β-lactamases (ESBLs) were represented by *bla*CXM-14 *and bla*CXM-15, *bla*SHV-1, and *bla*TEM-1. Globally, *bla*CXM-15 is the most widely distributed ARGs in GNB including the Gulf countries (22-24). Furthermore, the worrying carbapenemase *bla*NDM-5 of class B metallo-beta-lactamase (MBL), is present in KP1 isolate. This notorious ARGS is an offspring of *bla*NDM that was first described in 2012 which is carried horizontally through mobile genetic elements (MGEs) being capable of producing carbapenamses that can destroy all basic and advanced β-lactamases including cephalosporins and carbapenems but remain susceptible to monobactams, one of the only few therapeutic agents that are capable to overcome MBLs albeit critically vulnerable to other ESBLs . The notorious *bla*NDM-1 ARG is endemic in the region being one of the most prevalent genes amongst CREs (25, 26) .Nevertheless, blaNDM-5 has been reported mainly in CRE *E. coli* from Greece, Japan, China, and Latin America usually associated with highly virulent strains (27-29).. The ARGs were also been isolated from companion animals which emphasize the concept of one health approach integrating the impact of interactions between humans, animal, and the environment (30). The introduction of novel antimicrobials βlactam βblactamase inhibitors (BLBLIs) agents in the region such as ceftazidime-avibactam and ceftlozane-tazobactam that has activity against OXA-type CREs but remains vulnerable to MBLs such as NDM might be responsible for the shifting epidemiology that need further supporting surveillance over coming years.

Characteristically, the first (KP1) showed utmost resistance to novel combinations including β-lactam/β-lactamase inhibitors (BLBLIs) such as ceftazidime-avibactam, ceftolozane-tazobactam as well as meropenem-vaborbactam while the other two were susceptible to ceftazidime-avibactam but resistant to ceftolozane-tazobactam. Avibactam is a potent BLBLI that inhibits class A, C, and D including OXA-types but is incapable of inactivating enzymes encoded by class B β-lactamases such *bla*NDM and *bla*VIM as in KP1 , while ceftolozane is an advanced cephalosporin that overcomes many enzymes encoded by ARGs but remains susceptible to broad spectrum ones including OXA-types ARGs (31). The fact that the two other isolates KP2 and KP3 lacks *bla*NDM but harbour *bla*OXA 232 demonstrating broad BLBLIs discordance (KP2 and KP3 were sensitive to ceftazidime-avibactam but both resistant to ceftolozane-tazobactam), postulates that the ARG *bla*OXA 232 can be suppressed by avibactam but not tazobactam as previously observed (32). Similarly both Meropenem/vaborbactam and Imipenem/relebactam were inferior supporting the observation that the novel agent does not cover *bla*NDM and *bla*OXA type isolates as previously described (33).

The recommended management of CRE harbouring class B MBLs such as *bla*NDM is usually complex involving combination therapy with agents that are capable to overcome the genotypic resistance such as aztreonam-avibactam or cefiderocol with or without the addition of additional antimicrobials such as polymyxins, tigecycline, or eravacycline (34). It is also intriguing to notice that all PDR isolates were susceptible to cefiderocol one of the novel antimicrobials that demonstrated potent *in vitro* activity against XDR and PDR Gram-negative strains spanning all β-lactamase classes but needed supporting data towards clinical efficacy (35, 36). Cefidercol is a novel synthetic siderophore antimicrobial that hijack bacterial iron transporting mechanisms to traverse microbial cell wall and eventually lead to cell lysis through interference with cell wall synthesis . Its distinct ability to resist cell wall based βlactamases including class A, B , C and D in addition to its unique

ability to overcome bacterial efflux pumps and porin channels making it a promising a trojan horse capable of overcoming different mechanism of bacterial AMR. The antimicrobial is of significant potential interest since it has remarkable *in vitro* antimicrobial activity particularly for notorious organisms such as MDR-*pseudomonas aeruginosa* and carbapenem resistant *Acinetobacter baumannii* as well as *Stenotrophomonas maltophila* . Nevertheless, translation of the ASTs needs supporting clinical data since in some observational studies , cefidercol was linked to increased mortality such as the one observed with carbapenem resistant *Acinetobacter baumannii* Similarly, both eravacycline and omadacycline showed activity against two of the three PDR strains (KP2 and KP3 at Table 2) . These advanced and novel derivatives agents of the tetracycline group are of focused interest particularly for the coverage of resistant strains from intrabdominal infections albeit are limited for invasive diseases such as blood stream infections based on its pharmaco-kinetic and pharmacodynamic characteristics (36-38).

While polymyxins resistance is extremely rare in CRE (less than 5% in most regions), intriguingly the three isolates are colistin resistance (9). Polymyxins are not natural targets for ARGs since they act primarily and independently at cell wall levels of GNB destroying structural lipopolysaccharides . Reported resistance is mainly driven through plasmid-mediated mobile colistin resistance (*mcr* resistant gene) first described in China in 2015 in *E. coli* then described in most Enterobacterales (39). The fact that no *mcr*-related genes had been identified in the isolates, points toward other outer membrane resistance mechanisms. Previous studies of colistin-resistant *K. pneumoniae* lacking the *mcr* resistance gene explored the role of mutations in the two-component membrane system of the PhoPQ and PmrAB (40). Additionally, all isolates harbored Rifampin ADP-ribosyl transferase (*Arr*) which confers rifamycin resistance when used as an adjuvant therapy albeit not tested in the study. Furthermore, all the isolates harbored OXA-type carbapenemases such as *bla*OXA-48 and it is closely related to resistant gene *blaOXA*-232 which has five point mutations from bla_{OXA48} and both are similarly capable of inactivating carbapenems (41). It is worth pointing that OXA-type together with class B NDM carbapenemases are the most frequent carbapenemases in the Middle East and Gulf regions (21, 23).

Distinctively genomic characterization revealed mutations at the outer membrane porin permeability channels with the expressions of *K. pneumoniae* associated OMK35 that leads to decrease permeability to several hydrophilic antimicrobials such as β-lactams including carbapenems, fluoroquinolone, tetracycline, and its derivatives glycylcycline represented by tigecycline as well as rifamycin as well as being associated with increased virulence. Previous studies demonstrated restoration of the porin outer cellular mutations leads to restoring of lost antimicrobial activities (42).

In addition to the common ARGs, genotypic characterization revealed occurrence of other mechanism such as aminoglycosides modifying enzymes (AMEs), macrolide phosphotransferase (MPT), and fosfomycin thiol transferases resistance genes that might be linked to reported phenotypic patterns .For PDR- GNB, the co-occurrence and transmission of multiple resistance mechanisms is a well-recognized phenomenon that frequently leads to propagation of AMR (43).

Despite the study tries to elucidate underlying mechanism of resistance focusing on *Klebsiella pneumoniae*, it has some limitations which should be taken into considerations for the validity of scientific evaluation. It must be highlighted that two of the *Klebsiella pneumoniae* isolates were evaluated as colonizer rather than a true infection which limits its clinical evaluation particularly options for therapeutic interventions. Additionally, the genomic study focused on ARGs rather than combing that with virulence factor which was plausible not explored because of the small collection and paucity of invasive disease . Nevertheless, the relationship between bacterial AMR and virulence proved to be elusive even for comprehensive studies . Despite these limitations , the study continues to expand our knowledge for the fascinating study of mechanism of resistance particularly for GNB that should pave the way to overcome its challenges .

3. Material and methods

4.1. Identification and susceptibility testing

Phenotypic characterizations were performed using BD Phoenix™ automated system while bacterial identification and confirmation was performed using Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) of Bruker Daltonics MALDI Biotyper (Billerica, MA, USA) according to the manufacturer's recommendations. Additional ASTs for fosfomycin, cefiderocol, plazomicin, omadacycline, eravacycline, doxycycline, meropenem/vaborbactam, ceftazidime/avibactam, imipenem/relebactam, and ceftolozane/tazobactam were performed using MIC Test Strips (Liofilchem®, Diagnostics, Italy), while broth microdilution was used for colistin susceptibility testing (ComASP Colistin, Liofilchem, Roseto degli Abruzzi, Italy). *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Susceptibility reporting was based recommendations of the CLSI at the time (14). Since, there were no intermediate susceptibility category available for ceftazidime/avibactam against Enterobacteriaceae, isolates were therefore described as susceptible if the MIC was ≤ 8 mg/L and as non-susceptible if the MIC was >8 mg/L (14). PDR *K. pneumoniae* isolates were defined as having *in vitro* non-susceptibility to all routinely and conventionally tested anti-Gram-negative antimicrobial agents excluding additional advanced susceptibility tests (15). WGS was performed by Eurofins GATC Biotech GmbH, Konstanz, Germany using the Illumina HiSeq 2000 system (Illumina, San Diego, CA, USA).

4.2. Ethical approval

The study was approved by the Medical Research Centre (MRC) and Research and Ethics Committee (Protocol: MRC-04-22-522) at Hamad Medical Corporation, Doha, Qatar which abides by the local and international standards of ethics in medical research including, patients consent, data anonymity and management.

5. Conclusion:

The clinical, phenotypic, and genotypic characterization of three PDR *K. pneumoniae* revealed multiple risk factors for acquisition of healthcare associated MDR pathogens resulting in extensive resistance profiles while examining genomic studies revealed multiple underlying ARGs associated with phenotypic patterns. For PDR- *K. pnuemoniae*, novel antimicrobial agents such as cefiderocol, omadacycline and eravacycline are potential therapeutic agents that need further clinical evaluation.

Authors' contributions: HAH and MAS conceived and designed the study as well as analyzed results and drafted the initial manuscript. MAS, JMH and AMH performed the experimental work. SAJ participated in clinical data collection and reviewed the manuscript while EBI provided technical and scientific expertise. All authors read and approved the final manuscript.

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Ethical approval and Institutional Review Board Statement: The study was approved by the Medical Research Centre at Hamad Medical Corporation, Doha, Qatar which abides by local and international research standards.

Informed Consent Statement: The study received the needed institution ethical consent that protects patients' privacy and anonymity.

Data Availability Statement: We highlight that strictest confidence was maintained for data collection as well as access during the study. Data were not shared at any level with any individuals not authorized to access the research material. Data can be made available upon a reasonable request to the authors following permission from the Medical Research Center at HMC. We fully understand that the use of confidential data for personal purposes is prohibited.

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