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Role of outer membrane permeability, efflux mechanism, and carbapenemases in carbapenem-nonsusceptible *Pseudomonas aeruginosa* from Dubai hospitals: Results of the first cross-sectional survey

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ABSTRACT

Objectives: Carbapenem resistance in *Pseudomonas aeruginosa* is growing and results from variable mechanisms. The objectives of the current study were to investigate mechanisms of carbapenem resistance and genetic relatedness of *P. aeruginosa* isolates recovered in Dubai hospitals.
Methods: From June 2015 through June 2016, carbapenem-nonsusceptible *P. aeruginosa* were collected from 4 hospitals in Dubai, and subjected to antimicrobial susceptibility testing, molecular investigation of carbapenemases by PCR-sequencing, analysis of outer membrane porin OprD2 and multidrug efflux channel MexAB-OprM levels by qPCR, and fingerprinting by ERIC-PCR.
Results: Out of 1969 *P. aeruginosa* isolated during the study period, 471 (23.9%) showed reduced carbapenem susceptibility. Of these, 37 were analyzed and 32% of them produced VIM-type metallo-β-lactamases, including VIM-2, VIM-30, VIM-31, and VIM-42, while GES-5 and GES-9 co-existed with VIM in 5.4% of isolates. Outer membrane impermeability was observed in 73% of isolates and 75.6% displayed overproduced MexAB-OprM. ERIC-PCR revealed one large clone including most carbapenemase-producing isolates indicating clonal dissemination.
Conclusion: This is the first study on carbapenem-nonsusceptible *P. aeruginosa* from Dubai, incriminating VIM production as well as outer membrane permeability and efflux systems as resistance mechanisms. Further studies on carbapenem-nonsusceptible *P. aeruginosa* in Dubai are warranted for containment of such health hazard.

Introduction

Carbapenems, namely imipenem and meropenem, play a significant role in the treatment of infections caused by *Pseudomonas aeruginosa* (Khuntayaporn et al., 2013). However, the emergence and spread of carbapenem-nonsusceptible *P. aeruginosa* is quite alarming (Queenan and Bush, 2007). To date, different carbapenem resistance mechanisms have been identified in this organism including inducible chromosomal AmpC (Lister et al., 2009), reduced drug uptake due to mutant or lost outer membrane porins (OprDs), increased drug efflux due to over-expression of efflux pumps MexAB-OprM, and genetic acquisition of carbapenem-hydrolyzing enzymes (Li et al., 2012). More specifically, OprD2 is a 45–49 KDa outer membrane protein that

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serves as binding site for imipenem (Lister et al., 2009). Its deficient expression usually leads to basal level of multi-drug resistance, including imipenem resistance (Naenna et al., 2010). Another contributor to carbapenem resistance in *P. aeruginosa* is over-expression of a multi-drug efflux pump belonging to resistance-nodulation-cell division (RND) family, MexAB-OprM. This pump contributes significantly to intrinsic resistance and displays the broadest substrate profile among all *P. aeruginosa* efflux pumps. Its mutational overexpression leads to reduced susceptibility to fluoroquinolones, macrolides, chloramphenicol, tetracyclines, and most β-lactams including meropenem but not imipenem (Oliver et al., 2015; Choudhury et al., 2015). MexAB-OrpM efflux pump overproduction coupled to OprD loss makes the organism highly resistant not only to imipenem but also to meropenem and doripenem (Hammami et al., 2009). Although carbapenem resistance in *P. aeruginosa* is most likely related to the above two mechanisms, carbapenemases of Ambler classes A, B, and D have been described in this organism (Potron et al., 2015). Among class A, or extended-spectrum-β-lactamases, the first GES-type carbapenemase in *P. aeruginosa* was GES-2 described in South Africa (Poirel et al., 2001), followed by GES-5 from China (Wang et al., 2006), and more recently GES-18 from Belgium (Bebrone et al., 2013). Less frequent reports describe KPC-producing *P. aeruginosa* from USA (Poirel et al., 2010). The main carbapenemases in *P. aeruginosa* belong to Ambler class B metallo-β-lactamases. These include VIM enzymes, the first described in *P. aeruginosa* being VIM-1 (Lauretti et al., 1999), but the more prevalent nowadays being VIM-2 (Walsh et al., 2005), and IMP enzymes, of which about 32 variants were reported in this organism (Potron et al., 2015). More recently in 2011, NDM was described in *P. aeruginosa* isolates from Serbia (Jovcic et al., 2011), and was recovered later in India, Eygpt and Italy (Potron et al., 2015). Less common metallo-β-lactamases include SPM, GIM, and SIM first isolated in Brazil, Germany, and Korea respectively (Poirel et al., 2004; Castanheira et al., 2004; Lee et al., 2005). Additionally, literature has reported carbapenem-hydrolysing class D (oxacillinases) enzymes in *P. aeruginosa* including OXA-40 and OXA-198 (Potron et al., 2015). Therefore, it is evident that *P. aeruginosa* is genetically equipped with outstanding and variable machinery to be resistant to carbapenems.

In immunocompromised patients, carbapenem nosusceptible *P. aeruginosa* is an important pathogen which causes nosocomial infections, and it has emerged as a widespread Multi Drug Resistant (MDR) pathogen which requires antibiotic susceptibility testing on a regular basis (Biswal et al., 2014). For example, 34.1% *P. aeruginosa* isolated from neutropenic patients with hematologic diseases in China (Zhu et al., 2017) and 18.9% isolated from burn patients in an Indian survey were resistant to imipenem (Biswal et al., 2014). Also, in a Turkish survey of *P. aeruginosa* isolated from intensive care units, rates of carbapenem resistance were found to increase when monitored over four years (Alişkan et al., 2008). In pediatric cancer patients with febrile neutropenia, there was an alarmingly high resistance pattern of *Pseudomonas* spp. to carbapenems, with 50% resistant to imipenem and 25% resistant to meropenem in a recent survey from Eygpt (Kamel et al., 2018).

As far as Dubai is concerned, and to our knowledge, no previous reports did define carbapenem resistance in *P. aeruginosa*, although reports from some surrounding countries in the Arabian Gulf region are available (Al-Agamy et al., 2016; Zowawi et al., 2018). For example, previous studies reported 15% rates of carbapenem resistance of *P. aeruginosa* in Oman (Al-Yaqoubi and Elhag, 2008) and 41% in Lebanon (Hammoudi Halat et al., 2017). Likewise, other reports showed existence of other Gram-negative pathogens, such as *Enterobacteriaceae* with 4.6% rates of carbapenem resistance in Dubai (Moubareck et al., 2018), and 1.2% in Lebanon (Hammoudi Halat et al., 2017), with predominance of bla_{OXA-48} and bla_{NDM}, while some studies from Saudi Arabia reported bla_{VIM-4} (Memish et al., 2015). Carbapenem-nonsusceptible strains of *Acinetobacter baumannii* have been also reported from the region with a prevalence approximating 90% in Lebanon (Hammoudi et al., 2015), as well as from Saudi Arabia, Qatar, Oman, and Bahrain, with predominance of bla_{GES-11}, bla_{OXA-23} and other Ambler class D carbapenemases (Zowawi et al., 2015). With such evidence accumulating from surrounding countries, there is a need to investigate carbapenem resistance in *P. aeruginosa* from Dubai. As the second largest Emirate among the seven forming the United Arab Emirates, Dubai represents a major economic and touristic pivot of the Gulf region. The commercial status and rapid growth of Dubai has attracted a large proportion of expats and migrant workers from various geographical areas and diverse cultures, making it an appropriate venue for spread of bacterial resistance, especially in a quite versatile organism like *P. aeruginosa*. In light of such assortment, a study of *Pseudomonas* resistance to carbapenems in Dubai is mandatory. The objectives of the current study were to investigate involvement of carbapenemases, porin loss or downregulation, and efflux pump overactivity in carbapenem resistance in nosocomial *Pseudomonas* isolates from Dubai, and to analyze clonality of the studied strains.

Materials and methods

Isolates and data collection

Over a one year period, between June 2015 and June 2016, *P. aeruginosa* isolates with reduced susceptibility to either imipenem or meropenem at time of their isolation were collected from tertiary hospitals in Dubai, including two governmental (Dubai Hospital, DH, and Rashid Hospital, RH) and two private hospitals (American Hospital, AH, and Mediclinic City Hospital, MCH). DH and RH are 625- and 786-bed general medical/surgical specialized hospitals respectively, and both operate under the Dubai Health Authority, a government organization overseeing the health system of Dubai. On the other hand, AH is a 254-bed, acute care, general medical/surgical private hospital with a multi-specialty physician group practice. MCH is a 280-bed private hospital that accommodates patients with a wide range of health requirements, including specialist-focused treatment in cardiology, radiology, gynaecology, trauma, nuclear medicine, endocrinology, obstetrics, neonatal care and others. Isolates were delivered to Microbiology Research Laboratories of Zayed University, Dubai, and to Microbiology Laboratory of Saint-Joseph University, Beirut. Among received isolates, those with confirmed non-susceptibility to imipenem or meropenem according to CLSI 2017 criteria were included in the study.

Data on patient age, gender and nationality, type of clinical specimen from which *Pseudomonas* was recovered and brief disease information were accessed from patient medical files. However, data on chief medical complaints, clinical diagnosis, antibiotic therapy, travel history, and disease outcome following hospitalization were insufficient; hence relation of these parameters to carbapenem nonsusceptibility was not addressed within scope of the current study.

Susceptibility testing

Susceptibility testing to nine antimicrobial agents including imipenem, meropenem, piperacillin-tazobactam, ceftazidime, cefepime, amikacin, gentamicin, norfloxacin, ciprofloxacin, was done using the VITEK 2 Compact Analyzer. Susceptibility testing to colistin was done using broth microdilution. Results were interpreted according to CLSI 2017 criteria. Isolates were

Table 1
Minimum inhibitory concentrations, clones detected by ERIC-PCR, and genotypic profile of carbapenem resistance in studied *Pseudomonas aeruginosa* isolates.

Isolate number	Clinical specimen	Minimum inhibitory concentrations										Clone	Genotypic profile		
		Piperacillin/ Tazobactam	Ceftazidime	Cefepime	Imipenem	Meropenem	Amikacin	Gentamicin	Ciprofloxacin	Norfloxacin	Colistin		OprD2	MEX	Carbapenemase
AH1	Drain fluid	>128 /4	16	32	>16	>16	>64	>16	2	8	1	1	OprD2-	Mex+ +	VIM-30
AH2	Wound exudate	>128/4	>64	32	>16	>64	<2	<1	>4	8	1	1	Neg	Mex+ +	none
AH3	Sputum	32/4	4	4	>16	>16	<2	<1	0.5	2	1	6	OprD2-	Mex+ +	none
AH4	Sputum	<4/4	<1	<1	>16	4	4	4	1	2	1	1	Neg	Mex+ +	VIM-31
AH5	Sputum	8/4	4	8	>16	8	<2	<1	1	4	0.5	1	OprD2-	Mex+	none
AH6	Sputum	32/4	8	8	>16	>16	32	8	2	8	1	3	OprD2-	Mex+	none
AH7	Sputum	8/4	4	8	>16	8	16	>16	2	4	1	1	OprD2-	Mex+ +	VIM-30
AH8	Urine	<4/4	<1	<1	4	4	<2	<1	<0.25	<0.5	0.5	1	OprD2-	Mex+ +	none
AH9	Broncho-alveolar lavage	>128/4	>64	>64	>16	>16	32	8	2	4	1	1	OrpD2-	Mex+ +	none
AH10	Broncho-alveolar lavage	16/4	4	8	>16	>16	4	4	>4	8	0.5	1	OprD2-	Neg	none
AH11	Wound exudate	>128/4	>64	>64	>16	>16	>64	8	>4	>16	1	1	OprD2-	Mex+ +	none
AH12	Urine	>128/4	>64	>64	>16	>16	>64	>16	>4	>16	0.5	1	Neg	Mex+ +	none
AH13	Unknown	8/4	4	2	>16	8	<2	<1	<0.25	<0.5	0.5	5	OprD2-	Mex+ +	none
AH14	Unknown	8/4	4	2	>16	8	<2	<1	<0.25	<0.5	0.5	1	OprD2-	Mex+ +	none
DH15	Unknown	>128 /4	>64	>64	>16	>16	>64	>16	>4	>16	0.5	1	Neg	Mex+ +	none
DH16	Unknown	>128/4	16	8	>16	>16	>64	>16	>4	>16	1	1	OprD2-	Neg	VIM-42 + GES-5
DH17	Respiratory fluid	16/4	4	8	>16	>16	4	4	>4	8	0.5	3	Neg	Mex+ +	none
DH18	Blood	32/4	4	4	>16	>16	<2	>16	>4	>16	1	1	OprD2-	Mex+ +	VIM-2
DH19	Respiratory fluid	>128/4	>64	16	>16	8	<2	<1	<0.25	<0.5	0.5	1	OprD2-	Mex+ +	none
DH20	Wound exudate	>128/4	>64	>64	>16	>16	>64	>16	>4	>16	0.5	1	Neg	Mex+ +	VIM-2
DH21	Respiratory fluid	16/4	4	8	>16	>16	8	8	0.5	2	0.5	1	OprD2-	Neg	VIM-42
DH22	Respiratory fluid	17/4	16	32	>16	>16	16	8	1	2	1	1	OprD2-	Mex+	none
DH23	Ear	32/4	4	8	>16	>16	<2	2	1	2	1	1	Missing *OprD2*	Mex+ +	none
DH24	Urine	>128/4	>64	>64	>16	>16	8	4	<0.25	<0.5	1	1	OprD2-	Mex+ +	none
DH25	Respiratory fluid	32/4	8	8	>16	>16	<2	<1	0.5	2	1	1	Missing *OprD2*	Mex+ +	none
DH26	Wound	8/4	4	2	>16	4	4	4	<0.25	1	1	7	Neg	Mex+ +	VIM-30
DH27	Urine	>128/4	>64	>64	>16	>16	16	<1	>4	>16	1	1	OprD2-	Mex+ +	VIM-31
DH28	Respiratory fluid	8/4	2	<1	>16	4	<2	<1	1	2	1	4	OprD2-	Mex+ +	none
DH29	Urine	8/4	4	2	>16	4	<2	<1	<0.25	<0.5	1	1	Neg	Mex+ +	none

Table 1 (*Continued*)

Isolate number	Clinical specimen	Minimum inhibitory concentrations										Clone	Genotypic profile		
		Piperacillin/ Tazobactam	Ceftazidime	Cefepime	Imipenem	Meropenem	Amikacin	Gentamicin	Ciprofloxacin	Norfloxacin	Colistin		OprD2	MEX	Carbapenemase
DH30	Blood	>128/4	>64	>64	>16	>16	>64	>16	>4	>16	1	1	OprD2-	Mex+ +	none
DH31	Wound	32/4	16	8	>16	>16	>64	>16	>4	>16	0.5	1	OprD2-	Mex+	VIM-2
DH32	Respiratory fluid	<4 /4	2	<1	>16	4	8	4	<0.25	<0.5	1	1	OprD2-	Mex+ +	none
DH33	Wound	8/4	4	2	>16	4	<2	<1	<0.25	<0.5	1	1	OprD2-	Mex+ +	none
RH34	Unknown	>128/4	>64	>64	>16	>16	>64	>16	>4	>16	0.5	1	OprD2-	Mex+ +	none
RH35	Unknown	>128/4	>64	>64	>16	>16	>64	>16	>4	>16	1	2	OprD2-	Neg	none
MCH36	Foot ulcer	>128/4	>64	>64	>16	8	>64	>16	>4	>16	0.5	1	OprD2-	Mex+ +	VIM-2 + GES-9
MCH37	Sputum	>128/4	32	>64	>16	>16	>64	>16	>4	>16	0.5	1	OprD2-	Neg	VIM-2
MIC_{50}		<4/4 (S)	16 (I)	32 (R)	>16 (R)	>16 (R)	<2 (S)	<1 (S)	<0.25 (S)	<0.5 (S)	1 (S)				
MIC_{90}		>128/4 (R)	>64 (R)	>64 (R)	>16 (R)	>16 (R)	>64 (R)	>16 (R)	>4 (R)	>16 (R)	1 (S)				

DH = Dubai Hospital; RH = Rashid Hospital; AH = American Hospital; MCH = Mediclinic City Hospital.
OprD2-: down regulation of OprD2 porin.
Mex++: overexpression of *mexB* (increase by at least 3-fold).
Mex+: borderline expression of *mexB* (increase by 2 to 3-fold).
Neg: negative detection of OprD2 downregulation or MexB overexpression by qRT-PCR.

considered multidrug-resistant (MDR) if they were non-susceptible to at least one agent in three or more antimicrobial categories, extrensively drug resistant (XDR) if they remained susceptible to agents in two or fewer antimicrobial categories, and pandrug resistant (PDR) if they were non-susceptible to all agents in all antimicrobial categories (Magiorakos et al., 2012).

Detection of carbapenemase- and porin-encoding genes

Genomic DNA was extracted from samples by boiling method, and stored at −20 °C. Molecular investigation of carbapenemase-encoding genes was done by PCR-sequencing according to in-house protocols set by Institut Pasteur, France (Courvalin, 2011), as well as other previously designed conditions (Queenan and Bush, 2007; Poirel et al., 2011). Investigated genes belonged to Ambler class A, namely bla_{GES} and bla_{KPC} and Ambler class B, namely bla_{IMP-1}, bla_{IMP-2}, bla_{VIM}, bla_{NDM}, bla_{GIM}, bla_{SIM}, and bla_{SPM}. Moreover, the gene encoding the outer membrane porin OprD2 was investigated as previously described (El Amin et al., 2005).

Sequencing was performed to identify subtypes of bla_{GES} and bla_{VIM} genes as discussed previously (Courvalin, 2011).

RNA extraction and real-time PCR

Levels of expression of the genes *mexB* and *oprD2*, representing efflux pump MexAB-OprM, and outer membrane protein OprD2 respectively, were determined by quantitative real-time PCR (qRT-PCR). Total RNA was extracted and synthesized into cDNA, that was subjected to qRT-PCR using CFX96 Touch Real-Time PCR Detection System (Bio-Rad). A gene ecoding a 30S ribosomal protein, *rpsL*, was used as house-keeping control gene for normalizing transcription levels of target genes.

For isolation of RNA, isolates were cultivated overnight, diluted in brain heart infusion broth to logarithmic phase, identified by optical density (OD_{600}) of 0.5, then centrifuged. The RNeasy Protect Bacteria Mini Kit (Qiagen, Hilden, Germany) was used for RNA extraction from the pellet as per manufacturer recommendations. RNase-Free DNase (Qiagen, Hilden, Germany) was used to eliminate contaminating DNA, and cDNA was synthesized with Omniscript RT Kit (Qiagen, Hilden, Germany). For qRT-PCR, expression of *mexB* and *oprD2* was performed on cDNA using QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany), and expression levels were examined and compared to those of *rpsL*. The wild-type PAO1 of *P. aeruginosa*, was used as a positive control. The assay was performed in triplicate for each sample, using three independent RNA extractions, and the mean of three obtained quantities was considered as quantity of given gene expression for that sample. The $\Delta\Delta$Ct was used for determining gene expression level, and was calculated by subtracting ΔCt of sample from that of reference strain PAO1. The gene *mexB* was considered upregulated for an isolate when corresponding mRNA level was at least 3-fold higher than that of PAO-1 (reported as Mex++), and borderline if between 2- and 3-fold (reported as Mex+) (Cabot et al., 2011). The gene *opr*D2 was considered downregulated when corresponding mRNA level was at least 2-fold lower than that of PAO-1 (Ocampo-Sosa et al., 2012; Hocquet et al., 2006).

Investigation of clonal relatedness

Analysis of clonality of the studied isolates was performed by Enterobacterial Repetitive Intergenic Consensus-based PCR (ERIC-PCR) (Versalovic et al., 1991), using previously described conditions (Ferreira et al., 2011) but with annealing temperature of 58 °C. Banding patterns were converted by GelQuest/Sequentix® (Klein Raden, Germany) software into a binary matrix of either 1 or zero scores, and used to construct a dendrogram via unweighted

pair-group method using arithmetic averages (UPGMAs) clustering. The results for fingerprinting were analyzed using Dice coefficient. Isolates with more than 85% similarity were considered closely related and, therefore, were treated as clones; these isolates differed in one to two bands (Syrmis et al., 2004).

Results

Study samples and patients

A total of 1969 *P. aeruginosa* were isolated by the participating hospitals during the study period. Of these, 471 were carbapenem-nonsusceptible (overall prevalence of 23.9%). Thirty-seven isolates met the inclusion criteria and were subjected to further analysis. The largest proportion of samples was from Dubai hospital and American hospital, contributing with 14 and 19 samples respectively (Table 1). Specimens for recovery of carbapenem non-susceptible *P. aeruginosa* included urine, sputum, blood, respiratory samples, ear samples, drain fluid, foot ulcer samples, and wound exudates. Patients from were from different nationalities including nine from UAE, four from the nearby countries Kuwait, Saudi Arabia, Yemen, and Oman, and one from India.

Results of antimicrobial susceptibility testing

The results of antimicrobial susceptibility testing are shown in Table 1. Sixteen isolates (43%) were resistant to piperacillin/tazobactam with minimum inhibitory concentrations at which 90% of isolates were inhibited (MIC_{90}) superior or equal to 128/4 μg/mL. The MIC_{90} values of ceftazidime and cefepime were above 64 μg/mL, indicating high resistance. Thirty isolates (81%) were resistant to both imipenem and meropenem. Six isolates (AH8, DH26, DH28, DH29, DH32 and DH33) showed high imipenem resistance but intermediate resistance to meropenem with imipenem MICs greater than 16 μg/mL and meropenem MICs equal to 4 μg/mL. One isolate AH8 was both imipenem and meropenem intermediate with MICs of both carbapenems equal to 4. Regarding amikacin, gentamicin, ciprofloxacin, and levofloxacin, we observed variable sensitivities, but MIC_{90} values were superior to 64, 16, 4, and 64 μg/mL respectively, indicating resistant phenotypes. A discrepancy of MIC_{50}s and MIC_{90}s was noted for the latter agents (Table 1) with MIC_{50}s being susceptible while MIC_{90}s are resistant, probably indicating that our collection includes at least two subpopulations with wide variation in their MICs towards these agents (Schwarz et al., 2010). Four strains (11%) were MDR, and 14 (38%) were XDR, while none was characterized as PDR since they retained colistin sensitivity.

Results of carbapenemase and oprD2 *genes detection*

VIM metallo-β-lactamase was detected in 12 out of the 37 studied isolates (32.43%), and was the most prevalent among investigated carbapenemases. Sequencing of the VIM-positive isolates revealed VIM-2, VIM-30, VIM-31, and VIM-42 in 5, 3, 2, and 2 isolates respectively. Next to VIM, we detected the Ambler class A GES β-lactamase, with one VIM-2-positive isolate co-harboring GES-9, and one VIM-42-positive isolate co-harboring GES-5. It is worth noting that among the aforementioned GES enzymes, only GES-5 is a carbapenemase (Viedma et al., 2009), while GES-9 spares carbapenems (Poirel et al., 2005). The VIM-2 producing isolates were recovered from two different hospitals, as were those producing VIM-31; those producing VIM-30 were from the same hospital, and those producing VIM-42 were from a different one. Other investigated metallo-β-lactamases and KPC were not detected. The gene encoding the outer membrane porin gene *oprD2* was detected in 35 out of the 37 isolates and was missing in DH23 and DH25.

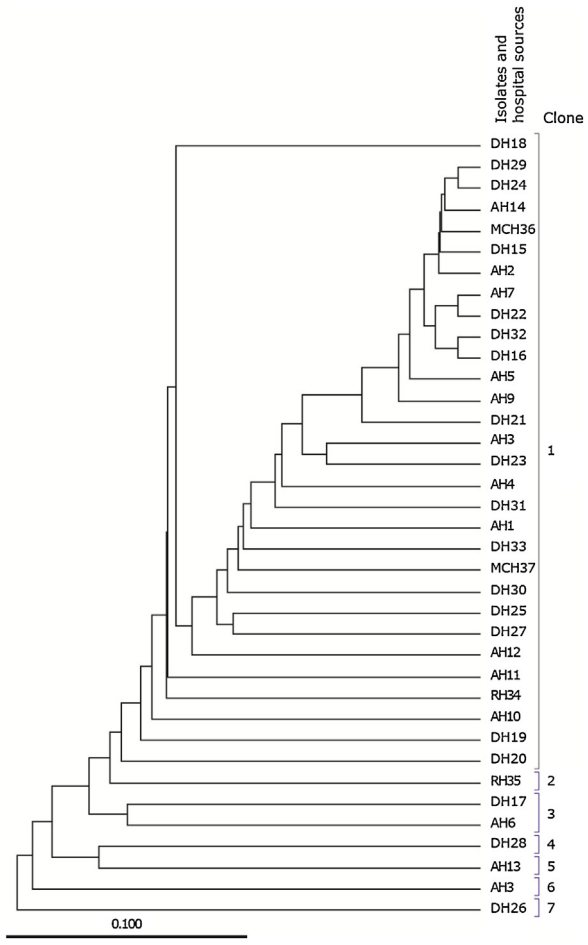

Figure 1. Dendrogram showing results of fingerprinting of the 37 studied isolates using ERIC-PCR at 85% similarity, with the 7 clones and hospital sources shown. DH = Dubai Hospital; RH = Rashid Hospital; AH = American Hospital; MCH = Mediclinic City Hospital.

Results of qRT-PCR

Although *oprD2* gene was present in all but two of our isolates, different levels of gene expression were detected by qRT-PCR. Twenty-seven isolates out of 37 (73%) displayed downregulation of *oprD2* mRNA when compared to level of gene expression of *oprD2* in the control strain PAO1 (Table 1). Also, 28 out of 37 isolates (75.6%) showed overproduction of *mex*B mRNA, indicating resistance resulting from efflux pump overexpression. The expression of *mex*B was borderline (2–3 folds higher than the reference strain PAO1) in 4 isolates, and hence efflux mechanism was not incriminated in their resistance to carbapenems.

Results of genotyping and qRT-PCR interestingly demonstrates that multiple mechanisms of resistance co-existed in 30 out of 37 of our isolates, with combinations of carbapenemases, OprD2 loss or downregulation, and/or MexB overexpression. In only seven studied strains, the detected determinants of resistance were single (2 isolates with OprD2 downregulation and 5 isolates with MexB overexpression).

Results of clonality analysis

ERIC-PCR fingerprinting analysis of the 37 isolates revealed 7 clones (Figure 1). Notably, 30 strains (81%) belonged to a single large clone, and they were recovered from the four participating hospitals, probably indicating an apparent clonal spread. This clone included 11 out of the 12 isolates with documented detection of the

different VIM and GES. The 6 remaining clones were smaller, including 1 or 2 strains, with a single VIM-30-positive isolate in clone 7. Deficiency of OprD2 gene expression was observed among 5 out of the 7 clones. Clone 3 included two strains where our tested carbapenemase genes were negative. Only two samples were recovered from blood culture (DH18 and DH30), and both belonged to clone 1 and shared downregulation of OprD2 and 3-fold overexpression of *mexB*. This clone also included samples from numerous other sites such as urine, respiratory fluid, and wound exudate, and its detection in the four hospitals may indicate a clonal inter-institutional spread.

Discussion

The global perturbing situation of carbapenem resistance in *P. aeruginosa* isolates observed in the last decade, together with obvious lack of reports from the Emirate of Dubai, both unveil need for a baseline investigation. This study was conducted in four centers located in Dubai to gain an overview of the status of susceptibility to carbapenem in *P. aeruginosa* over a 1-year period. The prevalence of non-susceptibility to carbapenems for *P. aeruginosa* was 23.9%. This prevalence is higher than that reported for Oman (15%) (Al-Yaqoubi and Elhag, 2008), but lower than that for Saudi Arabia (52%) (Al-Otaibi et al., 2016), and Lebanon (41%) (Hammoudi Halat et al., 2017). Concerning level of resistance to imipenem and meropenem, our data show high MIC_{50}s, superior to 16 μg/mL, for both carbapenems. Resistance to other β-lactams and other antibiotic classes was variable with few isolates exhibiting MDR phenotype, and about 40% being XDR. The absence of PDR isolates warrants follow up for colistin sensitivity status in Dubai.

To clarify the mechanisms of resistance to carbapenems in our isolates, carbapenemases were investigated. VIM metallo-β-lactamase appears to be the leading carbapenemase in this study. This carbapenemase has been detected in *P. aeruginosa* in close countries such as Lebanon (Hammoudi Halat et al., 2017), Kuwait, and Egypt (Zafer et al., 2015), with reports of VIM-2 (Kazmierczak et al., 2016); and Saudi Arabia, with reports of VIM-4 (Al-Agamy et al., 2016). VIM-31, detected in two studied isolates, is a new, mutant variant of VIM-2, first detected in *Enterobacter cloacae* (Bogaerts et al., 2012; Kupper et al., 2015). Interestingly, VIM-31 enzyme has weaker catalytic efficiency against β-lactam agents (Bogaerts et al., 2012), probably explaining low MIC of piperacillin-tazobactam, ceftazidime, and cefepime, depicted in isolate AH4. On the other hand, all these MICs substantially rise in isolate DH27, in which VIM-31 is accompanied with OprD2 downregulation. Two isolates showed presence of VIM-42, a variant of VIM-1 previously described in 2013 in *Klebsiella pneumoniae* from an Italian patient with urinary tract infection (Kazmierczak et al., 2016). The diversity of VIM carbapenemases in *P. aeruginosa* stresses on its importance as a large reservoir of bla_{VIM} gene cassettes (Sedighi et al., 2014).

The genetic platform of bla_{VIM} is capable of expression and acquisition of other genes, and may co-exist with one or more resistance determinants (Zhao and Hu, 2011). This scenario is demonstrated in our results, where two VIM-producing isolates produced either GES-5 or GES-9, both of which are ESBLs. GES-9 is a variant of GES-1 with broad hydrolysis spectrum for many β-lactams, but spares carbapenems and hydrolyzes aztreonam. GES-5, detected in isolate DH16, is a carbapenem-hydrolyzing ESBL first described in *P. aeruginosa* from Turkey (Iraz et al., 2014), and more recently from Saudi Arabia (Memish et al., 2015). In this latter report, no GES-5-harboring strains co-produced another carbapenemase, unlike our findings of GES-5 coexisting with VIM-42. The existence of GES-type β-lactamases with or without other enzymes in *P. aeruginosa* from the area was demonstrated elsewhere (Al-Agamy et al., 2016), and stresses the need for further investigation. GES-5 was earlier detected in far geographic areas, like China (Wang et al., 2006: 5), South Africa (Labuschagne et al., 2008), and Brazil (Pião et al., 2009), reflecting possibility of inter-country spread, given diverse nationalities with population flow into Dubai. We do not know whether such isolates have been recently transferred through international travel, or whether they existed since a long time and were not detected in the absence of surveillance for carbapenem resistant *P. aeruginosa*.

Genotypic investigation for *oprD2* gene by PCR showed its existence in 35 out of the 37 studied isolates, and a major proportion (27 isolates) showed downregulation of OprD2 by qRT-PCR, conferring decreased carbapenem permeability. This finding was demonstrated in another study from the region (Al-Agamy et al., 2016), and also earlier in *P. aeruginosa* carbapenem resistance literature (El Amin et al., 2005; Liu et al., 2013). Furthermore, OprD2 downregulation was detected in 5 out of 7 clones from different participating hospitals confirming the role of impermeability as a major contributor to resistance in *P. aeruginosa*.

In addition to permeability lesions and carbapenemases, the majority of our isolates displayed upregulated efflux pump activity. Previous documentation of such resistance mechanism in *P. aeruginosa* exists from the Arabian Gulf and elsewhere (Al-Agamy et al., 2016; Cabot et al., 2011). The existence of three genetic determinants of carbapenem resistance in a given isolate increased MIC of imipenem above 16 μg/mL.

Interestingly, in isolates AH10 and RH35, MIC of meropenem was above 16 μg/mL, although MexB overexpression was not detectable, while for strains AH2, AH12, DH15, DH17, and DH29, MIC of imipenem was above 16 μg/mL, although OprD down-regulation was not detectable. In these 7 strains, we did not detect carbapenemases. The patterns observed in these specific isolates do not conform with generally recognized patterns of resistance. This could be due to involvement of other factors like other efflux pumps or carbapenemases not tested in our experiments, but reported elsewhere (El Amin et al., 2005).

Our findings represent an addition to the global evidence indicating versatility and diversity of resistance mechanisms in this organism to carbapenems, with metallo-β-lactamases, permeability defects, and drug efflux, the latter two mechanisms being nontransferable. Further assessment is needed to scrutinize a probable role of certain Ambler class D enzymes and chromosomally encoded AmpC-type β-lactamases in emergence of such resistance (Potron et al., 2015).

The results of fingerprinting by ERIC-PCR suggest that most isolates (81%) probably originate from one clone disseminated in all four hospitals. With one exception, this clone included all VIM-producing isolates. With bla_{VIM} known to exist on a highly mobile genetic environment on class 1 integrons, transposons, and plasmids (Zhao and Hu, 2011), containment of such spread is needed. A horizontal spread of carbapenem-nonsusceptible *P. aeruginosa* in Dubai hospitals is suggested by the single VIM-producing isolate in clone 7. Many patients from whom samples were recovered had chronic illness, severe infections or cancer, and some had undergone invasive surgical procedures. A further understanding of clinical data would have been useful to comment on the clonality of the strains.

In conclusion, apparent mechanisms of carbapenem resistance in *P. aeruginosa* from Dubai are VIM and GES carbapenemases coupled with porin downregulation and efflux upregulation. With such scenario, and given the particular multicultural aspects of this geographic area, together with overwhelming resistance potential in *P. aeruginosa*, Dubai remains a hot spot for interesting

exploration in this field. Continuous epidemiologic and mechanistic surveillance would lead to further evidence on such baseline data and clarify carbapenem resistance in isolates that were negative to investigated mechanisms in this study.

Author contributions

C. Ayoub Moubareck designed the study, contacted the co-authors for collaboration, supervised the laboratory work, and revised and edited the manuscript. D. Hammoudi Halat contributed to analysis of results and drafting and correction of the manuscript. C. Akkawi contributed to molecular experiments. A. Nabi, M. AlSharhan, Z. AlDeesi, C. Peters, and H. Celiloglu contributed with study samples and reviewed and approved the manuscript. D. Karam Sarkis supervised the study, obtained funding for the research, and revised and approved the manuscript.

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Ethical approval

Ethical approval for the study was obtained from the Dubai Scientific Research Ethics Committee of Dubai Health Authority with the reference code DSREC-11.

Conflict of interest

None.

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