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ORIGINAL RESEARCH

Molecular Epidemiology and Mechanisms of High-Level Resistance to Meropenem and Imipenem in *Pseudomonas aeruginosa*

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Purpose: *Pseudomonas aeruginosa* possesses a large number of resistance mechanisms to different antimicrobials with carbapenems being the most powerful in treating resistant *P. aeruginosa*. Hence, it is imperative to explore different mechanisms of carbapenems-resistance in *P.* aeruginosa to achieve successful treatment through the design of new drugs acting on this interaction to combat against antimicrobial resistance.

Strains and Methods: A total of 634 P. aeruginosa clinical isolates were collected from various patient sources and their MIC levels were measured. Molecular evaluation of carbapenem resistance was assessed by investigating the presence of bla_{IMP1}, bla_{IMP2}, bla_{VIM1}, bla_{VIM2}, bla_{SPM} and bla_{NDM} genes and the gene expression of the following multi-drug efflux pump systems: MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM and its correlation with MIC. Isolates were typed by Random Amplified Polymorphic DNA (RAPD)-typing.

Results: Carbapenem resistance was detected in 32 (5%) isolates, which were all imipenem resistant (of which 29 were meropenem resistant). High-level resistance (≥64mg/mL) to imipenem was found in 27 (84.3%) isolates, and to meropenem in 28 (96.5%) isolates. The carbapenemase $bla_{VIM\text{-}1}$ was found in 31 isolates, while bla_{NDM} was detected in 4 isolates. None of the isolates possessed either $bla\text{-}_{VIM\text{-}2}$, $bla_{IMP\text{-}1}$, $bla_{IMP\text{-}2}$ or $bla_{SPM.}$ The majority of the isolates displayed over-expression of MexCD-OprJ (75%) followed by MexXY-OprM efflux pump (62%), while MexAB-OprM and MexEF-OprN efflux pumps were overexpressed in 21.8% and 18.7% of the isolates, respectively, with no down-regulation of *opr*D in any of the isolates. A strong correlation was found between CDJ efflux pump expression and meropenem, imipenem resistance (r=0.532, 0.654, p<0.001, <0.001) respectively. Four major clusters were detected by RAPD-typing: group 1(10 isolates), group 3 (9 isolates), group 2 (8 isolates) while the fourth group (4) included 4 isolates (12.5% polymorphism).

Conclusion: High-level carbapenem resistance reported in this study was allied to multiple mechanisms including carbapenemase production and efflux-pump over-expression. Threatening cross-infection is possible inside the hospital and stringent infection control measures are crucial.

Keywords: carbapenems, efflux pump, OprD and metallo-β-lactamases

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Introduction

Pseudomonas aeruginosa is a gram-negative opportunistic organism that causes severe global healthcare-associated infections such as respiratory tract infections, sepsis, urinary tract infections and surgical site infections, especially in immunocompromised

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patients.[1] The development of multidrug-resistant (MDR) strains that display resistance to nearly all antibiotics except for one or two classes is becoming an uppermost public health problem, increasing morbidity, mortality and length of hospital stay.[2] Unfortunately, *P.aeruginosa* harbors a wide array of MDR mechanisms, including the existence of outer-membrane barriers (porin *Opr*D), over-expression of multi-drug efflux pumps and endogenous antimicrobial inactivation.[3] Accordingly, the choice of an appropriate treatment is a formidable problem in hospitals in many regions worldwide.[4]

Imipenem, meropenem, and doripenem are members of the carbapenems (β lactam class) and are routinely used to manage *P. aeruginosa* infections.[3] The mechanism of action of carbapenems is the inhibition of the peptidoglycan-assembling transpeptidases (penicillin-binding proteins [PBP]) placed on the outer part of the plasma membrane.[5] Although this class of antibiotics has a great efficacy in treatment of MDR *P. aeruginosa* infections, an expansion of carbapenem-resistant strains has been detected in the recent years.[4]

Carbapenems resistance in *P. aeruginosa* could be attributed to a reduction in the outer membrane permeability, up-regulated expression of the efflux pumps genes and production of metallo-β-lactamases (MBL), which inactivate these drugs efficiently.[6]

The outer membrane protein *Opr*D permits the transport of amino acids, peptides and carbapenems. Downregulation of oprD generally causes resistance to imipenem and meropenem.[7] Another major mechanism of Carbapenem resistance in *P. aeruginosa* is decreasing the antibiotic concentration through efflux systems belonging to the resistance-nodulation- division (RND) family (MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM, MexJK and MexVW).[8] Meropenem is affected by high regulation of MexAB-OprM, MexCD-OprJ, and MexXY-OprM, whilst imipenem is not affected.[9] MexAB-OprM and MexXY-OprM pumps are the principal means of natural resistance against antimicrobial and disinfectant compounds. The MexAB-OprM system confers resistance against a wide range of antibiotics including tetracycline, chloramphenicol quinolones, trimethoprim and most β lactams and MexXY-OprM contributes to the resistance against aminoglycosides, tetracycline, and erythromycin.[10]

Carbapenems withstand relatively well hydrolysis by most of the beta lactamases; however, metallo-b-lactamases (MBL) can inactivate them.[11] This group of enzymes include imipenemase (IMP), Verona imipenemase (VIM), New Delhi MBL (NDM), Seoul imipenemase (SIM), Sao Paulo MBL (SPM), German imipenemase (GIM), Adelaide imipenemase (AIM), and Dutch imipenemase (DIM).[12] The genes that encode the production of MBLs are typically part of class 1 integron structures and are transmitted by mobile genetic elements.[13] Other non-metallo carbapenemases also exist, including Ambler class A β-lactamases: *K. pneumonia* carbapenemase (KPC) and Guiana Extended spectrum (GES) as well as class D β-lactamases: Oxa-48.[14]

The aims of this study were to evaluate the frequency of carbapenem resistance among *P. aeruginosa* isolated from different clinical specimens (urine, sputum, pus, etc.) in Minia governorate, Egypt and to investigate the correlation between the MICs obtained and overexpression of certain efflux pump genes, downregulation of outer membrane porin, *Opr*D and the presence of metallo-b-lactamases (MBL) genes.

Materials and Methods

This study was conducted at the Minia University Hospital (the largest tertiary hospital in Minia-Upper Egypt, receiving referrals from 9 districts and serving a population of more than 5 million) in the period between December 2017 to November 2018.

Bacterial Isolates

A total of 634 *P. aeruginosa* isolates were collected from different clinical specimens (160 urine, 103 sputum, 227 wound, 116 tracheal aspirates, 88 bloodstream infections, and 56 bronco-alveolar lavage samples) as part of the routine hospital laboratory procedure. Isolates were identified using standard laboratory methods including bacteriological and biochemical tests such as Gram stain, oxidase test, growth on cetrimide agar medium, the ability to grow at 42°C, O/F (Oxidation-Fermentation) test and pigment production. The identified *P. aeruginosa* isolates were stored at −70°C in trypticase soy broth (Merck, Darmstadt, Germany) supplemented with 10% glycerol until further investigation. *P. aeruginosa* ATCC 27853 was used as the reference strain throughout the study. A set of 32 carbapenem-sensitive clinical isolates were used as negative controls.

Antimicrobial Susceptibility Testing

The minimum inhibitory concentrations (MICs) of meropenem and imipenem were measured on Muller Hinton Agar plates (Oxoid, Basingstoke, UK) by the agar dilution method and interpreted according to CLSI breakpoint.[15] Meropenem and Imipenem were purchased from Himedia, Mumbai

(India). Antimicrobial profile against different anti-pseudomonas drugs was done for the carbapenem-resistant isolates using disc-diffusion method according to CLSI.[15]

Detection of Carbapenemase Genes

DNA Extraction and PCR

Total DNA from *P. aeruginosa* isolates was extracted by boiling method according to Rostami et al.[16] The tubes were stored at −20°C prior to being used in PCR amplification as a DNA template.[10]

All carbapenem-resistant *P. aeruginosa* isolates were screened by PCR for the bla_{IMP1}, bla_{IMP2}, bla_{VIM1}, bla_{VIM2}, bla_{SPM} and bla_{NDM} genes using specific primers and conditions listed in Table 1.

Efflux Pump and Porin Expression

RNA Extraction and Reverse Transcription to cDNA

For total RNA extraction, *P. aeruginosa* isolates were cultured in 1.5 mL LB broth and placed in a shaking incubator (180 rpm) at 37°C for 18–24 hrs. Tubes were incubated overnight at 37°C and then were centrifuged (8000rpm) at 4°C for 7 mins; afterwards the pellet was processed according to the manufacturer's instructions using the GeneJET RNA Purification Kit (Thermo-Scientific™, USA). To prepare cDNA, 5 µL of extracted RNA was added to15 µL deionized water and converted to cDNA in a total volume of 20µL according to the manufacturer's instructions using Revert Aid First Strand cDNA synthesis Kit (Thermo-Scientific™, USA). The cDNAs were stored at −20°C and were used within 1 week.

Table 1 Primers Used in This Study

Primer	Sequence (5′-3′)	Size (bp)	References
bla_{IMP1}	F: CTACCGCAGCAGAGTCTTTG R: AACCAGTTTTGCCTTACCAT	587	[17]
bla_{IMP2}	F: GTTTTATGTGTATGCTTCC R: AGCCTGTTCCCATGTAC	678	[18]
bla_{VIM1}	F:AGTGGTGAGTATCCGACAG R:ATGAAAGTGCGTGGAGAC	261	[19]
bla_{VIM2}	F: ATGTTCAAACTTTTGAGTAAG R: CTACTCAACGACTGAGCG	801	[20]
bla_{NDM}	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	[21]
bla_{SPM}	F: CCTACAATCTAACGGCGACC R: TCGCCGTGTCCAGGTATAAC	650	[22]
*opr*D	F:CTACGGCTACGGCGAGGAT R:GACCGGACTGGACCACGTACT	NA	[23]
*mex*A	F:AACCCGAACAACGAGCTG R:ATGGCCTTCTGCTTGACG		
*mex*C	F:GGAAGAGCGACAGGAGGC R:CTGCACCGTCAGGCCCTC		
*mex*E	F:TACTGGTCCTGAGCGCCT R:CAGCGGTTGTTCGATGA		
*mex*Y	F:CCGCTACAACGGCTATCCCT R:AGCGGGATCGACCAGCTTTC		[24]
*rps*L	F:GCAACTATCAACCGACTGGTG R:GCTGTGCTCTTGCAGGTTGTG		[25]

Real-Time (RT)-PCR to Measure *mex*A, *mex*C, *mex*E, *mex*Y and *opr*D Expression Levels

The expression of *mex*A, *mex*C, *mex*E, *mex*Y and *opr*D in the *P. aeruginosa* isolates that showed resistance to carbapenems (imipenem and meropenem) was determined by real-time PCR (RT-PCR). Primers that were used for the amplification of *mex*A, *mex*C, *mex*E, *mex*Y, *opr*D and *rps*L are specified in Table 1 and the housekeeping gene *rps*L was used as the normalizing gene. Expression levels of the above genes for clinical isolates of *P. aeruginosa* were compared to *P. aeruginosa* PAO1.

The quantification of transcripts was carried out by SYBRGreen PCR Master Mix (Thermo-Scientific™, USA). Applied Biosystems 7500-Fast Real-Time PCR system (Thermo-Scientific™-USA). The relative gene expression was calculated using the ΔΔCt method.[26] Expression of the house-keeping gene *rps*L was determined in parallel to normalize the transcriptional levels of target genes and they were furthermore standardized against target gene expression by the wild-type reference strain PAO1. Overexpression of the efflux systems MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM was considered when *mex*A, *mex*C, *mex*E or *mex*Y transcriptional levels were at least two-, two-, 10-and 4-fold higher than that of PAO1, respectively.[27,28] All the experiments were repeated three times.

Random Amplified Polymorphic DNA (RAPD) PCR

The isolated *P. aeruginosa* strains were sent for RAPD-genotyping using primer 272-AGCGGGCCAA as previously designated by Mahenthiralingam and co-workers.[29] The master mixture was prepared using 2.5 µL 10x PCR buffer; 2.5mM $MgCl_2$; 300µM of dNTPs; 1.7U Taq DNA polymerase; and 3 µL genomic DNA (40 ng) in a final volume of 25 µL using the following thermocycler

conditions: denaturation 5 mins at 95°C, annealing 5 mins at 36°C, and elongation 5 mins at 72°C, for 4 cycles and 31 cycles consisting of 94°C for 1 min, 45°C for 1min, and 72°C for 2 mins, followed by a final extension at 72°C for 10 mins. For reproducibility, each reaction was performed in triplicate. For analysis of RAPD products, PCR amplicons were separated on 1.% (w/v) agarose gels and the DNA band size was determined in comparison to 1Kb DNA ladders (250 bp–10,000 bp band sizes). Similarity between isolates was assessed according to Dice similarity coefficient and Unweighted Average Pair Group Method (UPGMA), using FreeTree and TreeView software. Only major reproducible bands of intensity were put into consideration for similarity matrix calculation. Cut-off values of ≥80% were used for determination of potential clonal relatedness.[30]

Statistical Analysis

Data were analyzed using SPSS statistical package version 20 (Chicago, USA). The following had been done accordingly: mean (X~) and standard deviation (SD) for quantitative data, Chi-squared test for categorical data and Bivariate Pearson correlation analysis for association analysis. For all tests, probability (p) was considered: non-significant if ≥ 0.05, significant if < 0.05, Highly significant if < 0.01 and very highly significant if <0.001. Grade of correlation or association were calculated as follows: 0.00 to 0.24: weak or no association, 0.25 to 0.49: fair association, 0.50 to 0.74: moderate association and 0.75+: strong association.

Results

Out of 634 *P. aeruginosa* isolates, 32 (5%) were imipenem resistant of which 29 were meropenem resistant. High-level resistance (≥64mg/mL) to imipenem was found in 27 isolates (84.3%) out of 32 isolates, while high-level resistance (≥64mg/mL) to meropenem was found in 28 (96.5%) out of the 29 meropenem-resistant isolates. The antimicrobial profile to different antimicrobials used for treating *P. aeruginosa* infection is illustrated in Table S1.

Carbapenemase genes were found as follows: 31 isolates were positive for $bla_{\text{VIM-1}}$, while bla_{NDM} was detected in 4 isolates, which co-harbored $bla_{\text{VIM-1}}$ also. None of the isolates possessed either bla-$_{\text{VIM-2}}$, $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$ or $bla_{\text{SPM.}}$

Regarding gene expression, most of the isolates showed over-expression of CDJ (75%) followed by XY efflux pump (62%), while ABM and EFN efflux pumps were overexpressed in 21.8% and 18.7% of the isolates, respectively. None of the isolates showed low expression of *opr*D (Table 2). There was a significant difference between sensitive and resistant isolates regarding XY and CDJ efflux pump expression, which were overexpressed in 14.3, 22.9% vs 62.1, 79.3% among sensitive and resistant groups, respectively (p <0.001) (Table 3). Only three of the isolates showed over-expression of the four tested efflux pumps. Expression levels of different genes are demonstrated in Table S2.

A highly significant fair association was found between XY efflux pump expression with meropenem, imipenem resistance (r=0.363, 0.327, p=0.003, 0.008) respectively. In addition, there was a very highly significant moderate correlation between CDJ efflux pump expression and meropenem, imipenem resistance (r=0.532, 0.654, p<0.001, <0.001) respectively (Table 4).

The carbapenem-resistant *P. aeruginosa* isolates were typed by RAPD-PCR analysis and Figure 1 illustrates their clustering. RAPD fingerprinting produced 6–10 fragments with band sizes between 250 bp and 3 kb. There were three major groups: group 1(10 isolates), group 3 (9 isolates), group 2 (8 isolates) while the fourth group (4) consisted of 4 isolates. Only one isolate (PA-15) was a unique isolate forming a separate cluster.

Discussion

In this study, carbapenem resistance was detected in 5% of *P. aeruginosa* isolates, with the majority showing high-level resistance to both imipenem and meropenem. Regarding the incidence of carbapenem resistance, our findings are lower than previously reported in the same region,[31] probably due to an improvement in abiding to infection control measures. Nevertheless, this is the first report of high-level resistance to carbapenems in Egypt, which is disturbingly snowballing in clinical *P.aeruginosa* isolates world-wide.[32] We further investigated the mechanisms of this high-level carbapenem resistance among the isolates and interestingly we found that 31 of the isolates (>96%) harbored at least one carbapenemase gene. This was in agreement with previous reports in Egypt,[33,34] which are all in agreement with finding that MBLs can overtake efflux pump up-regulation or loss of oprD as principal elements leading to carbapenem resistance.[35] However, our results show a dominance of blaVIM-1, which is not in agreement with earlier studies carried out in Egypt showing a dissemination of bla-VIM-2 instead or the universal bla-VIM genes.[34,36] Interestingly, we report here for the first time in Upper Egypt the emergence of New Delhi metallo-beta-lactamase −1 gene (bla-NDM) harboring isolates, which possessed bla-VIM-1 as well

Table 2 MIC and Characterization of Efflux Pump Overexpression and Carbapenemase Genes in the Carbapenem-Resistant *P.aeruginosa* Isolates

ID	Meropenem MIC (μg/mL)	Imipenem MIC (μg/mL)	mexA	mexC	mexE	mexY	OprD	Genotypic Profile (Different MBLs Genes)
PA- 1	2	16	-	-	-	-	-	bla_{VIM1}
PA- 2	256	256	-	+	-	-	-	bla_{VIM1}
PA- 3	256	256	-	+	-	+	-	bla_{VIM1}
PA- 4	256	256	-	-	-	+	-	bla_{VIM1}
PA- 5	256	256	+	+	-	+	-	bla_{VIM1}- bla_{NDM}
PA- 6	2	8	-	+	-	+	-	bla_{VIM1}
PA- 7	256	256	-	+	-	+	-	bla_{VIM1}
PA- 8	512	512	-	+	-	-	-	–
PA- 9	256	256	+	+	+	+	-	bla_{VIM1}
PA- 10	256	256	-	+	-	-	-	bla_{VIM1}
PA- 11	256	256	-	+	-	+	-	bla_{VIM1}
PA- 12	512	512	-	+	-	+	-	bla_{VIM1}
PA- 13	256	256	-	+	-	+	-	bla_{VIM1}
PA- 14	256	256	-	-	+	-	-	bla_{VIM1}
PA- 15	2	16	+	+	-	-	-	bla_{VIM1}
PA- 16	512	512	-	+	+	-	-	bla_{VIM1}- bla_{NDM}
PA- 17	256	256	-	+	-	-	-	bla_{VIM1}- bla_{NDM}
PA- 18	256	256	-	-	-	-	-	bla_{VIM1}
PA- 19	512	512	+	+	+	+	-	bla_{VIM1}
PA- 20	512	512	+	+	-	+	-	bla_{VIM1}
PA- 21	256	256	-	-	+	+	-	bla_{VIM1}
PA- 22	256	128	+	+	-	+	-	bla_{VIM1}
PA- 23	256	256	-	+	-	+	-	bla_{VIM1}
PA- 24	8	2	-	-	-	+	-	bla_{VIM1}
PA- 25	512	512	-	+	-	+	-	bla_{VIM1}
PA- 26	256	256	-	+	-	+	-	bla_{VIM1}
PA- 27	256	256	-	+	-	+	-	bla_{VIM1}
PA- 28	256	256	-	+	-	-	-	bla_{VIM1}
PA- 29	256	256	-	+	-	-	-	bla_{VIM1}-bla_{NDM}
PA- 30	256	256	+	+	+	+	-	bla_{VIM1}
PA- 31	256	256	-	+	-	+	-	bla_{VIM1}
PA- 32	256	256	-	-	-	-	-	bla_{VIM1}
Total No.			7	25	6	20	0	
Total percent (%)*			21.8	78	18.7	62.5	0	

Notes: *Percentages were calculated to total number of carbapenem-resistant *P.aeruginosa isolates*.
Abbreviations: ID, identification number; PA, *Pseudomonas aeruginosa*; MIC, minimum inhibitory concentration; ABM, MexAB-OprM; CDJ, MexCD-OprJ; EFN, MexEF-OprN; XY, MexXY-OprM; OprD, Outer membrane porin; -, absent; +, present; IMP1, bla_{IMP1}; IMP2, bla_{IMP2}; VIM1, bla_{VIM1}; VIM2, bla_{VIM2}; NDM, bla_{NDM}; SPM, bla_{SPM}.

and showed high-level resistance. There are no previous reports of bla-NDM in Egypt, except a case report of two isolates recovered from a tertiary hospital in 2012.[37] The patients who harbored the bla-NDM isolates had no reported contact with a foreign population or a history of travelling to or from India or any foreign country. The presence of this gene could be explained as described earlier by Zafer and co-workers as a result of antimicrobial abuse or unreported contact and cross-contamination with a positive case[37] (perhaps a contact with the previously described cases in Cairo could be the source, although not confirmed).

This occurrence of MBL-producing isolates can produce grave infections that are problematic to treat and their

Table 3 Correlation of Efflux Pump Gene Expression with Carbapenem-Resistant and Sensitive *P.aeruginosa* Isolates

Efflux Pump Gene Expression		Meropenem					Imipenem				
		Sensitive (No. = 35)		Resistant (No. = 29)		P value	Sensitive (No. = 32)		Resistant (No. = 32)		P value
		No.	%*	No.	%*		No.	%*	No.	%*	
*mex*Y	Not over- expressed	30	85.7	11	37.9	<0.001	28	87.5	13	40.6	<0.001
	Over- expressed	5	14.3	18	62.1		4	12.5	19	59.4	
*mex*A	Not over- expressed	25	71.4	23	79.3	0.469	23	71.9	25	78.1	0.564
	Over- expressed	10	28.6	6	20.7		9	28.1	7	21.9	
*mex*C	Not over- expressed	27	77.1	6	20.7	<0.001	0 26	0 81.2	7	21.9	<0.001
	Over- expressed	8	22.9	23	79.3		6	18.8	25	78.1	
*mex*E	Not over- expressed	29	82.9	23	79.3	0.717	0 26	0 81.2	26	81.2	1.000
	Over- expressed	6	17.1	6	20.7		6	18.8	6	18.8	

Notes: *Percentages were correlated to total No. of either carbapenem-resistant or sensitive *P.aeruginosa* isolates.

Table 4 Bivariate Correlation Analysis Between Meropenem, Imipenem Resistance and Efflux Pump Over-Expression

Efflux Pump		Meropenem	Imipenem
XY	(r) *P*	0.363 0.003	0.327 0.008
ABM	(r) *P*	−0.114- 0.370	−0.141- 0.267
CDJ	(r) *P*	0.532 <0.001	0.564 <0.001
EFN	(r) *P*	0.009 0.947	−0.031- 0.805

Note: Significant correlation at P value <0.05.
Abbreviation: r, Spearman's rho correlation.

existence in different sources in Egypt is of great country-wide importance.

Different efflux pumps are described in *P. aeruginosa*, among which MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM are the best-characterized systems that are correlated with antibiotic resistance in clinical *P. aeruginosa* isolates.[38] In our study, the majority of the isolates demonstrated over-expression of MexCD-OprJ and MexXY-OprM efflux pumps. A strong correlation was found between MexCD-OprJ over-expression and both meropenem and imipenem resistance, concurring with Bubonja-Sonje and co-workers.[39]

Although the MexXY-OprM system is known as a substantial element in aminoglycoside resistance in only *P. aeru*ginosa,[40] it is also over-expressed in multi-drug resistant *P. aeruginosa*[41] and similar reports to our study have shown its over-expression in carbapenem-resistant *P. aeruginosa* strains.[7,42,43]

MexAB-OprM over-expression was observed in 21.8% of the isolates, concurring with Xavier and co-workers.[44] In contrast, Dantas et al[7] and Chalhoub et al[32] reported a much higher frequency of MexAB-OprM over-expression in *P. aeruginosa* isolates. Surprisingly, no down-regulation of *opr*D was detected in this study, which could be claimed to the presence of mutations eg, in loops L2 and L3 as described by Muderris et al.[45] However, Chalhoub et al found no role for opr*D* mutation in high-level resistance compared to efflux pump overexpression.[32]

Finding bacterial genetic similarity and relatedness is crucial for cross-infection assessment, for which the value of various genotyping techniques has been recognized.[46,47] Of those techniques, RAPD-PCR is advantageous in being fast, simple, and reproducible in addition to having a high discriminatory power.[47]

In the present study, we detected 4 distinct RAPD clusters among 32 isolates (12.5% of polymorphisms), which unfortunately suggests possible hospital cross-contamination.

Conclusion

Our results demonstrate that high-level carbapenems resistance reported in this region is attributable to carbapenemase production as well as to concomitant efflux-pump over-production. A high probability of cross-acquisition is concluded from phylogenetic results suggesting an

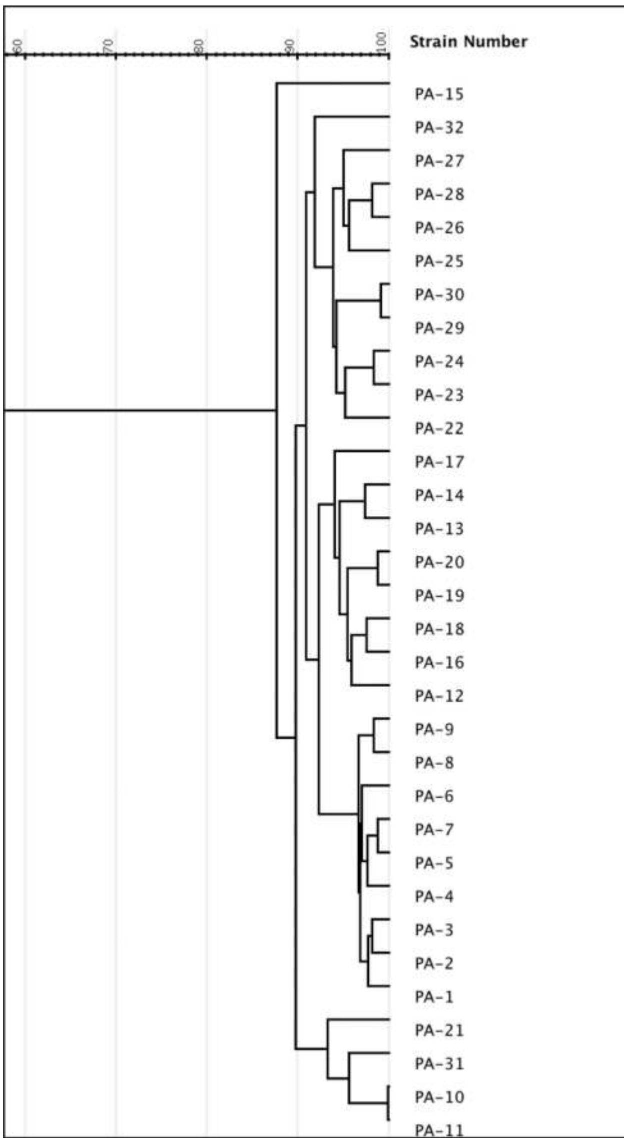

Figure 1 Dendrogram based on RAPD analysis of 32 *P.aeruginosa* isolates. The dendrogram was developed using gelJ software.
Abbreviation: PA, *Pseudomonas aeruginos*.

infection control problem, which necessitates more strict procedures.

Limitations: Studying the mutation of oprD was not done in this study due to lack of funding. There was no positive control for the over-producers.

Disclosure

The authors report no conflicts of interest in this work.

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