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Original article

Association between *MexA/MexB* efflux-pump genes with the resistance pattern among *Pseudomonas aeruginosa* isolates from Ain shams University Hospitals

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ABSTRACT

Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen that is a leading cause of many types of infections both healthcare associated infections (HAIs) or community acquired infections. In general, this organism is highly resistant to different classes of antimicrobials through different mechanisms that represents a major concern in treatment of infections in hospitals. **Aim of the study:** To detect the association between the presence of *MexA/MexB* genes and the resistance pattern among *P. aeruginosa* isolates from Ain Shams University Hospitals. **Methods:** A total of 60 isolates of *P. aeruginosa* were obtained from Main Microbiology Laboratory, Ain Shams University Hospitals. Phenotypic identification and antimicrobial susceptibility testing were performed followed by detection of *MexA/MexB* genes using polymerase chain reaction (PCR). **Results:** Most of isolates were isolated from urine samples 26 (43.3%), followed by sputum samples 14 (23.3%). Antimicrobial susceptibility showed highest maximum resistance to cefepime (97%), ceftazidime (90%), gentamycin (87%), Piperacillin (73%) and ciprofloxacin (60%). The least resistance was reported to meropenem (63%), imipenem (60%) and piperacillin/tazobactam (43%). 38 (63.3%) isolates were extensive drug resistance (XDR), 12 (20%) isolates were multi-drug resistance (MDR) and 10 (16.7%) isolates were non-MDR. *MexA* and *mexB* genes were detected in 56.7% (34 strains) and 46.7% (28 strains) of all tested isolates, respectively. According to our results, all strains that carry *mexB* gene carry *MexA* gene as well. **Conclusion:** Antimicrobial resistance among *P. aeruginosa* is widely spreading and significantly associated with presence of *MexA/MexB* gene.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is considered one of the most important pervading opportunistic pathogen causing community acquired and healthcare-associated infections (HAIs). The infections caused by this organism are difficult to cure due to high resistance caused by

different mechanisms either natural or acquired [1,2].

Antibiotic resistance is a crucial public health problem that increases both mortality and hospital stay [3,4]. The appearance of multidrug-resistant strains (MDR) and extensive drug-resistant (XDR) among *P. aeruginosa* is a concern because

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effective antibiotics against these resistant strains are limited [5].

Pseudomonas aeruginosa (*P. aeruginosa*) owns different resistance mechanisms by gene transfer through plasmids or transposons either through inherited or acquired means. These mechanisms result in changing the permeability of cell membrane, modification of the target site and efflux pumps [6].

One of the common antimicrobial mechanism is an efflux pump genes. One of these genes is *MexA/MexB-OprM* system which act by extruding different antibiotics outside the cytoplasmic membrane. These genes code for proteins that are responsible for cytoplasmic membrane protein, periplasmic linker protein and an outer membrane porin channel proteins. This system extrude antibiotics, like β -lactams, chloramphenicol, quinolones, macrolides, novobiocin, trimethoprim sulfamethoxazole, tetracyclines, even extending to nonantibiotic substances such as dyes, detergents, organic solvents and tea tree oil [7].

This efflux pump systems plays role in both intrinsic and acquired resistance, however other systems participate only in acquired resistance as *mexXY-oprM* [8].

So, in this work we will study the pattern of resistance among *P. aeruginosa* and association between resistance profile and presence of *MexA/MexB* genes.

Material and Methods

This research work was conducted on sixty *P. aeruginosa* isolates previously identified by VITEK2 (bioMérieux, Inc., Hazelwood, MO) that is a fully automated system. These isolates were retrieved from different clinical samples from Ain Shams University Hospital, Main Microbiology Laboratory, Cairo, Egypt. The study was approved by the Research Ethics Committee, Ain Shams University. (FWA 00017585), and from MASRI (FMASU R 140/2022).

Detection of antimicrobial susceptibility pattern in *P. aeruginosa* isolates

All isolates were subjected to antimicrobial susceptibility testing by disk diffusion method for the following antibiotics (Oxoid, England): aztreonam 30 μ g (ATM), cefepime 30 μ g (FEP), ceftazidime 30 μ g (CAZ), piperacillin(PIP) 100 μ g , piperacillin /tazobactam 110 μ g (TPZ), ciprofloxacin 5 μ g (CIP), gentamycin 10 μ g (GN), amikacin 30 μ g

(AK), and tobramycin 30 μ g (TOB). , imipenem (IPM) 10 μ g, meropenem(MEM) 10 μ g , and then results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [9].

Isolates that were non susceptible to at least one agent in three or more antimicrobial classes were defined as MDR while, isolates that non susceptible to at least one agent in all but two or fewer antimicrobial classes were defined as XDR [10].

Polymerase chain reaction (PCR)

Then all isolates were tested for the presence of *MexA/MexB* genes by using PCR. DNA extraction was performed by using QIAGEN DNA extraction Kit® (QIAGEN, USA), for purification of DNA from Bacterial cultures, then amplification of *MexA/MexB* genes was done using primers shown in **table (1)** by using Go Taq® Green Master Mix (Promega, USA).

By using a thermal cycler (Applied Biosystems, USA), the amplification conditions were adjusted as shown in **table (2)**.

Then the amplified products were visualized by using (2%) agarose gel electrophoresis. DNA in the gel was visualized using UV transilluminator and the product size of the genes were shown in **figure (1)**.

Statistical analyses

All collected data entered in Microsoft Office Excel worksheet and statistical analysis was done. Social Science (SPSS) version 16.0.

Results

A total of sixty *P. aeruginosa* isolates were collected from Main Microbiology Laboratory, Ain Shams University Hospitals. Most isolates were obtained from urine samples 26 (43.3%), followed by isolates obtained from sputum 14 (23.3%). 12 (20%) isolates were from pus samples, 4 (6.7%) were from CSF, 2 (3.3%) were from central lines and 2 (3.3%) were from blood.

All isolates were subjected to antimicrobial susceptibility testing, most of isolates were resistant to FEP (97%), CAZ (90%), CN (87%), PIP (73%) and CIP (60%). Approximately half strains were resistant to TOB (57%) and AK (50%). The least resistant was reported to MEM (63%), IPM (60%) and TPZ (43%). Resistance pattern to different antibiotics is shown in **figure (2)**.

Out of 60 *P. aeruginosa* isolates, 38 (63.3%) isolates were considered as XDR, 12 (20%)

isolates were MDR and 10 (16.7%) isolates were non-MDR according to definitions of Magiorakos et al. [12]

MexA and *mexB* genes were done by PCR to all isolates. *MexA* and *mexB* genes were detected in 56.7% (34 strains) and 46.7% (28 strains) of all tested isolates, respectively. According to our results, all strains that carry *MexB* gene carry *MexA* gene as well.

Regarding distribution of *MexA/MexB* genes in different clinical samples, *MexA* gene was found in 83.3%, 78.6% and in 50% of isolates

obtained from sputum, pus and urine respectively. *MexB* gene was found in 83.3%, 75% and in 46.1% of isolates obtained from sputum, pus and urine respectively.

Significant association was found between antimicrobial resistance pattern and studied genes, *MexA* gene was found in 30 (78.95%) out of 38 XDR and 4 (33.3%) out of 12 MDR respectively. However, *MexB* gene was found in 24 (63.16%) out of 38 XDR isolates and 4 (33.3%) out of 12 MDR isolates as shown in table (3)

Table 1. *MexA/MexB* genes primers.

Gene	Primer Sequence	Amplified product
MexA gene	Forward 5'CGACCAGGCCGTGAGCAAGCAGC3' Reverse 5'GGAGACCTTCGCCGCGTTGTCGC 3'	530 bp
MexB gene	Forward 5'GTGTTCCGGCTCGCAGTACTC 3' Reverse 5'AACCGTCGGGATTGACCTTG 3'	244 bp

Table 2. Amplification conditions.

Phases	Number of cycles	Temperature	Time
Initial hold	1	95 °C	2 minutes
Amplification	40		
Denaturation		95 °C	45 seconds
Annealing		55 °C	45 seconds
Extension		72 °C	1 minutes
Final extension	1	72 °C	10 minutes

Table 3. Association between antimicrobial resistance pattern and *MexA/MexB* genes.

		Antimicrobial resistance								Chi-Square	
		XDR		MDR		Non-MDR		Total			
		N	%	N	%	N	%	N	%	X ²	p-value
<i>MexA</i> 530BP	Positive	30	78.95	4	33.33	0	0.00	34	56.67	23.420	<0.001*
	Negative	8	21.05	8	66.67	10	100.00	26	43.33		
<i>MexB</i> 244BP	Positive	24	63.16	4	33.33	0	0.00	28	46.67	13.759	0.001*
	Negative	14	36.84	8	66.67	10	100.00	32	53.33		

Figure 1. Agarose gel electrophoresis of PCR-assay for identification of *MexA* and *MexB* gene. *MexA* gene was detected at 530 bp and *MexB* was detected at 244bp.

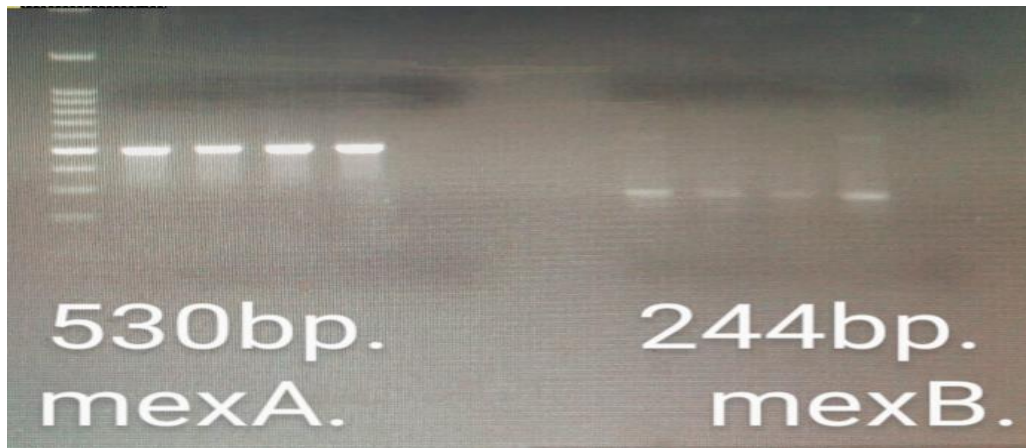


Figure 2. Susceptibility testing results of *P. aeruginosa*. to different antibiotics by the disc diffusion method

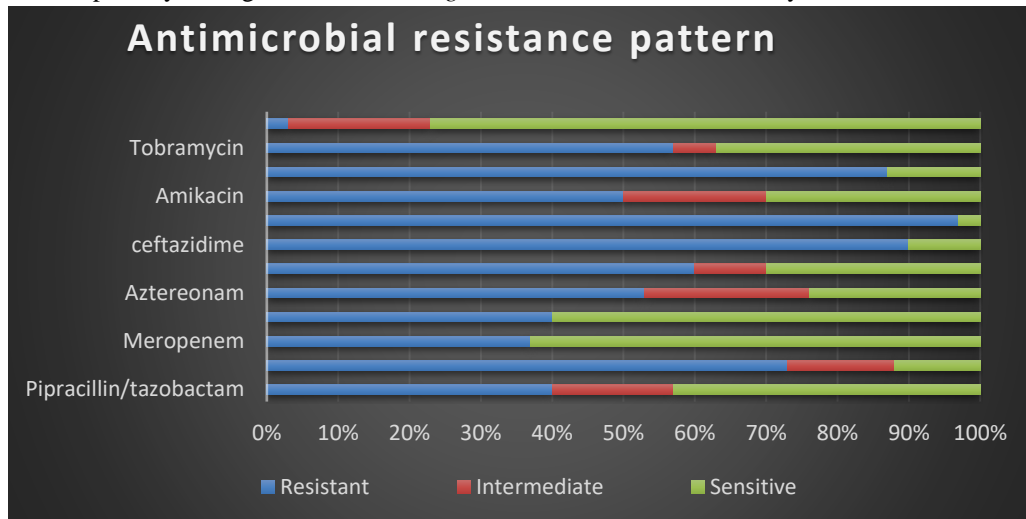


Figure 3. A plate of MacConkey agar showing non-lactose fermenting colonies of *P. aeruginosa*.



Figure 4. A plate of blood agar showing growth of *P. aeruginosa*.

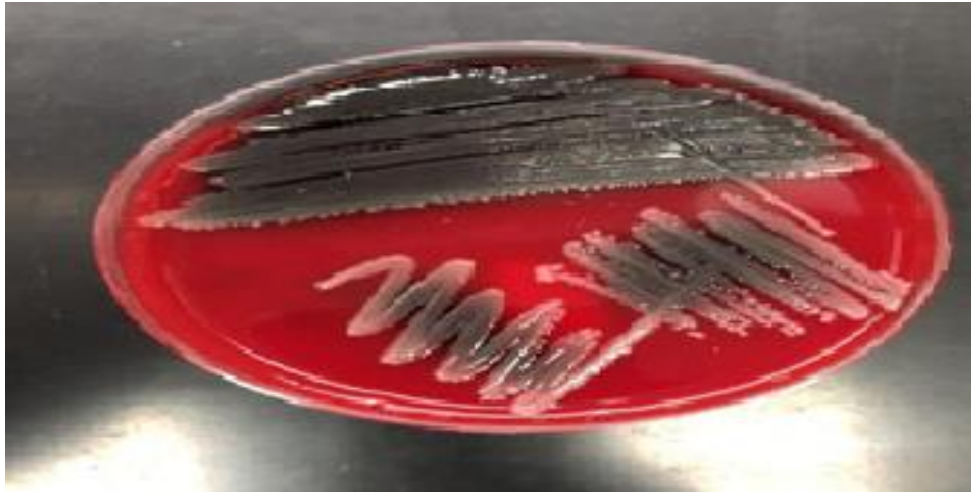
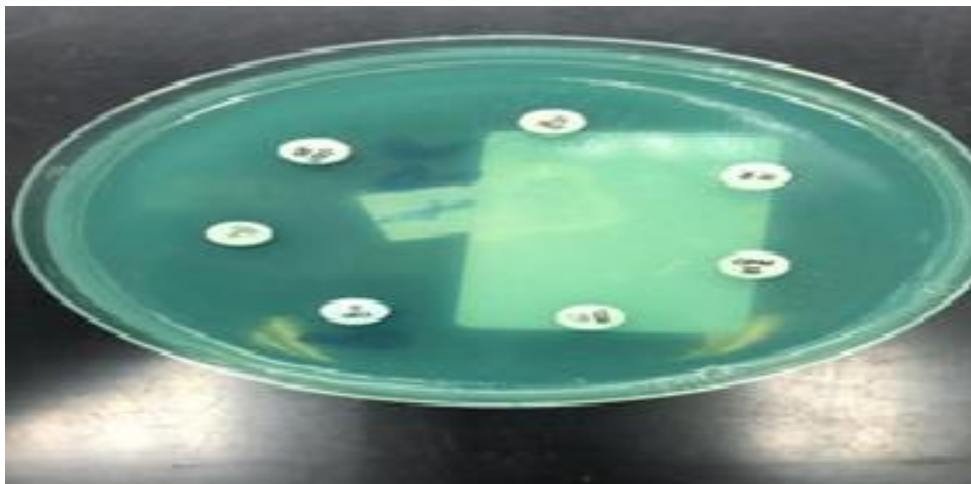


Figure 5. A plate of Muller-Hinton agar inoculated by *P. aeruginosa* showing extensive drug resistance.



Discussion

The emergence of XDR and MDR among *P. aeruginosa* is increasing and considered a major public health threat, one of major mechanisms involved in emergence of resistance in this organism is efflux pump [11,12].

This study aimed to detect the association between the presence of *MexA/MexB* genes and the resistance pattern among *P. aeruginosa* isolates from Ain Shams University hospitals

A total of 60 isolates of *P. aeruginosa* were included in this study, most of isolates were isolated from urine samples 26 (43.3%), followed by sputum samples 14 (23.3%), 12 (20%) isolates were isolated from pus samples, four (6.7%) were isolated from CSF, two (3.3%) were isolated from central lines and two (3.3%) were isolated from blood.

This finding goes in accordance with a study conducted in Egypt by **Abdallah et al.** [13] who reported that the most of *P. aeruginosa* were isolated from endotracheal samples (44%) followed by urine (36%) and the least from blood (2%). Also, **Mohamed et al.** [14] reported higher rate from respiratory samples (29%) followed by urine (27%).

On the other hand, a study done by **Wassef et al.** [15] reported higher rate of *P. aeruginosa* isolation from surgical site infections (37.5%).

In this study, all isolates were tested for antimicrobial susceptibility and the highest resistance was reported to FEP (97%), CAZ (90%), CN (87%), PIP (73%) and CIP (60%).

Similarly, a study performed by **AL-Zwaid et al.** [16] in Iraq reported high resistance to CAZ (93.6%) and FEP (77.2%) followed by 68% for PIP,

and 62% for CN while CIP resistance was detected in just (44%).

On the contrary, lower rate of PIP resistance was reported by **Vitkauskienė et al.** [17] who reported 37% and 27.3% resistance rate among *P. aeruginosa* isolates from the bloodstream of intensive care unit (ICU) and non-ICU patients.

In this study, the least resistance was reported to MEM (63%), IPM (60%) and TPZ (43%). This was in accordance to a study done by **Abed et al.** [18] in 2021, who reported lowest resistance to TPZ while higher resistance were found to CIP, CAZ and CN.

In correspondence, similar result was reported by a study done by **AL-Zwaid et al.** [16] who found that the lowest resistance was to MEM, imipenem and ciprofloxacin. In addition, **Bhandari et al.** [19] found lowest resistance to MEM and ofloxacin.

Our study showed that 38 (63.3%) isolates were XDR, 12 (20%) isolates were MDR and 10 (16.7%) isolates were non-MDR.

Similar results were reported in Egypt, **Hassuna et al.** [20] reported that the frequency of MDR was 22.5% among samples from ventilator associated pneumonia patients.

On the other hand, a study done by **Abdallah et al.** [13] reported that 68% of isolates were MDR. **Bhandari et al.** [19] reported that MDR strains were found in 54.84%. a study done by **Kishk et al.** [21] found that MDR stains was 70%.

The difference in antimicrobial susceptibility results may be attributed to the policy of antibiotic use in hospitals and different patients' comorbidity.

In this study, all isolates were tested for presence of *MexA* and *MexB* genes, *MexA* gene were detected in 56.7% (34 strains) and *MexB* gene in 46.67% (28 strains). 46.67% (28 strains) contained both *MexA* and *MexB* genes.

Similarly, **Abdallah et al.** [13] stated that out of 35 meropenem resistant isolates, *MexA* gene was detected in 54.2% while *MexB* gene was detected in 51.4% and both genes were detected in 40%. Another study done by **Murugan et al.** [22] detected higher frequency of efflux pump genes among tested isolates where (51%) for *MexA* and (46.5%) for *MexB*, and (40.5%) for *Opr M*.gene.

On the other hand, **Kishk et al.** [21] declared that *MexA* gene was detected in 88.2% of

isolates, while *MexB* gene was detected in 70.5% and both genes were detected in 80 strains (58.8%). Another study done by **AL-Zwaid et al.** [16] found *MexA* in 83.5% and *MexB* in 63.29% of isolates.

A recent study done in Nepal by **Bhandari et al.** [19] stated that both *MexA* and *MexB* genes were detected in 71% of *P. aeruginosa* isolates.

This current study elaborates the role of efflux pump as resistance mechanism in *P. aeruginosa* and this may give chance for further studies to evaluate the role of efflux pump inhibitors in combating antimicrobial resistance. Also we recommend working on a larger scale to define the genotypic characteristics of *P. aeruginosa* in Ain Shams University Hospitals

Conclusion

In conclusion, the current study recognized high prevalence XDR and MDR among *P. aeruginosa* isolates in Ain Shams University Hospitals. The emergence of high resistance pattern among *P. aeruginosa* is alarming and mandates the optimization of antibiotic use and all isolates that harbour efflux pump system (*Mex A/B* system) are XDR and MDR which explains the mechanism of antimicrobial resistance in *P. aeruginosa*.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

All authors have made substantial contributions to the design of the study. Clinical isolates were provided by Dr. Dalia Hosni Abdel Hamid. The identification and molecular tests were performed by Dr Yasmin Mohamed Ahmed, Dr Shimaa A. Abdel Salam and Dr Fatma el Zahraa Youssef fathy. Data analysis and interpretation were contributed to all the authors. Drafting the article was performed by all authors. Revising the draft critically for important intellectual and scientific content was carried out by all the authors. All the authors provided final approval of the version to be published.

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