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ORIGINAL ARTICLE

Phenotypic Detection of Efflux Pumps in Drug Resistant *Pseudomonas Aeurginosa* Isolated from Suez Canal University Hospitals

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ABSTRACT

Background: *Pseudomonas aeruginosa is considered as one of the top five pathogens of nosocomial diseases worldwide. Infections caused by P. aeruginosa are often severe and life threatening and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of emergence of antibiotic resistance during therapy. What adds to the problem of P. aeruginosa nosocomial infections is the emergence of multi-drug resistant (MDR) strains that develop resistance by various mechanisms like multi drug resistance efflux pumps, production of β-lactamases, aminoglycoside modifying enzymes and decrease outer membrane permeability. Efflux pumps contribute to multidrug resistance as they expel different types of antibiotics and chemicals.* ***Objectives:*** *Detection of the role of the efflux pump in multidrug resistant P. aeurginosa isolates from Suez Canal university hospital (SCUH) in Ismailia.* ***Methodology****: This study included 307 hospitalized patients of both sexes and from all age groups. A forty nine P. aeruginosa strains were isolated from blood, urine, sputum and pus. Antibiotic susceptibility tests were done by using disc diffusion method. For detection of the role of efflux pump in MDR, strains proved to be MDR were further tested by MIC (agar dilution susceptibility method) before and after addition of efflux pump inhibitor carbonyl cyanide m-chlorophenyl hydrazone (CCCP).* ***Results:*** *The isolation rate of P. aeruginosa was 15.9% out of a total number of 307cases. The isolation rate was highest from cases in the ICU (26.5%), while it was lowest from internal medicine department (8.2%). 57.1% of the isolates were multidrug resistant; the efflux pump mediated resistance was proved for ciprofloxacin and meropenem.* ***Conclusion:*** *Our study proved the role of efflux pump mechanism in mullti-drug resistance by P.aeruginosa isolates to ciprofloxacin and meropenem. Other studies proved the role of this mechanism in resistance to carbapenem, levofloxacin, ciprofloxacina and meropenem. Understanding the mechanisms by which these pumps act and how to overcome its activity opens the door for restoring the antibiotic activity and constitute a promising target for novel antibacterial agents.*

INTRODUCTION

Hospital acquired infections (HAIs) are significant clinical and economic burden worldwide. An estimated 5-10% of all hospitalizations are complicated by a nosocomial infection. HAIs have been shown to be a significant economic burden in both developed and developing countries [1,2].

Pseudomonas aeurginosa is considered one of the most opportunistic human pathogen especially in immunocompromised patients and one of the top five pathogens of nosocomial diseases worldwide[3]. The infections caused by *P. aeurginosa* are frequently severe and hard to treat, due to the high antibiotic resistance rate [4].

Pseudomonas aeruginosa represents a phenomenon of antibiotic resistance, and demonstrates practically all known enzymic and mutational mechanisms of bacterial resistance; often these mechanisms exist simultaneously, thus conferring combined resistance to many strains [5].

Efflux pump mechanism contributes to multidrug resistance (MDR) as they expel different types of antibiotics and chemicals such as dyes, organic solvents, detergents, molecules needed for the cell–cell communication and metabolic products. The efflux pump transporter in *P. aeruginosa* belongs to the resistance nodulation division (RND) family and composed of three parts, the transporter, the linker, and the outer membrane pore that ensures that the extruded compound does not remain in the periplasm, hence, avoiding its return to the cytosol [6].

The inhibition of efflux pump can be achieved by different mechanisms including the following: (1) interference with the regulatory steps needed for the expression of the efflux pump, (2) chemical changes in the antibiotic structure hence hindering its attachment as the specific substrate, (3) disruption of the assembly of

the efflux pump-components, (4) inhibition of the substrate (antibiotic) binding by either competitive or non-competitive binding using other compounds, (5) blocking the outer most pores responsible for the efflux of antibiotic compound, (6) interference with the energy required for the pump activity [7].

Hence, understanding the mechanisms by which these pump act and how to overcome its activity opens the door for restoring the antibiotic activity and constitute a promising target for novel antibacterial agents. The new direction for other chemotherapeutics is the use of efflux pump inhibitors (EPIs) [8].

The aim of the study is to detect the role of the efflux pumps in multidrug resistant *P. aeruginosa* isolates from Suez Canal university hospitals in Ismailia, as one of the mechanisms of resistance in this organism.

METHODOLOGY

This study was carried on 49 *P. aeruginosa* strains that were isolated from 307 specimens collected from admitted patients at the Burn Unit, Urology, Internal Medicine, Surgery wards, Intensive Care Unit (ICU) and nursery ICU in SCUH. According to the site of infection, the specimens included blood, pus, urine and sputum.

Antibiotic Susceptibility testing:

The susceptibility testing of the isolated *P. aeruginosa* was performed using disc diffusion method and strains proved to be MDR were further tested by minimal inhibitory concentration (MIC).

Disc diffusion method:

Routine antibiograms were determined by a disc diffusion method on Muller Hinton agar plates and interpreted according to the method of Clinical Laboratory Standards Institute (CLSI, 2013).

The following antibiotic discs (Oxoid, England) were used:

- Cephalosporins: ceftazidime (30 µg) and cefepime (30 µg).
- Carbapenems: meropenem (10µg) and imepenem (10µg).
- Aminoglycosides: gentamicin (10 µg), tobramycin (10 µg) and amikacin (30µg).
- Quinolones: ciprofloxacin (5µg) and levofloxacin (5µg).
- Penicillins: Pipracillin-tazobactam (110µg).
- Monobactams: azetronam (30µg).

Minimum Inhibitory Concentration Method (MIC):

Strains proved to be MDR were further tested by MIC (Agar dilution susceptibility method). The antibiotics used for the MDR strains were ciprofloxacin, meropenem, cefotaxime and gentamicin.

Investigating the role of efflux pump in antibiotic resistance

The MICs of four antibiotics of different groups (ciprofloxacin, meropenem, cefotaxime and gentamicin) for the MDR *P. aeruginosa* isolates were detected. The MICs were repeated in the presence of concentration of 10 µM of the efflux pump inhibitor carbonyl cyanide m-chlorophenylhydrazone (prepared by adding 2mg of CCCP to one liter of the agar). The reduction in MIC of a certain antibiotics with CCCP is an indication of presence of an efflux system which might play a role in resistance to this antibiotic.

RESULTS

Table 1 shows the isolation rate of *P. aeruginosa* from different hospital wards.

Twenty eight strains (57.1%) of *P. aeruginosa* isolates were proved to be multidrug resistant (table 2).

There was reduction in the MIC of ciprofloxacin and meropenem for 11 out of the 28 isolates after the addition of CCCP (by 1-2 dilutions for meropenem and 1-3 dilutions for ciprofloxacin), which prove the role of efflux pumps in the resistance to these drugs. There was no change in the MIC of cefotaxime and gentamicin for all the isolates after CCCP addition (table 3).

Table 1. The isolation rates in different hospital wards

Clinical ward	No.	Percent
ICU	13	26.5%
Surgery	8	16.3%
Burn unit	7	14.3%
NICU	6	12.2%
Urology	6	12.2%
Pediatrics	5	10.2%
Internal medicine	4	8.2%
Total	49	100.0%

Table 2. Incidence of MDR *P. aeruginosa* among the isolated strains

Variable	No. (%)
MDR	28 (57.1%)
Non MDR	21 (42.9%)
Total	49 (100.0%)

Table 3. Effect of adding CCCP on antibiotic resistance pattern of MDR *P. aeruginosa* isolates

Str. No	Ciprofloxacin		Meropenem		Cefotaxime		Gentamicin	
	MIC (mg/L)	MIC in presence of 10 µM CCCP (mg/L)	MIC (mg/L)	MIC in presence of 10 µM CCCP (mg/L)	MIC (mg/L)	MIC in presence of 10 µM CCCP (mg/L)	MIC (mg/L)	MIC in presence of 10 µM CCCP (mg/L)
1	0.25	0.25	1	1	8	8	1	1
2	0.25	0.25	2	2	8	8	1	1
3	0.25	0.25	**32**	**16**	8	8	0.25	0.25
4	0.25	0.25	0.5	0.5	8	8	1	1
5	0.25	0.25	0.25	0.25	8	8	1	1
6	**1**	**0.5**	**8**	**4**	8	8	4	4
7	0.25	0.25	**16**	**4**	8	8	4	4
8	0.25	0.25	0.5	0.5	8	8	1	1
9	0.25	0.25	**16**	**8**	64	64	2	2
10	**8**	**4**	2	2	256	256	128	128
11	0.25	0.25	1	1	64	64	4	4
12	**8**	**2**	**2**	**1**	64	64	512	512
13	**16**	**4**	0.5	0.5	128	128	256	256
14	**64**	**16**	1	1	64	64	512	512
15	0.25	0.25	**16**	**8**	8	8	2	2
16	0.25	0.25	**32**	**16**	256	256	2	2
17	**8**	**4**	**8**	**2**	128	128	256	256
18	**256**	**128**	0.5	0.5	128	128	512	512
19	64	64	**1**	**0.5**	16	16	512	512
20	**256**	**64**	0.5	0.5	128	128	512	512
21	0.25	0.25	8	8	8	8	1	1
22	0.25	0.25	**64**	**16**	16	16	1	1
23	**4**	**2**	1	1	64	64	64	64
24	0.25	0.25	1	1	8	8	2	2
25	0.25	0.25	2	2	8	8	2	2
26	0.25	0.25	0.5	0.5	8	8	2	2
27	**64**	**8**	0.5	0.5	64	64	8	8
28	**4**	**2**	**2**	**1**	64	64	8	8

*Strains that shows decrease in MIC after addition of CCCP are bolded

DISCUSSION

In the present study, *P. aeruginosa* was isolated at a rate of 15.9% from cases of nosocomial infections in SCUH, Ismailia. Gad et al. [9], in Minia Egypt, isolated the organism at a rate of 18% from cases of nosocomial infections' while in the study of Mahmoud *et al.* [(10)] at Menofia university hospital, it was found to account for 19% of nosocomial infections [10]. Lower isolation rate (6.67%), was reported by some studies as Khan *et al.* [11] in Pakistan.

In our study, the highest isolation rates of *P. aeruginosa* were from ICU, Surgery Department and burn unit (26.5%, 16.3% and 14.3% respectively). Ikeno et al. [12] found the organism to be responsible for 16% of nosocomial pneumonia cases, 11% of hospital acquired urinary tract infections, 8% of surgical wound infections, and 10% of bloodstream infections. Gad and his colleagues [9] found that acquired infection rates in ICU were 5-10 times higher than hospital acquired rates in general ward patients. Mahmoud *et al.* [10] recorded high incidence of *P. aeruginosa* infections in burn unit at Menofia university hospital.

Regarding the isolation rates from different clinical presentations, we reported that the highest isolation rate of *P. aeruginosa* was from pus of infected wound and burns (36.7%), while it was 30.6% from urine, 18.4% from sputum and 14.3% from blood. Also, Mahmoud et al. [10] reported isolation rate of *P. aeruginosa* from burn of 38.8%, while it was 27.7% from urine, 14.8% from sputum, 5.5% from surgical wounds and 3.7% from blood.

In our research, high resistance rates exhibited by *P. aeruginosa* against cefepime (98%), ceftazidime (93.3%) were detected. Mahmoud et al. [10] has reported similar results (98.2% for cefepime and 91.2% for ceftazidime).

We reported high prevalence of MDR *P. aeruginosa* strains (57.1%). Different rate of MDR *P. aeruginosa* isolates has been reported elsewhere in other studies (60% and 30% by Unan and Gnsern [13] in Turkey and Zahra and Murray [14] in Iran respectively).

The study proved that there is a role of efflux pump-mediated resistance to ciprofloxacin and meropenem (there is a decrease in the MICs after addition ofCCCP for meropenem by 1-2 dilutions and ciprofloxacin by 1-3 dilutions), while no role was detected for gentamicin and cefotaxime. Gad et al. [9] also detected the role of efflux pumps in ciprofloxacin and meropenem resistance by the use of CCCP.

Pai et al. [15] reported the role of efflux system in carbapenem resistance in *P. aeruginosa*. Lomovskaya et al. [16] also detected that Levofloxacin had an eight fold decrease in MIC in the presence of an EPI for all strains expressing the mexAB-oprM.

CONCLUSION

The problem of *P. aeruginosa* nosocomial infections is due to the emergence of multi-drug resistant strains that develop resistance by various mechanisms like efflux pump that contribute to multidrug resistance by expelling different types of antibiotics and chemicals.

Our results clarified the role of efflux pump mechanism in mullti-drug resistance by *P. aeruginosa* isolates to some drugs as ciprofloxacin and meropenem. Other studies proved the role of this mechanism in resistance to carbapenem, levofloxacin, ciprofloxacin and meropenem.

Understanding the mechanisms by which these pumps act and how to overcome its activity opens the door for restoring the antibiotic activity and constitute a promising target for novel antibacterial agents.

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