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ORIGINAL ARTICLE

Efflux Pump Inhibition effect of Curcumin and Phenylalanine Arginyl *β*-Naphthylamide (PA*β*N) against Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Burn Infections in Tanta University Hospitals

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Key words:

Curcumin, Efflux pump, MDR Pseudomonas aeuroginosa, Phenylalanine arginyl β-naphthylamide (PAβN), Efflux pump inhibitors

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ABSTRACT

Introduction*: Multidrug resistant (MDR) Pseudomonas aeuroginosa strains are very important pathogens causing hospital acquired infections especially in intensive care units. One of the mechanisms of developing drug resistance is efflux pump through which bacteria extrude antibiotics. If these efflux channels are blocked or inhibited, increased drug concentration can be obtained inside a bacterial cell with optimal drug dose. This study was aimed to investigate the role of curcumin and phenylalanine arginyl β-naphthylamide(PAβN) as efflux pump inhibitors (EPIs).* ***Materials and Methods****: A total of 40 Pseudomonas aeruginosa isolates were taken from burn wounds. Antibiotic susceptibility was performed using disc diffusion test, then minimum inhibitory concentration (MIC) against selected antibiotics before and after adding PAβN (20mg/L, 50mg/L) and curcumin (from 5 to 50µg/ml) was tested.* ***Results****: MDR isolates showed significant reduction in MIC after adding curcumin (50µg/ml) and PAβN (20mg/L) with selected antibiotics, while no change in MIC was observed when were used alone, indicating their efflux pump inhibitor activity.* ***Conclusion****: curcumin and PAβN potentiated the effect of antibiotics and thus change their susceptibility pattern which can be attributed to efflux pumps inhibition. Further genotypic studies may be needed to confirm.*

INTRODUCTION

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic organism that accounts for up to 11% of nosocomial infections especially in burn infections also, it causes severe infections, especially in patients with cystic fibrosis or those hospitalized in intensive care units[1].

The recent increase in occurrence of multi-drug resistant (MDR) isolates of *P. aeruginosa* (*MDRPA)* raises serious problems. *MDRPA* can be defined when the strain is resistant to 3 or 4 of the following antibiotic classes: penicillins /cephalosporins /monobactams, carbapenems, aminoglycosides, and fluoroquinolones. This may be due to several resistance mechanisms as a result of multiple genetic events, i.e. (chromosomal mutations or horizontal transfers of resistance genes). Other mechanisms may include; active efflux, impermeability resulting from porins loss, plasmid-encoded b-lactamases/carbapenemases or aminoglycosides-modifying enzymes, and enzymatic or mutation-associated changes in antibiotics targets[2]. Antibiotic selection pressure represents the leading risk factor for *MDRPA* acquisition[3].

Colistin (polymyxin E) remains the best treatment of all *MDRPA* isolates, and until now it is the last available option to treat infections caused by these strains, however, the emergence of colistin resistance has been reported in *P. aeruginosa*, which may announce the spread of pan-resistant strains in the near future[4].

The active efflux pumps are very important methods contribute to the high resistance to antibiotics used in treatment of different *P. aeruginosa* infections [5]. Efflux pumps responsible for multidrug resistance as they expel different types of antibiotics and chemicals such as dyes, organic solvents, detergents, molecules needed for the cell–cell communication, biocides, and metabolic products. So, understanding the mechanisms by which these pumps act and how to overcome these mechanisms helps us for restoring the antibiotic activity and give us a promising target for novel antibacterial agents [6].

The bacterial multidrug efflux transporters can be divided into five classes: (1) small multidrug resistance (SMR), (2) major facilitator superfamily (MFS), (3) resistance nodulation cell division (RND), (4) multidrug and toxic compound extrusion (MATE), and (5) ATP-binding cassette (ABC). Those five classes obtain energy required for the active transporting either from H+ protons (RND, SMR, and MFS), Na+ dependant (MATE), or by hydrolysis ATP (ABC) [7].

The efflux pump transporter in *P. aeruginosa* belongs to the (RND) family. It consists 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[8]. There are 12 types of RND efflux systems including for example MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM, MexPQ-OpmE, MexMN-OprM, and MexVW-OprM that differ in their substrates [9]. MexAB-OprM is considered to be the most important efflux-pump mediating antibiotic resistance in *P. aeruginosa* because it transports a broad range of antibiotics [10]. MexA is the membrane fusion protein; MexB is the inner membrane transporter; and OprM is the outer membrane channel. MexAB-OprM is associated with resistance to fluoroquinolones, chloramphenicol, erythromycin, azithromycin, novobiocin, and certain β-lactams and lastly over-expression is linked to colistin resistance [11].

Phenylalanine arginyl *β*-naphthylamide (PA*β*N) are the group of peptidomimetic molecules that was introduced as one of the widely used efflux pump inhibitors (EPIs) for *P.aeruginosa*overexpressing MexAB pump, The mechanism of efflux pump inhibition of these inhibitors is through competitive inhibition mechanism, where the efflux pumps recognize them as a substrate instead of the target antibiotics (quinolones mainly ciprofloxacin and levofloxacin) and as long as the pumps expel these inhibitors outside the cells, the antibiotic remains intracellular and their concentration hence increased thus restoring its antibacterial activity. Taking into consideration that PA*β*N has a differential behavior, meaning that it can compete with certain antibiotics and not the other depending on the nature of the efflux pump and the large substrate-binding site [12]. It was also shown that PA*β*N can restore the activity of other unrelated antibiotics such as chloramphenicol and macrolides; hence, it can be considered a broad spectrum efflux pump inhibitor[13].

Curcumin (CUR) is a phenolic compound derived from the rhizomes of the plant *Curcuma longa* that is cultivated in India and Asia and is a constituent of the food ingredient turmeric. The compound has been studied widely and has been shown to have significant antimicrobial, anti-inflammatory, anti-cancer and anti-oxidant properties [14]. Evidence has been reported that CUR acting as EPIs *in vitro* against *MDRPA*, and thus the reduced minimum inhibitory concentration (MIC) of several antibiotics against these isolates was due to efflux-pump inhibition [15]. The mechanism by which CUR is antibacterial is through antibacterial inhibition of polymerization of the essential prokaryotic cell division protein FtsZ thus preventing cytokinesis [16].

The advantage of EPIs is the difficulty to develop bacterial resistance against them, but the disadvantage is their toxic property hindering their clinical application. The structure activity relationship of these compounds showed other derivatives from PA*β*N that are higher in their activity with higher solubility in biological fluids and decreased toxicity level. This raises further questions on how can we treat *P. aeruginosa* infections[5].

METHODOLOGY

1. Patients:

The present study was carried out in Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University, and was carried out on 115 patients admitted to burn wards of Tanta University Hospitals during the period of the research (from Jan. 2016 to Dec.2016). Written informed consents were obtained from all participants in this research, a code number was given for each specimen to maintain privacy of participants and confidentiality of the data, and Procedures that used in this research were completely non-invasive.

Patients' demographic data like age, sex, underlying diseases, onset, course and duration of illness and antibiotic course of treatment, were collected. Infected burn wound discharges were collected under complete aseptic conditions using sterile swabs then; samples were transported as rapid as possible to the Microbiology and Immunology Department Laboratory. Samples were then processed for isolation and identification of *Pseudomonas aeruginosa* using routine standard methods: Gram stain, culture on the following media (nutrient agar for detection of pigment production, MaCconkey's agar for detection of non-lactose fermenting colonies, blood agar), biochemical tests included (oxidase test, triple sugar iron agar, sugar fermentation reactions). The isolated *Pseudomonas aeruginosa* strains were stored at -70°C in 15% glycerol until further processing. Repeated freezing and thawing was avoided.

Antibiotic susceptibility testing

Primarily, antibiotic susceptibility testing was done on using Kirby-Bauer disc diffusion method in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines. The following antibiotic disks (Oxoid) were used: meropenem (10µg/disc), carbenicillin (100µg/disc), ceftazidime (30µg/disc), gentamicin (10µg/disc) and ciprofloxacin (5µg/disc). Then MICs of tested antibiotics were detected using Agar dilution method.

2. Detection of MICs of tested antibiotics using Agar dilution method

Antibiotic powders were purchased from Sigma Co., Egypt. Preparation of turbidity standard equal to McFarland 0.5 $BaSO_4$ was done as follows [17]**:** a 1.175% (wt/vol) barium chloride dihydrate ($BaCl_2.2H_2O$) solution (0.048 mol l−1 $BaCl_2$) and a 1% (vol/vol) sulfuric acid (H_2SO_4) solution (0.18 mol l−1, 0.36 N) were prepared. 0.5 ml of the 1.175% $BaCl_2$ solution to 99.5 ml of the 1% H_2SO_4 solution with constant stirring to get a suspension was added. 4–6 ml was putted into screw-capped glass tubes that have

the same size as those for preparing the bacterial suspensions. Tubes were Sealed tightly and stored in the dark at room temperature. Muller Hinton agar (MHA) medium (Sigma Co, Egypt) was prepared according to the manufacturer's instructions. For preparation of the inoculum: The colonies were suspended in Brain Heart Infusion (BHI) broth and incubated for 24 hours. The turbidity was adjusted to McFarland 0.5 standard, then 2µl of the suspension was delivered into the surface of the MHA medium in the form of spots. The surface was allowed to dry and plates were incubated for 24 hours. The surface of every MHA medium was divided into 20 squares for inoculating the 20 strains in one plate in the form of spots.

Stock solutions of the antibiotics were prepared and stored in the dark (wrapped in aluminium foil) at 4 °C in sealed containers as follow: before weighing the antibiotic; the container was left to warm at room temperature for about 2 h to avoid condensation of water on the powder. Stock solution was prepared at a concentration of 10 mg \ml, the following formula was used for calculating the right amount of antibiotic to be weighed,

$W = \frac{(C \times V)}{P}$, Where, W = weight of antimicrobial agent in milligram to be dissolved; V = desired volume (ml); C = final concentration of stock solution (µg ml−1); P = potency (µg mg−1). Sterile containers and spatula were used for weighing the antibiotics. All tested antibiotics were dissolved in sterile distilled water, except ceftazidime dissolved in saturated NAHCO3 solutionthen stored at −70 °C

A series of dilutions of antibiotics ranging from 2048 µg/ml to 2µg/ml were made from stock solution in MHA as shown in (Table1), which was allowed to equilibrate in water bath at 48°C- 50°C. The agar and antibiotics were mixed thoroughly and poured into petri dish in a depth of 4mm. The agar was allowed to solidify at room temperature and stored at 4-8°C.

3. Detection of MIC of tested antibiotics after addition of PAβN

Two concentrations of PABN (New test co, Alex) (20 mg/L and 50mg/L) were prepared and mixed with each antibiotic separately of the following dilutions (ranging from 2048 µg/ml to 2µg/ml), were added to the corresponding amount of MHA that was allowed to equilibrate in water bath at 48°-50°C. Media was poured in petri dishes, allowed to settle and MIC testing was carried out. MHA was prepared even with the two concentrations of PAβN, without antibiotics, so as to find whether it has any antimicrobial effect when used alone or not [18].

4. Detection of MIC of tested antibiotics after addition of curcumin

Curcumin (New test co, Alex) was dissolved in dimethyl sulfoxide (Sigma Co, Egypt), and a stock concentration of 10 mg/ml was stored at -20°C. Final test concentrations consisted of 50, 30, 20, 15, 10, and 5µg/ml of curcumin solution. Appropriate dilutions (ranging from 2048 µg/ml to 2µg/ml) of each antibiotic solution along with curcumin at concentration mentioned above, to determine its EPI activity, were added to the corresponding amount of MHA that was allowed to equilibrate in water bath at 48°-50°C. Media was poured in petri dishes, allowed to settle and MIC testing was carried out. MHA was prepared even with the same concentrations of CUR, without antibiotics, so as to find whether it has any antimicrobial effect when used alone or not [19].

The MICs of antibiotics were interpreted as the lowest concentration (µg/ml) of the antibiotic that prevents visible growth of a microorganism under defined conditions.

Table 1: Preparation of antibiotic dilution range

Amount of antibiotic solution added (µl) stock solution (10mg/ml)	*Volume of MHA (ml)*	*Final concentration of antibiotic in 20 ml of medium used in each plate (µg/ml)*
2048	17.952	1024
1024	18.976	512
512	19.488	256
256	19.744	128
128	19.872	64
64	19.936	32
32	19.968	16
16	19.984	8
8	19.992	4
4	19.996	2

RESULTS

Out of total 115 burn wound swabs, 70 *Pseudomonas aeuroginosa* strains were isolated. Out of them, 40 (57.14%) were resistant to at least one antibiotic and 10 (14.29) were observed to be resistant against all the selected antibiotics namely meropenem, carbenicillin, gentamicin, ceftazidime and ciprofloxacin according to MIC results. After increasing the concentration of PAβN from 20mg/L to 50 mg/L with those selected antibiotics, the increase in sensitivity was only (from 2.5%-10%) which was not significant

statistically (table 5). So the concentration of 20mg/L was considered appropriate for the present study. Isolates whose MIC dropped within the sensitive range after addition of PAβN (20 mg/L), were considered to have efflux pump mediated resistance mechanism (table 3). No major effect of curcumin with antibiotics was observed till concentration of 15µg/ml. When the concentration was increased from 20- 50µg/ml increased sensitivity of strains was observed (table 6). Best results were detected at a concentration of 50µg/ml. None of the *MDRPA* strains was susceptible to curcumin alone at any concentration. There were no significant differences between the effect of addition of PAβN 20 mg/L and curcumin 50 µg/ml on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains of Carbenicillin, Meropenem, and Ceftazidime (P values were non-significant) but there were significant differences with Gentamicin and Ciprofloxacin (P values were significant).

Table 2: Antibiotic Susceptibility pattern of *Pseudomonas aeruginosa strains* as determined by MIC:

Antibiotic	*Total no of sensitive strains after MIC testing % n=40*	*Total no of resistant strains after MIC testing %*
Meropenem	**30(75.0%)**	**10(25.0%)**
Carbenicillin	**25(62.5%)**	**15(37.5%)**
Ceftazidime	**23(57.5%)**	**17(42.5%)**
Gentamicin	**19(47.5%)**	**21(52.5%)**
Ciprofloxacin	**17(42.5%)**	**23(57.5%)**

Table 2 showed that 10(25.0%) of *P. aeuroginosa* strains were resistant to meropenem, 15(37.5%) strains were resistant to carbenicillin, 21(52.5%) strains were resistant to gentamicin, 17(42.5%) strains were resistant to ceftazidime and 23(57.5%) strains were resistant to ciprofloxacin according to MIC results. Interpretation was done according to CLSI guidelines 2013 for meropenem and gentamicin: Sensitive ≤ 4µg/ml; Intermediate susceptibility 8µg/ml; Resistance ≥ 16µg/ml; for carbenicillin: Sensitive ≤ 128 µg/ml; Intermediate susceptibility 256 µg/ml; Resistance ≥ 512 µg/ml; for ceftazidime: Sensitive ≤ 8µg/ml; Intermediate susceptibility 16µg/ml; Resistance ≥ 32µg/ml; for ciprofloxacin: Sensitive ≤ 1µgm/ml; Intermediate susceptibility 2µgm/ml; Resistance ≥ 4µgm/ml. Note: In this data intermediate susceptibility was considered as sensitive.

Table 3: Effect of addition of PaβN (20 mg/L) on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains

Antibiotic	*Total number of sensitive strains after addition of PAβN (20 mg/L) (%)*	*Number of strains showing resistance due to efflux pump(%)*
Meropenem	32(80.0%)	2(2/10=20%)
Carbenicillin	29(72.5%)	4(4/15=26.7%)
Ceftazidime	27(67. 5%)	4(4/17=23.5%)
Gentamicin	23(57.5%)	4(4/ 21=19%)
Ciprofloxacin	20(50.0%)	3(3/23=13%)

Table 3 showed that the number of sensitive strains to Meropenem increased from 30(75.0%) to 32(80.0%) after addition of PAβN (20 mg/L) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 2(2/10=20%) , the number of sensitive strains to Carbenicillin increased from 25(62.5%) to 29(72.5%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 4(4/15=26.7%) , the number of sensitive strains to Ceftazidime increased from 23(57.5%) to 27(67. 5%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 4(4/17=23.5%), the number of sensitive strains to Gentamicin increased from 19(47.5%) to 23(57.5%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 4(4/ 21=19%), the number of sensitive strains to Ciprofloxacin increased from 17(42.5%) to 20(50.0%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 3(3/23=13%).

Table 4: Effect of addition of PAβN (50 mg/L) on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains

Antibiotic	*Total number of sensitive strains after addition of PAβN (50 mg/L) %*	*Number of strains showing resistance due to efflux pump (%)*
Meropenem	35(87.5%)	5 (5/10=50%)
Carbenicillin	31(77.5%)	6 (6/15=40%)
Ceftazidime	28(70%)	5 (5/10=50%)
Gentamicin	27(67.5%)	6 (6/21=28.57%)
Ciprofloxacin	21(52.5%)	4 (4/23=17.39%)

Table 4 showed that the number of sensitive strains to Meropenem increased from30(75.0%) to 35(87.5%) after addition of PaβN (50 mg/L) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 5 (5/10=50%), the number of sensitive strains to Carbenicillin increased from 25(62.5%) to 31(77.5%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 6 (6/15=40%), the number of sensitive strains to Ceftazidime increased from 23(57.5%) to 28(70%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 5 (5/10=50%), the number of sensitive strains to Gentamicin increased from 19(47.5%) to 27(67.5%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 6 (6/21=28.57%), the number of sensitive strains to Ciprofloxacin increased from 17(42.5%) to 21(52.5%) and thus the Number of strains showing resistance to this antibiotic due to efflux pump were 4 (4/23=17.39%).

Table 5: Comparison between the effect of addition of PAβN (20 mg/L) and PaβN (50 mg/L) on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa strains.*

Antibiotic	*Total number of sensitive strains after addition of PaβN20 mg/L (%)*	*Total number of sensitive strains after addition of PAβN50 mg/L (%)*	X^2	*P value*
Meropenem	32(80.0%)	35(87.5%)	0.832	0.363
Carbenicillin	29(72.5%)	31(77.5%)	0.273	0.606
Ceftazidime	27(67. 5%)	28(70%)	0.062	0.809
Gentamicin	23(57.5%)	27(67.5%)	0.854	0.356
Ciprofloxacin	20(50.0%)	21(52.5%)	0.048	0.823

Table 5 showed that there were no significant differences between the effect of addition of the two concentrations of PAβN on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains (P values were non-significant)

Table 6: Effect of addition of Curcumin at cocentrations from 5 µg/ml to 50 µg/ml on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains

Antibiotic	*sensitive strains with Cur (5µg/ml)*	*sensitive strains with Cur (10µg/ml)*	*sensitive strains with Cur (15µg/ml)*	*sensitive strains with Cur (20µg/ml)*	*sensitive strains with Cur (30µg/ml)*	*sensitive strains with Cur (50µg/ml)*
Meropenem	30 (75%)	30 (75%)	30 (75%)	31 (77.5%)	31 (77.5%)	35 (87.5%)
Carbenicillin	25 (62.5%)	25 (62.5%)	26 (65%)	28 (70.0%)	30 (75%)	32 (80.0%)
Ceftazidime	23 (57.5%)	23 (57.5%)	25 (62.5%)	25 (62.5%)	27 (67.5%)	29 (72.5%)
Gentamicin	19 (47.5%)	19 (47.5%)	19 (47.5%)	23 (57.5%)	23 (57.5%)	35 (62.5%)
Ciprofloxacin	17 (42.5%)	17 (42.5%)	17 (42.5%)	24 (60%)	29 (72.5%)	37 (92.5%)

Table 6 showed that the curcumin start to affect Meropenem sensitivity at concentration 20µg/ml, the curcumin start to affect Carbenicillin sensitivity at concentration 15µg/ml, the curcumin start to affect Ceftazidime sensitivity at concentration 15µg/ml, the curcumin start to affect Gentamicin sensitivity at concentration 20µg/ml, the curcumin start to affect Ciprofloxacin sensitivity at concentration 20µg/ml and with all the tested antibiotics the number of sensitive strains increased as the concentration of the curcumin increased above the concentration which started to affect the sensitivity.

Table 7: Comparison between the effect of addition of PAβN (20 mg/L) and Curcumin at cocentrations (50 µg/ml) on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains

Antibiotics	*Total number of sensitive strains after addition of PAβN20 mg/L (%)*	*Total number of sensitive strains after addition of curcumin 50 µg/ml (%)*	X^2	*p. value*
Meropenem	32(80.0%)	35(87.5%)	0.832	0.363
Carbenicillin	29(72.5%)	32(80.0%)	0.623	0.431
Ceftazidime	27(67. 5%)	29(72.5%)	0.243	0.626
Gentamicin	23(57.5%)	35(87.5%)	9.032	0.003*
Ciprofloxacin	20(50.0%)	37 (92.5%)	17.642	0.001*

Table 7 showed that there were no significant differences between the effect of addition of PAβN 20 mg/L and curcumin 50 µg/ml on antibiotic Susceptibility pattern of *Pseudomonas aeruginosa* strains of Carbenicillin, Meropenem, and Ceftazidime (P values were non-significant) but there were significant differences with Gentamicin and Ciprofloxacin (P values were significant).

DISCUSSION

P. aeruginosa is very important nosocomial organism usually associated with healthcare associated infections, especially in critically ill or immunocompromised patients. The exact prevalence of *MDRPA* is not yet well established, however, rates of resistance increased for imipenem, quinolones and for third generation cephalosporins by 15-23%, 15-32%, and 16-25% respectively [20].

Mesaros et al. [21], showed that PAβN may be used for phenotypic screening for presence of efflux pump activity in clinical isolates of *P.aeruginosa* along with genotypic methods. Another study observed 4 to 8-fold reduction of meropenem MICs among Acinetobacterbaumanii using PAβN as an efflux pump inhibitor [22].

Among the 40 strains of *MDRPA* included in our study, antibiotic resistance was observed to be contributed to efflux pump for Meropenem in 2(2/10=20%) strains, for Carbenicillin in 4(4/15=26.7%), for Ceftazidime in 4 (4/17=23.5%), for Gentamicin in 4(4/ 21=19%), and for Ciprofloxacin in 3(3/23=13%). Total resistance due to efflux pump was 17 (17/40=%). Less Nearly similar results were found by Nidhi et al.[23], who performed a study on 170 strains of *P. aeruginosa.* In their study, antibiotic resistance was observed to be due to efflux pump for Meropenem in 13 (13/50=26%) strains, for Carbenicillin in 12(12/61=19.7%), for Ceftazidime in 15 (15/71=21.1%), for Gentamicin in 13 (13/87=14.9%), and for Ciprofloxacin in 9(9/98=9.18%).

Also, our study revealed that curcumin was responsible for reduction of MIC of MDR *P. aeruginosa* isolates to the level of susceptibility for the five tested antibiotics. In a study made by Ballard and Coote[24], addition of curcumin restored the antibiotic susceptibility of *P. aeruginosa* strains that overexpresses the efflux-pump MexAB-OprM. Our results were in agreement with Negi et al.[15] who found similar results. This also agrees with Nidhi et al.[23] study that showed that curcumin was responsible for reduction of MIC of 30% (9/30) of MDR *P.aeruginosa*.

We noted that EPI activity of CUR increased by increasing the concentration. The best concentration of curcumin as EPI in our study was 50 µg/ml. On the other hand, addition of PAβN restores the antibiotic susceptibility of the tested *MDR P. aeruginosa* strains toward the five tested antibiotics but there was no significant difference in the effect of the two different concentration used. Bonert et al.[25] and Mesaros et al.[21] found similar results. So, we can conclude that it is better to use PAβN as EPI at concentration of (20 mg/L). When comparing PAβN (20 mg/L) and CUR (50 µg/ml), we found that there were no significant differences between the effect of addition of PAβN (20 mg/L) and CUR (50 µg/ml) on antibiotic Susceptibility of *Pseudomonas aeruginosa* strains to Carbenicillin, Meropenem, and Ceftazidime (P values were non-significant) but there were significant differences with Gentamicin and Ciprofloxacin (P values were significant). Similar results were observed by Nidhi et al.[23] who found that 3 resistant isolates to gentamicin and ciprofloxacin became sensitive after adding curcumin and not after adding PAβN, these observations indicate that curcumin is inhibiting the expression of efflux pump which are not inhibited by PAβN and thus this indicates the potential use of curcumin as an adjunct to antibiotics in treatment of drug resistant *P. aeruginosa* infection.

CONCLUSION

The mechanism by which curcumin and PAβN has potentiated the effect of antibiotics and thus change their susceptibility pattern in our study appears to be due to efflux pump inhibition which needs further genotypic studies.

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