Egyptian Journal of Medical Microbiology Volume 29 / No.1 / January 2020 27-35 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Efflux pump inhibition versus quorum sensing inhibition in hospital acquired multidrug resistant *Pseudomonas aeruginosa* isolates in Mansoura University Hospitals

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Key words:
MDR P. aeruginosa, quorum sensing inhibitors, efflux pump inhibitors

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ABSTRACT

Background: *It is necessary to search for alternative strategies such as quorum sensing inhibitors (QSIs) and efflux pump inhibitors (EPIs) for treatment of multidrug resistant (MDR) Pseudomonas aeruginosa infections.* ***Objectives:*** *1- Assessing and comparing the anti-biofilm activities of the EPI: carbonyl cyanide m-chlorophenylhydrazone (CCCP) and the QSI: baicalein against MDR strong biofilm forming P. aeruginosa isolates. 2- Assessing the presence of mexB and mexY genes encoding the MexAB-OprM and the MexXY-OprM efflux pumps, respectively in MDR P. aeruginosa isolates.* ***Methodology:*** *Wound samples were collected, cultured and antimicrobial susceptibility was done to select MDR P. aeruginosa and MDR ciprofloxacin resistant P. aeruginosa isolates (MDR^{CIP-R} P. aeruginosa). The effects of CCCP and baicalein on the minimum inhibitory concentration (MIC) of ciprofloxacin were assessed. Biofilm eradication assay was done to compare the effects of CCCP and baicalein on preexisting premature and mature biofilms of MDR^{CIP-R} P. aeruginosa isolates. The mexB and mexY genes were detected by PCR in isolated MDR P. aeruginosa.* ***Results:*** *Seventy one P. aeruginosa isolates were MDR and 53 of them were also resistant to ciprofloxacin (MDR^{CIP-R}). The sub-MIC concentrations of CCCP and baicalein resulted in ciprofloxacin MIC decrease factor (MDF) of ≥ 4 in 62.2%, and 52.8% MDR^{CIP-R} P. aeruginosa isolates, respectively. The biofilm eradication assay indicated that ciprofloxacin combined with either CCCP or baicalein was more effective in eradicating both premature and mature biofilms than either drug alone. However, baicalein alone was superior to CCCP alone in eradicating both premature and mature biofilms. The mexB and mexY genes were detected in 100% and 93% MDR P. aeruginosa isolates, respectively.* ***Conclusion:*** *The use of anti-biofilm agents as EPIs and QSIs with antibiotics could be an effective strategy for treating MDR P. aeruginosa infections.*

INTRODUCTION

Pseudomonas aeruginosa is an important opportunistic pathogen that can cause a wide range of human infections. *P. aeruginosa* is unique in its tolerance to antibiotics and continues to cause serious hospital acquired infections worldwide[1].

The intrinsic antimicrobial resistance of *P. aeruginosa* is mainly due to the expression of efflux pump systems. Clinically important efflux pumps of *P. aeruginosa* belong to resistance nodulation-cell division (RND) family which includes MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM. In *P. aeruginosa*, the best characterized examples of adaptive resistance are the formation of biofilm and the generation of persisters, which result in persistent infection and poor prognosis. The intrinsic resistance of *P. aeruginosa* to various antibiotics is even more prominent when this organism is found growing in a biofilm[2,3].

Persisters are phenotypic variants that aren't genetically resistant to antibiotics but are tolerant to high concentrations of antibiotics. While the majority of *P. aeruginosa* cells can be killed by antibiotics, persisters can remain viable due to the existence in a dormant state that shuts down the synthesis of the antibiotic targets[4]. Persisters don't proliferate in the presence of antibiotics, but they resume growth once the antibiotics are removed and are believed to be responsible for the persistence of chronic infections[5].

The incidence of infections caused by MDR *P. aeruginosa* strains has increased, so it is necessary to search for alternative treatment strategies including: QSIs, EPIs, lectin inhibitors, iron chelators, phage therapy, and antimicrobial peptides[6].

The EPIs which specifically target RND superfamily pumps could play an important role in increasing antibiotic efficacy. CCCP has been reported to be an efficient EPI in *P. aeruginosa*, as it can potentiate fluoroquinolone activity in resistant *P. aeruginosa* strains[7]. The CCCP is an ionophore that disturbs

electrochemistry gradient inhibiting these MDR efflux pumps. CCCP has become an important laboratory tool for investigating efflux systems but its cellular toxicity against mammalian cells limited its use to laboratories only[8].

The QSIs represent another promising alternative strategy. They attenuate bacterial pathogenesis by interfering with the intercellular communication system termed: quorum sensing (QS), which has been reported to be essential for creation of mature, differentiated biofilms[9]. Baicalein, a major flavonoid monomer extracted from the roots of *Scutellaria baicalensis*, has been described as a medicine in China and is used clinically for the treatment of upper respiratory tract infections and is found to have neuroprotective, antitumor, and antioxidant activities. Baicalein has remarkable synergy with tetracycline and β-lactams in the treatment of methicillin-resistant *S. aureus*[10]. Moreover, baicalein can significantly attenuate the QS-controlled virulence factors of *P. aeruginosa*[11].

To best of our knowledge, no studies compared the antibiofilm activity of QSI and EPI against MDR *P. aeruginosa* isolates.

This study aimed at: i- Selection of of MDR *P. aeruginosa* and MDR ciprofloxacin resistant *P. aeruginosa* isolates (MDR^{CIP-R} *P. aeruginosa*) from nosocomial wound infections. ii- Assessing and comparing the anti-biofilm activities of the EPI: CCCP and the QSI: baicalein against MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates. iii- Assessing the presence of *mexB* and *mexY* genes encoding MexAB-OprM and MexXY-OprM efflux pumps, respectively in MDR *P. aeruginosa* isolates.

METHODOLOGY

Study design:

This study is an observational cross-sectional study, which was conducted after the approval of the Institutional Review Board, Faculty of Medicine, Mansoura University. The study was carried out in the Microbiology Diagnostics and Infection Control Unit, Medical Microbiology and Immunology Department, Mansoura Faculty of Medicine, over a period of 24 months from December 2016 till November 2018.

Clinical samples and bacteriological examination:

Wound swabs were taken from nosocomial wound infections among patients admitted to Mansoura University Hospitals, and cultured on blood and MacConkey's agar media. *P. aeruginosa* isolates were identified by Gram-stained films, colony morphology and biochemical reactions[12].

Antimicrobial susceptibility testing:

The antimicrobial susceptibility of the *P. aeruginosa* isolates was carried out by the disc-diffusion method (Modified Kirby- Bauer method). The antibiotic discs were selected according to the Clinical Laboratory Standards Institute's (CLSI) guidelines[13]**.** The antibiotics used were piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, colistin, polymyxin B, gentamycin, tobramycin, amikacin, ciprofloxacin, and levofloxacin. *P. aeruginosa* strains were categorized as MDR when resistance is observed in ≥1 agent in ≥3 antibiotic categories[14].

Assessing the MIC of ciprofloxacin, CCCP and baicalein against MDR^{CIP-R} P. aeruginosa isolates

The MIC of ciprofloxacin, CCCP and baicalein against MDR^{CIP-R} *P. aeruginosa* was determined with reference to CLSI standards[15] using broth-microdilution method with an inoculum of 1×10^5 CFU/mL in Muller–Hinton broth. The final tested drug concentrations ranged from 16 to 1,024 µg/mL, 8 to 512 µg/mL and 0.5 to 32 µg/mL in serial two-fold dilutions for baicalein, CCCP and ciprofloxacin, respectively.

Determining the effect of sub-MICs of CCCP and baicalein on ciprofloxacin susceptibility of MDR^{CIP-R} P. aeruginosa isolates

The MIC of ciprofloxacin was determined in the presence of sub-MIC of CCCP and baicalein (½ the MIC was used for each isolate). Subsequently, the MDF of each isolate was calculated according to the following formula MDF= MIC of CIP without (CCCP or baicalein) / MIC of CIP with drug (CCCP or baicalein). An MDF value of 4 or more was considered as a significant effect[16].

Microtiter biofilm assay:

Briefly, MDR^{CIP-R} *P. aeruginosa* broth cultures (1×10^7 CFU/ml) were used to inoculate wells of sterile 96-well flat bottom polystyrene microtiter plates. Sterile culture medium was taken as a negative control. After incubation, wells were washed, air-dried, and biofilms which remain adherent were then stained with 0.1% crystal violet solution for 10 min, then the wells were rinsed again to remove excess stain and plates were air-dried. Then 200 µl of 95% ethanol was added to stained wells and dye was allowed to solubilize by incubating for 15 min. The absorption at 620 nm was determined using a microtiter plate reader. The test was performed in triplicate and the average of the three optical density (OD) was taken[17]. The ability to form biofilm was scored as:

- Strong biofilm producer when $OD_T > 4 \times OD_C$
- Moderate biofilm producer when $2 \times OD_C < OD_T \leq 4 \times OD_C$
- Weak biofilm producer when $OD_C < OD_T \leq 2 \times OD_C$
- Non-biofilm producer when $OD_T \leq OD_C$

Microtiter biofilm eradication assay:

Briefly, broth cultures of MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates (1×10^7 CFU/ml) were used to inoculate wells of sterile 96-well flat bottom polystyrene microtiter plates. Sterile culture medium was taken as a negative control. After 24 hr (for testing premature biofilm eradication) and 5 days (for testing mature biofilm eradication) incubation at 37°C, the

wells were washed, air-dried. The drug (CCCP or baicalein) alone (in ½ MIC concentration for each isolate), ciprofloxacin antibiotic alone (in concentration of 1 µg/ml) and a combination of both (ciprofloxacin antibiotic + either CCCP or baicalein) were added to the wells and incubated with the preexisting biofilm at 37°C for 24 hr without shaking. After incubation, the wells were washed to remove non-adherent cells. The biofilm was quantified by staining with crystal violet following the method described earlier[11].

Detection of the mexB and mexY genes in the isolated MDR strains:

Chromosomal DNA extraction was carried out using "GeneJET Genomic DNA Purification Kit" (Thermo Fisher Scientific, EU). For amplification of *mexB* and *mexY* genes sequences, 2 µl of DNA template was used per reaction (final volume 25 µl). The 23 µl reaction mixture contained: 12.5 µl 2x PCR Master mix solution (i-Taq™) (iNtRON biotechnology,EU), 1.5 µl of both forward and reverse primers (listed in table I)[18], and 7.5 µl RNase-free water. The gene-specific PCR amplification was accomplished using DNA thermal cycler (Peltier-Effect Cycling - MJ Reasercs, INC.). The program used for amplification involved 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; a final extension step of 7 min at 72°C[19]. From each reaction, an aliquot was evaluated on a 1.5% agarose gel stained with ethidium bromide and compared with the molecular weight marker "Simply Load™ 100 bp DNA ladder" (Lonza, USA).

Table 1: Forward and reverse primers of *mexB* and *mexY* genes, and amplicon size

Primer	Sequence (5/-3/)	Amplicon size (bp)
mexB-F	GTGTTCGGCTCGCAGTACTC	244
mexB-R	AACCGTCGGGATTGACCTTG	
mexY-F	CCGCTACAACGGCTATCCCT	246
mexY-R	AGCGGGATCGACCAGCTTTC	

Statistical Analysis

Statistical analysis was done using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). The correlation between two variables was evaluated using Chi-square "$\chi 2$" or Fischer's exact and Student *t* tests, with $P \leq 0.05$ considered significant.

RESULTS

During the period of study, 364 clinical samples were collected. The total number of all isolated pathogens was 312. One hundred *P. aeruginosa* strains were identified corresponding to 32.1% of all isolated nosocomial pathogens. *P. aeruginosa* was considered the most common isolated organism during the study period.

The most effective antibiotics against the *P. aeruginosa* isolates were colistin (100%) and polymyxin B (98%), whereas the least effective antibiotics were cefepime (20%) and ceftazidime (16%) (figure 1). Also, 71% of *P. aeruginosa* isolates were MDR (figure 2).

Among 71 MDR *P. aeruginosa* isolates, 53 isolates were also resistant to ciprofloxacin (MDR^{CIP-R} *P. aeruginosa*) (figure 3), and were selected for testing the antibacterial activity of CCCP (as a representative of EPI), and baicalein (as a representative of QSI). The ciprofloxacin MIC of the 53 MDR^{CIP-R} *P. aeruginosa* isolates showed that 23 isolates (43.4%) were ciprofloxacin resistant (MIC ranging from ≥ 4 to <32 µg/ml), 20 isolates (37.7%) were highly resistant (MIC ≥ 32 µg/ml), whereas 10 (18.9%) exhibited intermediate resistance (MIC ranging from >1 to <4 µg/ml) (table 2).

The MIC of CCCP for MDR^{CIP-R} *P. aeruginosa* showed that most common MIC encountered was 128 µg/ml (17 isolates corresponding to 32.08%) followed by 64 µg/ml (13 isolates corresponding to 24.53%), 256 µg/ml (11 isolates corresponding to 20.75%), 32 µg/ml (7 isolates corresponding to 13.21%), and 16 µg/ml (5 isolates corresponding to 9.43%) (table 3). The MIC of baicalein for MDR^{CIP-R} *P. aeruginosa* showed that majority of isolates was 1024 µg/ml (36 isolates corresponding to 67.93%) followed by >1024 µg/ml (11 isolates corresponding to 20.75%), and 512 µg/ml (6 isolates corresponding to 11.32%) (table 4). The sub MIC concentrations of CCCP were associated with MDF value of ≥4 more than the sub MIC concentrations of baicalein but that wasn't statistically significant (P = 0.167) (table 5).

Regarding biofilm-forming ability among MDR^{CIP-R} *P. aeruginosa* isolates, 12 (22.6%), 20 (37.7%), and 15 (28.3%) were weak, moderate, and strong biofilm-formers, respectively, whereas 6 (11.3%) were unable to form biofilm (table 6). The MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates were selected for the microtiter biofilm eradication assay. Regarding premature biofilm eradication assay, our results showed that of the 15 MDR^{CIP-R} strong biofilm forming isolates 10 isolates (66.7%) were eradicated by combination of both CCCP and ciprofloxacin (P= 0.002), 11 isolates (73.3%) were eradicated by combination of both baicalein and ciprofloxacin (P= 0.001), 6 isolates (40%) were eradicated by baicalein alone (P= 0.031), and none were eradicated by ciprofloxacin alone or CCCP alone. As regards premature biofilm eradication assay, our results showed that of the 15 MDR^{CIP-R} strong biofilm forming isolates 10 isolates (66.7%) were eradicated by either combination CCCP + ciprofloxacin or baicalein + ciprofloxacin (P= 0.002), 8 isolates (53.4%) were eradicated by baicalein alone (P= 0.008), and none were eradicated by ciprofloxacin alone or CCCP alone. Degrees of biofilm eradication are shown in tables 7& 8.

By PCR, *mexB* gene was detected in all MDR *P. aeruginosa* isolates and *mexY* gene was detected in 66 (93%) isolates only (figure 4& 5).

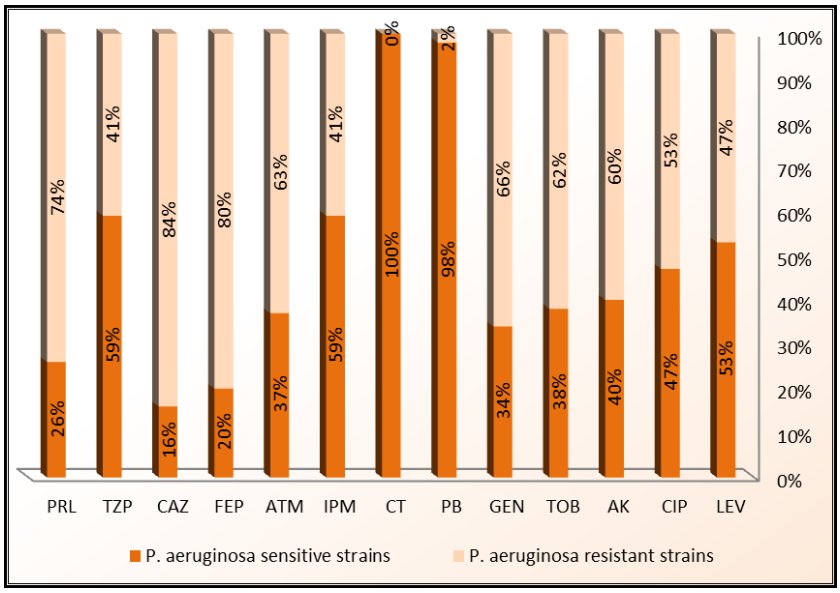

Fig. 1: Antibiotic sensitivity pattern of the isolated *P. aeruginosa* by disc-diffusion method. PRL: piperacillin; TZP: piperacillin/tazobactam; CAZ: ceftazidime; FEP: cefepime; ATM: aztreonam; IPM: imipenem; CT: colistin; PB: polymyxin B; GEN: gentamycin; TOB: tobramycin; AK: amikacin; CIP: ciprofloxacin; LEV: levofloxacin.

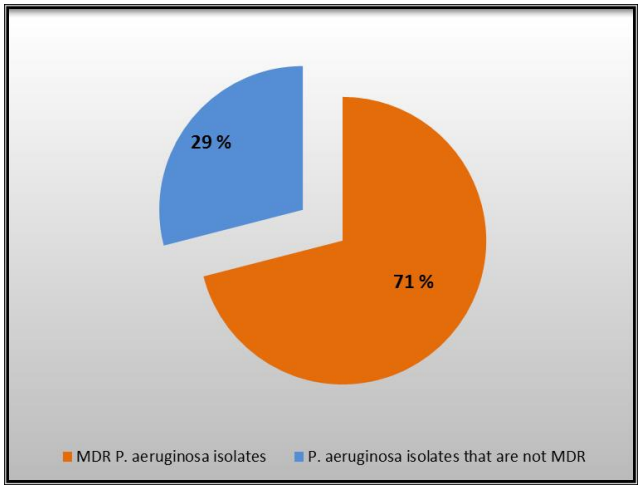

Fig. 2: Frequency of MDR *P. aeruginosa* among all isolated *P. aeruginosa*

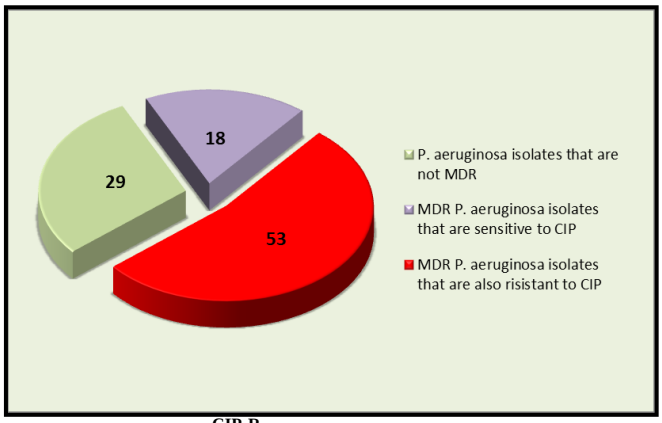

Fig. 3: Frequency of MDR$^{\text{CIP-R}}$ *P. aeruginosa* among *P. aeruginosa* isolates

Table 2: The MIC of ciprofloxacin for the isolated MDR^{CIP-R} *P. aeruginosa* isolates as determined by the broth microdilution method.

MIC results	MDR^{CIP-R} *P. aeruginosa* isolates (n = 53)	
	No	%
Intermediate resistant (MIC > 1 - < 4 µg/ml)	10	18.9
Resistant (MIC ≥ 4 - < 32 µg/ml)	23	43.4
Highly resistant (MIC ≥ 32 µg/ml)	20	37.7

Table 3. The MIC of CCCP for the isolated MDR^{CIP-R} *P. aeruginosa* as determined by the broth microdilution method.

MIC results	CCCP (n = 53)	
	No	%
16 µg/ml	5	9.43
32 µg/ml	7	13.21
64 µg/ml	13	24.53
128 µg/ml	17	32.08
256 µg/ml	11	20.75

Table 4: The MIC of baicalein for the isolated MDR^{CIP-R} *P. aeruginosa* determined by the broth microdilution method.

MIC results	Baicalein (n = 53)	
	No	%
512 µg/ml	6	11.32
1024 µg/ml	36	67.93
>1024 µg/ml	11	20.75

Table 5: The effect of sub MIC concentrations of CCCP and baicalein on MDF of ciprofloxacin in MDR^{CIP-R} *P. aeruginosa* isolates.

		MDF after Baicalein		Total
		MDF < 4	MDF ≥ 4	
MDF after CCCP	**MDF < 4**	7	13	20
	MDF ≥ 4	18	15	33
Total		25	28	53

Table 6: Biofilm-forming ability among MDR^{CIP-R} *P. aeruginosa* isolates.

Biofilm production	MDR^{CIP-R} *P. aeruginosa* isolates (n=53)	
	No	%
No biofilm formation	6	11.3
Weak biofilm formation	12	22.6
Moderate biofilm formation	20	37.7
Strong biofilm formation	15	28.3

Table 7: Degrees of premature biofilm eradication among MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates

	MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates (n=15)									
	CIP alone		Sub MIC of CCCP		CIP + Sub MIC of CCCP		Sub MIC of baicalein		CIP + Sub MIC of baicalein	
	No	%	No	%	No	%	No	%	No	%
Isolates that remained strong biofilm formers	15	100	15	100	5	33.4	9	60	4	26.6
Isolates that become moderate biofilm formers	0	0	0	0	4	26.6	5	33.4	6	40
Isolates that become weak biofilm formers	0	0	0	0	5	33.4	1	6.6	5	33.4
Isolates that become non biofilm formers	0	0	0	0	1	6.6	0	0	0	0

Table 8: Degrees of mature biofilm eradication among MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates

	MDR^{CIP-R} strong biofilm forming *P. aeruginosa* isolates (n=15)									
	CIP alone		Sub MIC of CCCP		CIP + Sub MIC of CCCP		Sub MIC of baicalein		CIP + Sub MIC of baicalein	
	No	%	No	%	No	%	No	%	No	%
Isolates that remained strong biofilm formers	15	100	15	100	5	33.4	7	46.6	5	33.4
Isolates that become moderate biofilm formers	0	0	0	0	6	40	5	33.4	7	46.6
Isolates that become weak biofilm formers	0	0	0	0	4	26.6	2	13.4	2	13.4
Isolates that become non biofilm formers	0	0	0	0	0	0	1	6.6	1	6.6

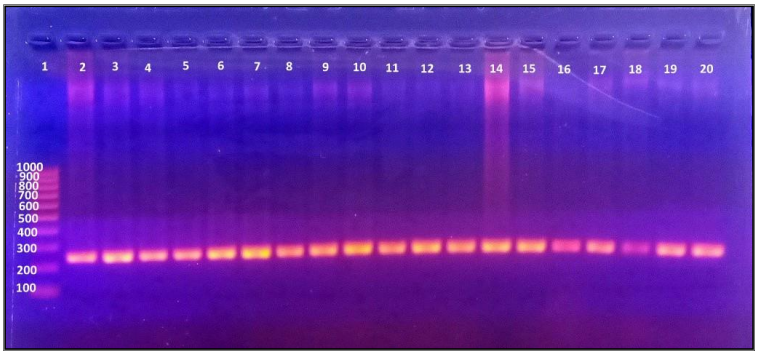

Fig. 4: *mexB* gene in the isolated MDR *P. aeruginosa* strains

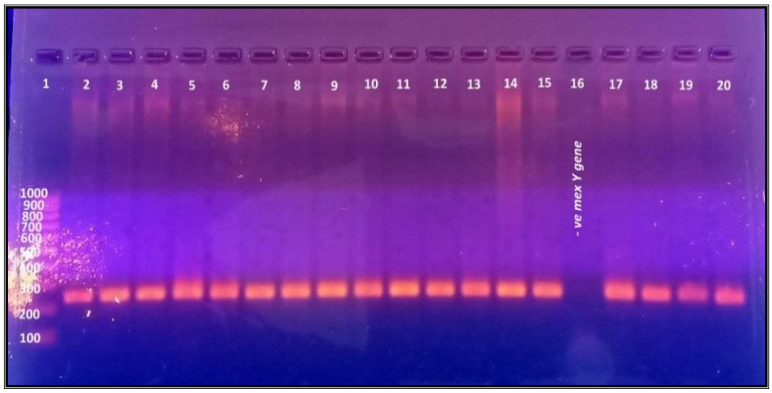

Fig 5: *mexY* gene in the isolated MDR *P. aeruginosa* strains

DISCUSSION

This study was conducted to compare the activity of efflux pump inhibition and quorum sensing inhibition against MDR^{CIP-R} *P. aeruginosa* isolates. *P. aeruginosa* strains represented 32.1% of the isolated pathogenic organisms and was considered the most common microorganism isolated during the study period. Similarly, *P. aeruginosa* was the commonest pathogen isolated from wound infections (38%) in another study in India[20].

In this study, the most effective drugs against *P. aeruginosa* infections were colistin (100%) and polymyxin B (98%), while the least effective drugs were cefepime (20%) and ceftazidime (16%). Our results are near to results of Khoravi & Mohammadian[21], who reported that the most effective antibiotic against *P. aeruginosa* was polymyxin B (96%), and the least effective one was cefepime (20%). Also, a study in Egypt[22] reported that the most effective antibiotic against *P. aeruginosa* was colistin (84%), whereas the least effective ones belonged to 3rd generation cephalosporins. In contrast to our findings, El Zowalaty & Gyetvai[23] reported in their study that all tested isolates of *P. aeruginosa* were susceptible to piperacillin, meropenem, and amikacin, and the least effective antibiotic was gentamicin (12.5% sensitivity). The variations in antibiotic resistance patterns among different studies can be explained by selection pressure of drugs used according to local hospital policy.

In our study, 71% of *P. aeruginosa* isolates were MDR. This high prevalence of drug resistant organisms in our study may be attributed to the prolonged antibiotic consumption, and non-adherence to hospital policy of antibiotics. Similar to our rates, Goli et al[24] reported that 68% of their isolated *P. aeruginosa* were MDR, and they also reported the emergence of colistin resistance (2% of their isolates were colistin resistant). Lower rates of MDR *P. aeruginosa* were reported in a study in Iran, where 38% of *P. aeruginosa* isolates were resistant to almost all available antibiotics except for colistin[25].

Ciprofloxacin is considered to be a clinically relevant antimicrobial agent for the treatment of *P. aeruginosa* infections. However, it isn't capable of eradication of biofilm-associated *P. aeruginosa* infections alone[26]. So, CCCP (as a representative of EPI) and baicalein (as a representative of QSI) were investigated to examine whether the antibacterial and anti-biofilm activities of ciprofloxacin were increased or not when used in combination with either drug.

In our research, the sub MIC concentrations of CCCP and baicalein resulted in MDF value of ≥4 in 33 (62.2%), and 28 (52.8%) MDR^{CIP-R} *P. aeruginosa* isolates, respectively. Both drugs are able to reverse resistance in *P. aeruginosa*. In addition, the sub MIC concentrations of CCCP were associated with MDF value of ≥4 more than the sub MIC concentrations of baicalein but that wasn't statistically significant. Choudhury et al[27] in India, found a reduction in MIC of all MDR *P. aeruginosa* isolates when CCCP was added to ciprofloxacin, with an MDF value of ≥ 4 in 58.4% of their isolates. Also, Adabi et al[28] found that by using CCCP, a reduction in MIC was observed in 61% of MDR *P. aeruginosa* isolates, with 46% of them showing MDF value of ≥ 4. Lower rates were reported by Talebi-Taher et al[29], who found a reduction in MIC of all MDR *P. aeruginosa* isolates when CCCP was added to ciprofloxacin, with MDF value of ≥ 4 in 34% of the isolates. However, this effect demonstrated in our study, may not imply to different classes of antibiotics. A study demonstrated that baicalein didn't alter the susceptibility *P. aeruginosa* strains toward tobramycin[30].

In our research, ciprofloxacin combined with either CCCP or baicalein was more effective in eradicating both premature and mature biofilms than either drug alone. However, baicalein alone was superior to CCCP alone in eradicating both premature and mature biofilms. Also, ciprofloxacin alone or CCCP alone were not able to eradicate premature and mature biofilms. The study done by Ikonomidis et al[31] reported that *P. aeruginosa* biofilm formation showed no statistically significant difference in the presence of sub-MIC concentrations of CCCP, relatively to the untreated culture and both produced biofilms. These findings were in agreement with ours. Luo et al[11], in their study on the wild-type PAO1 strain, reported that sub-MIC levels of baicalein detached both the premature and mature biofilms in a concentration-dependent manner. Also, Rajkumari et al[32] found that the presence of sub-MIC concentration of baicalein decorated gold nanoparticles significantly attenuated the biofilm formation in *P. aeruginosa* PAO1. Luo et al[33] tested the ability of a combination of baicalein plus an antibiotic (either levofloxacin, amikacin, or ceftazidime) to disrupt preformed biofilms and they found that these combinations decreased biofilm mass compared with baicalein or antibiotics alone after short-term (24 hrs) and long-term (96 hrs) treatment.

The MexAB-OprM and the MexXY-OprM efflux systems are the major pumps in wild-type cells, where they contribute to the intrinsic antimicrobial resistance, and their over-expression is responsible for the acquired resistance in *P. aeruginosa*. Since they mediate resistance to many clinical in-use antibiotics, including fluoroquinolones[2], we chose those two pumps to investigate the existence of encoding genes.

The present study showed the presence of the *mexB* gene encoding the MexAB-OprM efflux pump in all 71 (100%) MDR *P. aeruginosa* isolates and the *mexY* gene encoding the MexXY-OprM efflux pump in 66 (93%)

MDR *P. aeruginosa* isolates. In a study done by Naserpour Farivar et al[34], they found that all MDR *P. aeruginosa* isolates were positive for the presence of *mexB* and *mexY* genes. Also, Talebi-Taher et al[29] reported that all MDR *P. aeruginosa* isolates in their study were positive for the presence of *mexA* gene as a representative of MexAB-OprM efflux pump.

On the other hand, Goli et al[35] drew attention to other genes that may be involved in the high rate of MDR *P. aeruginosa* demonstrated in burn isolates. In their study, they reported that the presence gene cassettes carried with class 1 integrons had more significant role than the efflux pumps in conferring high level resistance to some antibiotics.

CONCLUSION

The intrinsic resistance of *P. aeruginosa* to various antibiotics is even more prominent when this organism is found growing in biofilms which also increase the expression of efflux pumps. Therefore, the use of EPIs and QSIs can significantly contribute to the treatment of patients infected with MDR strains. Both EPI and QSI are able to reverse antibiotic resistance in *P. aeruginosa.* Ciprofloxacin combined with either CCCP or baicalein was more effective in eradicating both premature and mature biofilms than either drug alone. However, baicalein alone was superior to CCCP alone in eradicating both premature and mature biofilms.

Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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