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ORIGINAL ARTICLE

Influence of Diclofenac and Pheniramine on the Virulence of Different Clinical Isolates of *Pseudomonas aeruginosa*

Heba E. AboElfarh, Mohammed M. EL-Sokkary, El-Sayed E. Habib
Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Egypt

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P. aeruginosa; Resistance; Antibiotics; Diclofenac; Virulence

Corresponding Author:
Heba Elsaeed Aboelfarh
Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Egypt
Tel.: 01010985512
heba.aboelfarh.elsaeed@gmail.com

ABSTRACT

Objectives: *This study aimed to examine the effect of commonly used non-antibiotic drugs e.g. diclofenac and pheniramine,* ***Background:*** *Treatment of Pseudomonas aeruginosa infections, on antibiotic resistance and the virulence of this pathogen. The antibiotics: gentamicin, cefepime, ciprofloxacin and meropenem were investigated in this study.* ***Methodology:*** *In this work we selected the following final concentrations: 1,4µg/ml for diclofenac and 0,173µg/ml for pheniramine to be used in combination with antibiotics or alone for investigation of their effects on antibiotic resistance and virulence of 20 isolates of P. aeruginosa.* ***Results:*** *The drugs either increased or decreased antibiotic resistance in only 3 isolates of the 20 isolates which indicated that the investigated drugs did not affect the antibiotic resistance when used in combinations. Interestingly, our study demonstrated that both diclofenac and pheniramine increased the haemolytic activity of the investigated isolates. On the other side, no overall final increasing or decreasing effect could be observed regarding the effect of diclofenac or pheniramine on the proteolytic activity of the investigated isolates. The results were confirmed by Real time PCR diclofenac showed a significant down- regulation of virulance genes namely; algD, plch and toxA apperently in case of combination with ciprofloxacin and to a lower extent when combined with gentamicin.* ***Conclusion:*** *Resistance of P. aeruginosa to Gentamicin and Ciprofloxacin may be successfully affected by combining these antibiotics with Diclofenac.*

INTRODUCTION

Pseudomonas aeruginosa is Gram-negative opportunistic bacteria, particularly in immunocompromised patients and capable of causing many life-threatening infections [1].This infection is usually associated with high mortality and morbidity owing to increasingly frequent infections caused by multidrug resistant (MDR) strains with limited therapeutic options [2]. The induced intrinsic resistance is conferred by production of antibiotic inactivating enzymes, efflux pumps expression and low outer membrane permeability. Acquired resistance may be related to mutational changes or resistance genes acquisition through horizontal transfer by mobile genetic elements, such as plasmids, transposons or integrons [3]. For these reasons, since 2017, the World Health Organization listed *P. aeruginosa* as one of the critical priority pathogens to encourage development, research and improve into new anti-bacterials [4].

Infectious diseases treatment, in which extensive and strong inflammatory process is reported, nonsteroidal anti-inflammatory drugs (NSAID) represent a group of widely used antipyretic and analgesic agents. Diclofenac is a frequently used NSAID which acts by inactivating cyclooxygenase and inhibiting prostaglandins synthesis [5]. Several studies suggested that diclofenac could inhibit the proliferation of many microorganisms, including *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Candida albicans*, and *Escherichia coli* [5]. Especially in combination with β-lactams, diclofenac antibacterial effects have been noted and used often during the preoperative period[5]. Similarly, pheniramine maleate is another NSAIDs that was reported to has good antimicrobial activity with growth inhibition of *Staphylococcus aureus* and *P. aeruginosa* [6,7].

Recently, synergistic drug screening and drug repurposing have become promising ways to combat infections caused by MDR pathogens [8]. For the above observations, this work aimed to evaluate the impact of combination of, diclofenac and pheniramine with different antimicrobial drugs (gentamicin, ciprofloxacin, cefepime and meropenem) and whether this combination *in vitro* improves or abrogates the activity of these drugs *P. aeruginosa* strains.

METHODOLOGY

Collection, isolation, and identification of *P. aeruginosa* clinical isolates:

Sixty-five clinical isolates of *P. aeruginosa* (P1-65) were obtained from Mansoura International Hospital, University Hospital, Burns and Cosmetics Centre, Chest

Hospital, Urology and Nephrology Centre and Paediatric University Hospital, Mansoura, Egypt.

All isolates were identified as *P. aeruginosa*, based mainly on their growth on cetrimide agar plate (0.03% cetrimide), oxidase test, motility test, pyocyanin pigment production, in addition to the ability of the isolates to grow at 42°C [9]. A reference strain PAO1, obtained from the MIRCENS center (Faculty of Agriculture, Ain Shams University, Egypt), was included as a positive control in all biochemical tests. After the identification and purification of isolates, they were cultured on nutrient broth and stored in 20% v/v glycerol stock at −80 °C until further use.

Experimental protocol designed in this study complies with the ethical guidelines adopted by the Ethics Committee in the Faculty of Pharmacy, Mansoura University, which approved this study.

Antibiotic susceptibility testing of *P. aeruginosa* clinical isolates:

According to the Clinical Laboratory Standards Institute guidelines (CLSI 2016), isolates sensitivity to antibiotics was determined by disc diffusion method. For each isolate, fresh MHB suspension of overnight isolated colonies culture was adjusted to match turbidity of 0.5 McFarland. Sterile cotton swab was dipped in the suspension and spread on Muller-Hintor agar plates (MHA; Oxoid, Hampshire, UK). Then the plates were allowed to dry and the antibiotic discs (Oxoid, Hampshire, UK); gentamicin (10µg), cefepime (30 µg), ciprofloxacin (5µg), and meropenem (10µg) were placed followed by incubation for 24 h at 37°C. The clearance zones diameters around antibiotic discs were measured and interpreted as sensitive (S) and resistant (R).

Determination of MIC of different antibiotics against *P. aeruginosa* clinical isolates in presence or absence of diclofenac and pheniramine:

The MICs of the antibiotics with and without drugs against 20 randomly selected *P. aeruginosa* and standard PAO1 strain were determined by the broth microdilution method (CLSI, 2016). Overnight culture of isolated colonies in Muller-Hinton broth (MHB, Oxoid, Hampshire, UK) was prepared and the turbidity was adjusted to 0.5 McFarland. The prepared suspension was diluted with MHB to have an approximate cell density of 5×10^6 CFU/mL. The plates were incubated at 37 °C for 24 hrs and the MIC was determined as the lowest concentration of antibiotic that inhibits any visible growth.

The effect of diclofenac (voltaren ampoule, 75 mg/3ml) and pheniramine (avil ampoule, 45.5 mg/2ml) on antibiotic sensitivity of *P. aeruginosa* was also investigated. Either diclofenac or pheniramine were included at concentrations of (1,4µg/ml) and (0,173µg/ml) respectively in the examined experiment for broth microdilution. Each experiment was performed as triplicate wells in each plate. In addition to positive control wells (culture of each isolate in MHB) and negative control wells (MHB medium only), another control of either diclofenac or pheniramine together with each bacterial isolate was also included to make sure that these drugs did not affect bacterial growth.

Phenotypic impact of diclofenac and pheniramine on some virulence factors of *P. aeruginosa*:

To evaluate the effect of diclofenac and pheniramine on the protease activity of 25 isolates of *P. aeruginosa*, the skim milk agar method was performed as described previously[12]. The turbidity of overnight cultures for each isolate in MHB was adjusted to match that of 0.5 McFarland. The turbidity-adjusted cultures were centrifuged and the supernatant of each bacterial isolate (20 µL) was incubated with skim milk (1.25%, 100 µL) and 1.5 µL of either diclofenac (Final concentration 1,4µg) or pheniramine (Final concentration 0,173µg/ml) in eppendorf tube at 37°C for 15 min. The degree of clearance of skimmed milk indicated the protease activity and it was calculated by measuring the turbidity at OD600 nm. The value was taken by the average of two readings for each isolate and compared to negative control tube without drug.

Hemolysin test was used to investigate the impact of diclofenac and pheniramine on the hemolytic activity of 20 isolates of *P. aeruginosa*. The turbidity of overnight cultures for each isolate in MHB was adjusted to match that of 0.5 McFarland. The turbidity-adjusted cultures were centrifuged and the supernatant of each bacterial isolate (75 µL) was incubated with 75 µL fresh sheep red blood cells (RBCs) in the wells of microtiter plate for 2 h at 37°C. Before incubation of the plate, 1.5 µL of either diclofenac (Final concentration 1,4µg/ml) or pheniramine (Final concentration 0,173µg/ml) was added to the reaction mixture for each isolate. Positive control wells were composed of 75 µL SDS (0.2% in MHB) + 75 µL fresh sheep red blood cells, while negative control ones comprised 75 µL MHB + 75 µL fresh sheep red blood cells. Each experiment was performed as three readings in each plate. To ensure that the drugs did not exhibit any hemolytic activity, 75 µL of MHB containing each drug (at the same final concentrations) were added to 75 µL fresh sheep red blood cells then inoculated at 37°C for 2 h. In all experiments, hemolytic activity was determined by measuring hemoglobin release at OD630 nm after incubation of the plates [13].

Conventional Polymerase Chain Reaction protocol and cycling conditions:

PCR amplification of *pseudomonas aeruginosa* (PS) genes, namely (*algD, plcH, toxA, aprA* and *lasB*) were performed using the following reaction mixture: 12.5 µl Dream Taq Green PCR Master Mix (2X), 1 µl of forward primer (10 µM), 1 µl of reverse primer (10 µM), 1 µl of bacterial DNA template and 9.5 µl of nuclease free water were added for a total of 25 µl per

reaction. Negative control reaction (without bacterial DNA templates) was also performed. The cycling conditions included; initial denaturing at 95 °C for 5 min, then (denaturation at 95 °C for 30 sec, annealing (Table 1) for 30 sec and extension at 72 °C for 1 min) for 40 cycles and final extension cycle at 72 °C for 5 min.

Table 1: Amplification primers used in this study for amplifying different genes among *Pseudomonas aeruginosa* isolates

Primer name	Oligonucleotide sequence (5`-3`)	Amplicon size (bp)	Annealing temp.
16srRNA-F 16srRNA-R	CAAAACTACTGAGCTAGAGTACG TAAGATCTCAAGGATCCCAACGGCT	215	67
algD-F algD -R	CGCCGAGATGATCAAGTACA AGGTTGAGCTTGTGGTCCTG	126	55
plcH -F plcH -R	GAAGCCATGGGCTACTTCAA AGAGTGACGAGGAGCGGTAG	307	55
toxA -F toxA -R	GGAGCGCAACTATCCCACT TGGTAGCCGACGAACACATA	150	55
AprA-F aprA -R	GTCGACCAGGCGGCGGAGCAGAT GCCGAGGCCGCCGTAGAGGATGTC	993	63
lasB -F lasB -R	GTTGCGATCATGGGTGTT GCCGTTGTGGAATTGCTC	165	49

F: forward primer R: reverse primer

Quantitative Polymerase Chain Reaction protocol:

The extracted cDNA was subjected to Realtime PCR reaction in a reaction mix containing 1.5 L of each forward and reverse primer (10 µm each),12.5 µL (2x) SYBR Green PCR master mix (Willowfort Co., Birmingham, UK.) and nuclease-free water were added to complete the final volume of 25 µL. All real-time reactions were carried out using MyGo real-time PCR machine under the following conditions: 95 °C for 5 min, followed by 45 cycles of 95 °C for 20 s, annealing temperature (Table 1) for 20 s, and finally 72°C for 40s. Ct values and melting curve detection were obtained using MyGo real-time PCR machine software. The relative abundance of each microbial species was calculated as a relative unit normalized to the total bacteria of the corresponding sample, using the $2^{-\Delta Ct}$ method (where ΔCt = the average Ct value of each target - the average Ct value of total bacteria) [14].

Different strains in addition to PAO1 standard strain were selected in this study. The strains were tested under 6 different conditions as presented in table 2.

Table 2: Different drugs and antimicrobial combinations tested in this study.

Number	Drug	Antibiotic	Concentration
1	-	ciprofloxacin	512 µg/ml
2	pheneramine	ciprofloxacin	0,173µg/ml,512 µg/ml
3	diclofenac	ciprofloxacin	1,4 µg/ml,512 µg/ml
4	-	Gentamicin	2048 µg/ml
5	pheneramine	Gentamicin	0.173µg/ml,2048µg/ml
6	diclofenac	Gentamicin	1,4 µg/ml,2048 µg/ml

Statistical analysis:

Effect of each drug on virulence factors was analysed by GraphPad Prism software version 8 using *ANOVA* test followed by student *t* test at $P<0.05$ for significance.

RESULTS

Antibiotic susceptibility of *P. aeruginosa* isolates:

Disc diffusion method was performed to detect the sensitivity of *P. aeruginosa* isolates to different 4 antibiotics (gentamicin, cefepime, meropenem, and ciprofloxacin) High level of resistance was found with

these antibiotics. Starting with 65 isolates of *P. aeruginosa*, the resistance rates of gentamicin, cefepime, meropenem, and ciprofloxacin were 73.8% (48 isolates), 61.53% (40 isolates), 46.15% (30 isolates) and 43.07% (28 isolates), respectively. Antimicrobial resistance in relation to the source of isolation was investigated in this study (figure 1). As a result, in case of meropenem, most resistant isolates were detected in oral samples (20%).

In case of cefepime, non of the isolated strains were found sensitive in both ETA and tube swab samples. However, higher percentages were detected in oral and urine samples identified by 22%, 12% respectively. Interestingly, the highest resistance percentages were found in case of gentamicin with 25%, 12% and 11% resistance of isolates from oral swab, throat swab and urine respectively , on the other hand the highest sensitivity of our isolates were detected in case of ciprofloxacin, where the isolated strains were 61% or lower.

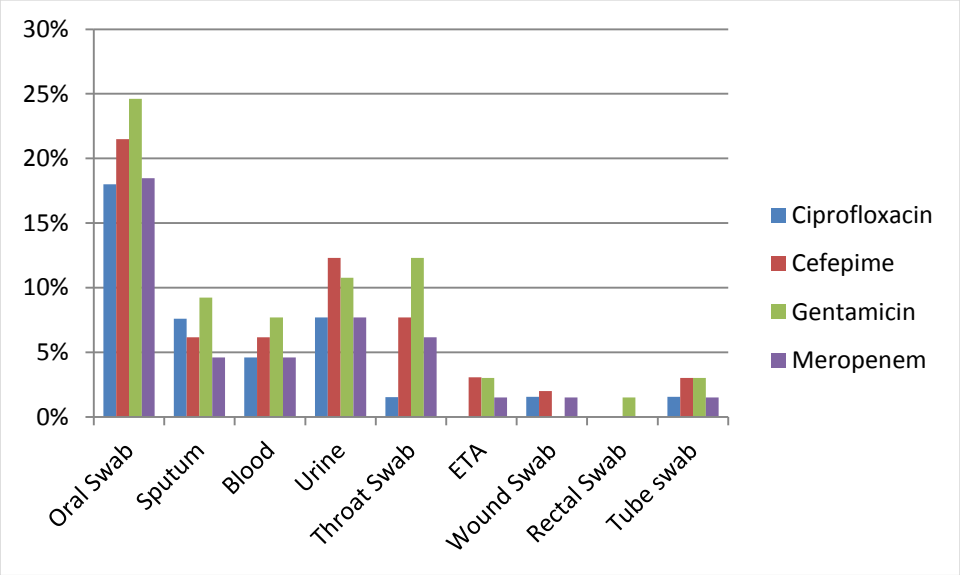


Fig. 1: Antimicrobial resistance in relation to the source of isolation

Effect of diclofenac and pheniramine on antibiotic susceptibility testing of *P. aeruginosa* clinical isolates:

Using different antibiotic-drug combinations against resistant *P. aeruginosa* isolates, diclofenac and pheniramine showed different changes in antimicrobial activity. Neither diclofenac nor pheniramine was found to affect the growth of any of the investigated bacterial isolates. Regarding combination of diclofenac with either of the antibiotics of this study **(Table 3)**, the resistance to ciprofloxacin in presence of diclofenac was decreased as indicated by the decrease of MIC in three isolates (P15, P36, P37), While the resistance to cefepime in presence of diclofenac was decreased as indicated by the decrease of MIC in three isolates (P14, P36, P37).Also the resistance to gentamicin in presence of diclofenac was decreased as indicated by the decrease of MIC in two isolates (P15, P40).

While the resistance to ciprofloxacin in presence of diclofenac was increased as indicated by the increase of MIC in presence of two isolates (P3, P45), and the resistance to cefepime in presence of diclofenac was increased as indicated by the increase of MIC in presence of two isolates (P2, P40). In addition to the resistance to gentamicin in presence of diclofenac was increased as indicated by the increase of MIC in presence of one isolate (P45).As well as the resistance to meropenem in presence of diclofenac was increased as indicated by the increase of MIC in presence of one isolate (P15).

Concerning combination of pheniramine with either of the antibiotics in this work **(Table 3)**, the decrease of resistance to ciprofloxacin in presence of pheniramine by the decrease of MIC in one isolate (P15). Also the resistance to cefepime in presence of pheniramine was decreased as indicated by the decrease of MIC in two isolates (P14, P44), and the resistance to gentamicin in

presence of pheniramine was decreased as indicated by the decrease of MIC in two isolates (P15, P44). However the resistance to ciprofloxacin in presence of pheniramine was increased as indicated by the increase of MIC in presence of one isolates (P10). Also the resistance to cefepime in presence of pheniramine was increased as indicated by the increase of MIC in presence of one isolate (P2). The resistance to meropenem in presence of pheniramine was increased as indicated by the increase of MIC in presence of two isolates (P15, P40).

Table3: MIC values of different antibiotics for different isolates of *P. aeruginosa* in presence of absence of diclofenac or pheniramine.

MIC values (µg/ml)												
	Ciprofloxacin			**Cefepime**			**Gentamicin**			**Meropenem**		
Isolate	**N**	**+D**	**+P**	**N**	**+D**	**+P**	**N**	**+D**	**+P**	**N**	**+D**	**+P**
P1	32	32	32	128	128	128	512	512	512	32	32	32
P2	32	32	32	256	512	512	2048	2048	2048	2048	2048	2048
P3	0.5	4	0.5	4	4	4	8	8	8	1	1	1
P4	0.5	0.5	0.5	4	4	4	8	8	8	1	1	1
P5	64	64	64	64	64	64	16	16	16	512	512	512
P6	0.5	0.5	0.5	4	4	4	2	2	2	1	1	1
P10	32	32	64	32	32	32	64	64	64	16	16	16
P14	64	64	64	1024	512	512	128	128	128	256	256	256
P15	64	32	32	64	64	64	512	256	256	128	256	256
P27	0.5	0.5	0.5	8	8	8	2	2	2	1	1	1
P33	2	2	2	16	16	16	8	8	8	4	4	4
P36	64	32	64	1024	512	1024	8	8	8	2048	2048	2048
P37	64	32	64	512	128	512	64	64	64	256	256	256
P39	32	32	32	128	128	128	2048	2048	2048	128	128	128
P40	32	32	32	32	64	32	8	4	8	256	256	512
P41	32	32	32	4	4	4	64	64	64	64	64	64
P42	0.5	0.5	0.5	4	4	4	2	2	2	1	1	1
P43	0.5	0.5	0.5	16	16	16	256	256	256	16	16	16
P44	0.5	0.5	0.5	512	512	32	16	16	8	16	16	8
P45	0.5	1	0.5	256	256	256	8	16	8	16	16	16
PAO1	0.5	0.5	0.5	4	4	4	2	2	2	1	1	1

N: In absence of diclofenac or pheniramine, **+D:** In presence of diclofenac, **+P:** In presence of pheniramine.

Phenotypic effect of diclofenac and pheniramine on some virulence factors of *P. aeruginosa*:

Regarding the effect of diclofenac on the proteolytic activity of *P. aeruginosa* isolates **(Table 4)**, diclofenac was found to decrease the proteolytic activity in 13 isolates of the investigated strains, while it was increased in 12 isolates. On the other side, diclofenac was found to decrease the hemolytic activity in 7 isolates, while it was increased in 18 isolates. Concerning the impact of pheniramine on the proteolytic activity of *P. aeruginosa* isolates **(Table 4)**, pheniramine was found to decrease the proteolytic activity in 10 isolates of investigated isolates, while it was increased in 14 isolates. However, pheniramine was found to decrease the hemolytic activity in 7 isolates while it was increased in 18 isolates.

Table 4: Impact of diclofenac or pheniramine on proteolytic and hemolytic activity of *P. aeruginosa* isolates

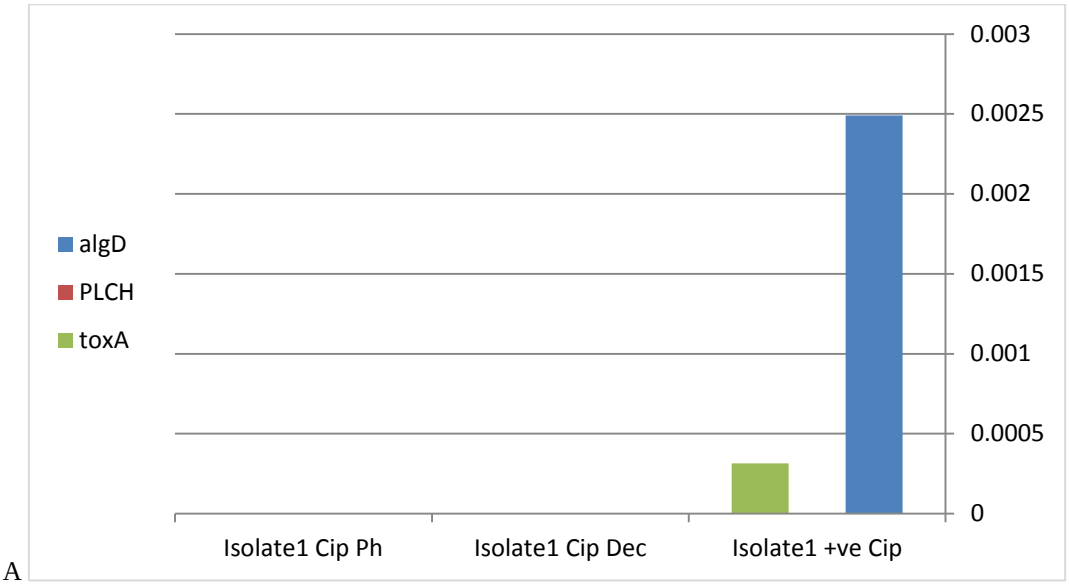
Isolate	Proteolytic activity			Hemolytic activity		
	Activity (OD600 nm in absence of drugs	% Increase (+) or decrease (-) in activity in presence of Diclofenac	% Increase (+) or decrease (-) in activity in presence of Pheniramine	Activity (OD630 nm in absence of drugs	% Increase (-) or decrease (+)in activity in presence of Diclofenac	% Increase (-) or decrease (+) in activity in presence of Pheniramine
P2	+0.24	-151.5	+33.5	+0.522	+9.1	-34.1
P4	+0.195	-43.6	-113.3	+0.558	-2.5	-0.4
P9	+0.395	+7.8	+4.2	+0.141	-61.7	+3.5
P18	+0.3995	-1.6	-15.8	+0.615	-1.0	-4.1
P19	+0.41115	+16.9	+68.2	+0.6425	+0.7	-12.7
P22	+0.335	+4.2	0	+0.5965	-5.4	-10.2
P23	+0.154	+47.4	+55.2	+0.6575	+5.9	-1.3
P26	+0.4365	+23.6	+0.3	+0.484	-10.3	+23.1
P28	+0.189	-52.9	-19.6	+0.7	-18.1	-11.9
P30	+0.154	-207.1	-2.3	+0.4175	-33.2	-60.1
P31	+0.367	+15.5	+18.5	+0.5175	-42.9	-27.5
P32	+0.284	-4.1	-10.4	+0.23	-130.9	-163.5
P33	+0.2065	-67.1	-8.2	+0.319	-120.1	-35.0
P36	+0.5	+16.4	-4.3	+0.7435	-1.3	+11.6
P37	+0.0785	-121.7	-94.3	+0.6395	-2.7	+6.0
P38	+0.12	-21.3	+32.9	+0.5965	+6.5	-6.8
P39	+0.2525	+12.7	+3.6	+0.572	+74.0	+44.8
P40	+0.341	-10.4	-30.6	+0.4535	-16.6	-27.0
P42	+0.479	-15.0	+11.0	+0.2925	-117.9	-117.4
P43	+0.3835	-0.8	+10.3	+0.238	-163.9	-151.1
P44	+0.3335	-18.9	-3.9	+0.215	+36.0	+21.4
P45	+0.223	+56.3	+56.7	+0.775	8.3-	-7.0
P47	+0.1995	+35.1	+51.6	+0.738	-13.3	-5.9
P55	+0.4185	+14.8	+31.4	+0.7095	+8.5	+61.7
P57	+0.413	+30.4	+5.1	+0.622	-17.0	-10.5
Negative control	**+0.354**			**+0.6033**		

Molecular identification of some virulence factors:

Detection of GDP-mannose 6-dehydrogenase (algD), **Hemolytic phospholipase C (***plch) and* exotoxin *(toxA). (algD, plch and toxA)* by PCR is performed for molecular identification of *P. aeruginosa*. The genes were detected at different percentages in the 20 isolates in 88%, 94%**,** 93,7%, in case of *algD, plch and toxA* respectively.

Down regulation of the expression of virulence genes:

A significant decrease in the expression of *algD, plch* and *toxA* was observed in case of declofenac when combined with ciprofloxacin (Figure 2) and to a lower extent when combiend with gentamicin (Figure 3). In case of pheneramine a significant increase in the expression of virulance factors could be observed in case of gentamicin, however a significant decrease could be observed in case of pheniramine when combined ciprofloxacin in the two strains investigated.



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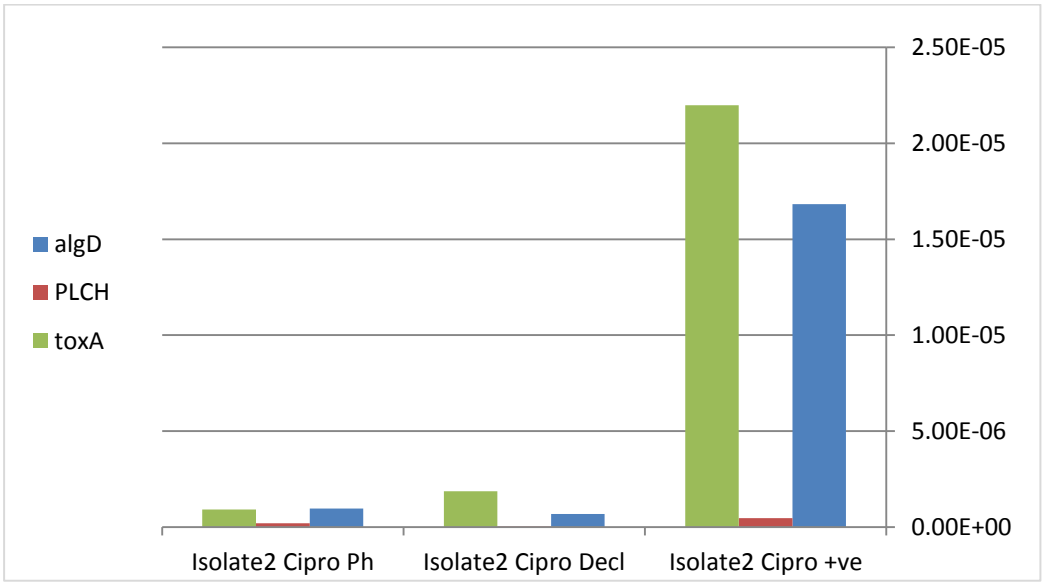

B

Fig. 2: Effect of different drugs in addition to ciprofloxacine on *P. aeruginosa* virulence factors genes *toxA, plch and algD*, assessed by real time PCR for 2 different isolates 1 and 2, figures a and b respectively.

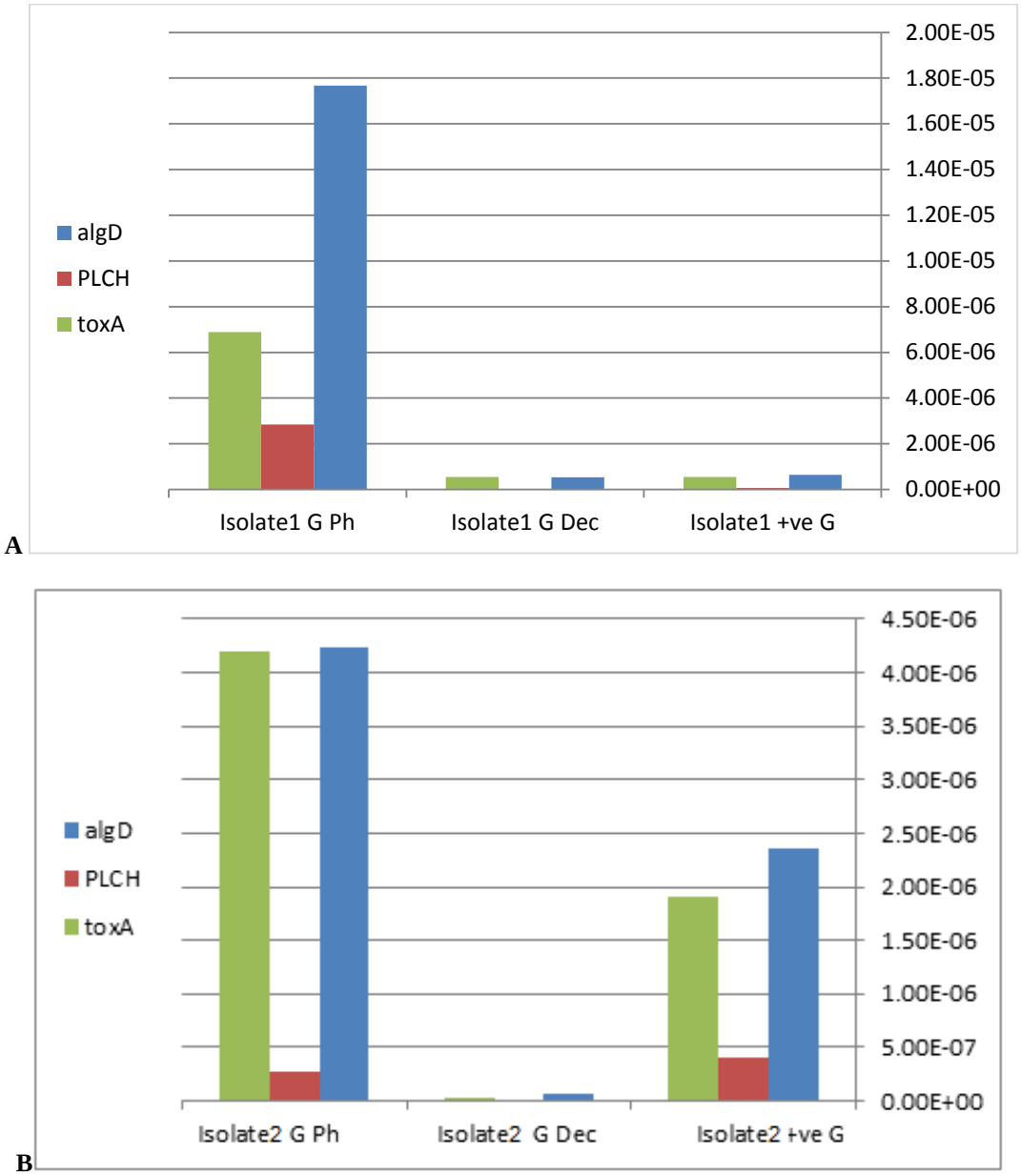

Fig. 3: Effect of different drugs in addition to gentamicin on *P. aeruginosa* virulence factors genes *toxA*, *plch* and *algD*, assessed by real time PCR for 2 different isolates 1 and 2, figures c and d respectively The y-axis in all figures represent relative abundance which is the percent composition of an organism of a particular kind relative to the total number of organisms in the area.

DISCUSSION

We examined the effect of the commonly used non-antibiotic drugs, for treatment of manifestations of *P. aeruginosa* infections, on antibiotic resistance and virulence of this pathogen. The antibiotics: gentamicin, cefepime, ciprofloxacin and meropenem were investigated in this study, while the proteolysis and haemolysis were selected as virulence factors for investigation.

We selected the following final concentrations: 1,4 µg/ml for diclofenac and 0,173µg/ml for pheniramine. As this concentration is Peak plasma of the active metabolite hydroxy[10,11].

Unexpectedly, the impact of drugs on antibiotic resistance did not show sound results as only 3, 2, 1, or even no isolates of the 20 isolates were affected in each combination which indicates that the investigated drugs did not affect the antibiotic resistance when used in combinations. Previous studies demonstrated the effect

of drugs (diclofenac and pheniramine) on antibiotic resistance of *P. aeruginosa.* [15,16].

Interestingly, diclofenac decreased the proteolytic activity in 13 isolates of investigated strains by 100%, 50-100%, 5-10% and 5% in 3, 3, 4, and 3 isolates respectively. On the other hand, diclofenac increased the proteolytic activity in 12 isolates of investigated strains by 50-100%, 10-50%, 5-10% and 5% in 2, 8, 1 and 1 isolates respectively. Regarding pheniramine, it was found to decrease the proteolytic activity in 10 isolates of investigated strains by 00%, 50-100%, 10-50 %, 5-10% and 5% in 1, 2, 3, 1 and 3 isolates respectively. On the other hand, pheniramine increased the proteolytic activity in 14 isolates of investigated strains by 50-100%, 10-50 %, 5-10% and 5% in 5, 5, 1, and 3 isolates respectively. These results indicate that no overall final increasing or decreasing effect could be observed regarding the effect of either diclofenac or pheniramine on the proteolytic activity of the investigated isolates.

Concerning the effect of drugs on haemolytic activity of the investigated strains, diclofenac decreased the haemolytic activity in 7 isolates of investigated strains by 50-100%, 10-50 %, 5-10% and lower than 5% in 1, 1, 4, and 1 isolates respectively. On the other hand, diclofenac increased the haemolytic activity in 18 isolates of investigated strains by 100%, 50-100%, 10-50 %, 5-10% and 5% in 4, 3, 5, 2 and 4 isolates respectively. Regarding pheniramine, it was found to decrease the haemolytic activity in 7 isolates of investigated strains by 50-100%, 10-50 %, and 5% in 2, 3, and 2 isolates respectively. On the other hand, pheniramine increased the haemolytic activity in 18 isolates of investigated strains by 100%, 50-100%, 10-50%, 5-10% and 5% in 3, 2, 7, 3 and 3 isolates respectively. These results demonstrate that either diclofenac or pheniramine increased the haemolytic activity of the investigated isolates. Similarly, variable results were observed using molecular methods where a significant decrease in the expression of *algD, plch* and *toxA* was observed in case of diclofenac when combined with ciprofloxacin and with a lower extent when combined with gentamicin. However, in case of pheneramine a significant increase in the expression of virulance factors could be observed in case of gentamicin compared to ciprofloxacin were a significant decrease could be observed.

Other studies demonstrated the effect of drugs on virulence factors of *P. aeruginosa*.[17,18].

CONCLUSION

In conclusion, the impact of drugs on antibiotic resistance did not show sound results. On the other hand, promising results were obtainted in case of diclofenac when combined with antibiotics with a negative influence on the virulance activity. We recommend future studies to be done to study the effect of other non-antibiotic drugs on antibiotic resistance and virulence of *P. aeruginosa*.

Declarations

This study has been approved by faculty of pharmacy Mansoura University Ethics Committee according to Helsinki declaration.

The authors declear no conflict of interest by publication of this manuscript.

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NO competing interest upon publication

All data are available upon request from the corresponding author

Authors' contributions:

MMAE: Conceptualization and study design MMAE and EEH: Supervision, HEA and MMAE: Methodology and data analysis HEA MMAE and EEH: validation, writing, reviewing and editing of manuscript . All authors approved the final version of the article.

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