Research article

Bacterial growth and antibiotic sensitivity of *Proteus mirabilis* treated with anti-inflammatory and painkiller drugs

Mohsin Rasheed Mohsin[1], Bahaa Abdullah Laftaah AL-Rubaii[2]

[1,2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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Corresponding author: **Mohsin Rasheed Mohsin**. Email: mohsin.rasheed.bio@gmail.com; Alanimohsin@gmail.com

ABSTRACT

Introduction and Aim: *Proteus mirabilis* is a Gram-negative bacterium that is responsible for urinary tract infections (UTI), especially catheter-associated UTI. In this study, we investigated whether certain painkillers and anti-inflammatory drugs, when used alongside antibiotics, could lower *P. mirabilis* growth and consequently lower resistance to antibiotics.

Materials and Methods: Urine samples (n=119) were collected from Baghdad hospitals. *Proteus* spp was identified using the Vitek-2 compact system and PCR amplification of the 16SrRNA gene. Antibiotic susceptibility test was performed using the Vitek-2 system. The Kirby-Bauer method was used to investigate the effects of different concentrations of chemical compounds (Dexamethasone, Nefopam, Olfen, paracetamol and Piroxicam) alone and synergism with antibiotics on extensively drug resistant (XDR) isolates.

Results: 35 out of the 119 urine samples tested were found to be positive for *P. mirabilis*. Antibiotic susceptibility test revealed the isolates to be resistant to minocycline (71.40%), ticarcillin (68.57%), trimethoprim/ sulfamethoxazole (65.70%) and ticarcillin/clavulanic (57.10%). Antibiotics in combination with Olfen (75 mg/2 ml) and Paracetamol (500 mg/5ml) drugs showed complete growth inhibition of *P. mirabilis* when compared to controls.

Conclusion: Drugs dexamethasone, paracetamol, nefopam and olfen tested had no direct effect on bacterial growth of *P. mirabilis* when used alone. However, different concentrations of the drug in combination with antibiotics exerted a synergistic or antagonist effect on growth of *P. mirabilis*.

Keywords: *Proteus mirabilis* Antibiotic resistance; Paracetamol; Inhibition zone; PCR.

INTRODUCTION

Proteus mirabilis a member of the *Enteriobacteriacea* family, is a Gram-negative, facultative anaerobic, rod-shaped bacterium, noted for its urease production and swarming motility in specific 'bulls-eye' pattern on agar media (1). *P. mirabilis* predominantly occurs as a part of the microflora of human and animal gastrointestinal tract (2) and is an opportunistic pathogen, that causes infections of the gastrointestinal, urinary, and respiratory tracts as well as infections of the eye, ear, nose, skin, throat, burns, and wounds, and wounds (2,3). Asymptomatic bacteriuria is common, especially in the elderly and those with type 2 diabetes, although *P. mirabilis* can cause asymptomatic infections of the urinary tract, such as cystitis and pyelonephritis (4, 5). Urosepsis, which develops from a bacteremia, can be fatal in patients with these diseases. Further, urolithiasis (the production of urinary stones) can result from a *P. mirabilis* infection (6). *P. mirablis* is most noted for catheter associated urinary tract infection and has been implicated in neonatal meningoencephalitis, empyema, and osteomyelitis (7, 8). Several virulence factors including urease production, motility and adhesion mediated by flagella and fimbria, toxins such as hemolysin and Proteus toxic agglutinin, Lipopolysaccharide (LPS), and metal acquisition and biofilm development have been implicated in the pathogenesis of *P. mirabilis* (9). It is also known to be resistant to several antibiotics including benzylpenicillin, oxacillin, tetracycline, and macrolides used for its treatment (10).

MATERIALS AND METHODS

Isolation and identification

From October 2021 to February 2022, 119 urine samples were collected in sterilized containers from Baghdad Teaching Hospital, Ghazi Al- Hariri Hospital for Surgical Specialties and Teaching Laboratories at Medical City, Baghdad, Iraq. Each urine sample was inoculated onto MacConkey agar (Himedia/India) and incubated at 37ºC for 24hr. Typical colonies developed were picked and sub-cultured on nutrient agar and incubated overnight at 37ºC. Colonies developed were identified as *P. mirabilis* using the Gram-negative identification Kit (GN Kit) and by the Vitek-2 System. Molecular confirmation of *P. mirabilis* was done by PCR using specific 16SrRNA primers: *16SrRNA*-F: 5_AGAGTTTGATCCTGGC TCAG_3 and, *16SrRNA*-R: 5'CTACGGCTACCTT GTTACGA 3' (11).

Antibiotic susceptibility test using the Vitek-2 system

P. mirabilis isolates were tested for their antibiotic susceptibility using the Vitek-2 system. Susceptibility testing was done against 16 antibiotics which included amikacin, aztreonam, cefalexin, ceftazidime, cefepime,

ciprofloxacin, gentamicin, imipenem, meropenem, minocycline, tazobactam, ticarcillin, ticarcillin/ clavulanic acid, tobramycin, trimethoprim, and piperacillin. The isolates were considered sensitive, intermediate or resistant based on the interpretation given by the Vitek-2 system.

The effect of different drugs (painkiller or anti-inflammatory) on the antibiotic susceptibility

This test was performed by Kirby-Bauer method (12). The drugs Dexamethasone, Nefopam, Olfen, Paracetamol and Piroxicam were used in the experiment. The drugs were serial diluted separately using distilled water to obtain four different concentrations (C1-C4) as shown in Table 1.

To test the effect of these drugs on *P. mirabilis*, fresh bacterial colonies were picked and inoculated onto 4 tubes containing 2ml of nutrient broth and incubated for 24 h at 37º C. To the overnight grown bacterial culture, 1ml of each drug concentration was added, mixed well and further incubated at the same conditions. The bacterial growth was spread plated onto Muller Hinton agar plates using cotton swabs. The control tube was prepared in the same way but contained no drugs. The antibiotics disks were placed on the plates using sterile forceps, and further incubated for 24 h at 37ºC. The diameter (mm) of the inhibition zone was measured and compared with standards as mentioned by Clinical and Laboratory Standards Institute (CLSI), 2021(Table 2).

Effect of some drugs (painkillers and anti-inflammatory) on bacterial growth

To test the inhibitory effect of drugs on bacterial growth, agar well diffusion method was used. Briefly, the bacterial inoculum was spread on Muller Hinton agar plate by using cotton swabs. To wells (0.5 mm) made in the agar plates, 50 µl of the drug suspension (C1-C4) was added and then the plates incubated for 24h at 37ºC. The diameter (mm) of the inhibition zone was measured as mentioned previously.

RESULTS

Isolation and identification

Of the 119 urine samples tested, 35 were found positive for *P. mirabilis*. Preliminary identification of the bacterium was based on the appearance of pale colonies and characteristic swarming movement (bull's-eye pattern) on both blood and MacConkey agar (Fig.1). Further identification was based on biochemical tests typical for *P. mirabilis* on Vitek-2 compact system (Fig. 2). Molecular confirmation using 16SrRNA gene PCR, yielded the expected band size for *P. mirabilis* (Fig. 3).

Table 1: Concentrations of each drug used in the study

Painkillers and anti-inflammatory compounds used	Concentration used			
	C1	C2	C3	C4
Nefopam (painkiller)	20mg/2ml	20mg/4ml	20mg/8ml	20mg/16ml
Paracetamol (painkiller)	500mg/5ml	500mg/10ml	500mg/20ml	500mg/40ml
Piroxicam (anti-inflammatory)	20mg/1ml	20mg/2ml	20mg/4ml	20 mg/8ml
Dexamethasone (anti-inflammatory)	8mg/2ml	8mg/4ml	8mg/8ml	8mg/16ml
Olfen (anti-inflammatory)	75mg/2ml	75/4ml	75mg/8ml	75mg/16ml

Table 2: Zone diameter interpretation standards as per Clinical and Laboratory Standards Institute 2021

Antimicrobial agent (µg/Disc)	Diameter of zone inhibition (mm)		
	Resistant	Intermediate	Sensitive
Cefotaxime (30)	≤ 22	23-25	≥ 26
Trimethoprim (5)	≤10	11-15	≥16
Meropenem (10)	≤ 19	20-22	≥ 23
Imipenem (10)	≤ 19	20-22	≥ 23
Ciprofloxacin (5)	≤ 21	22-25	≥ 26
Gentamicin (10)	≤ 12	13-14	≥ 15
Ampicillin (10)	≤ 13	14-16	≥ 17
Tetracycline (30)	≤ 11	12-14	≥ 15

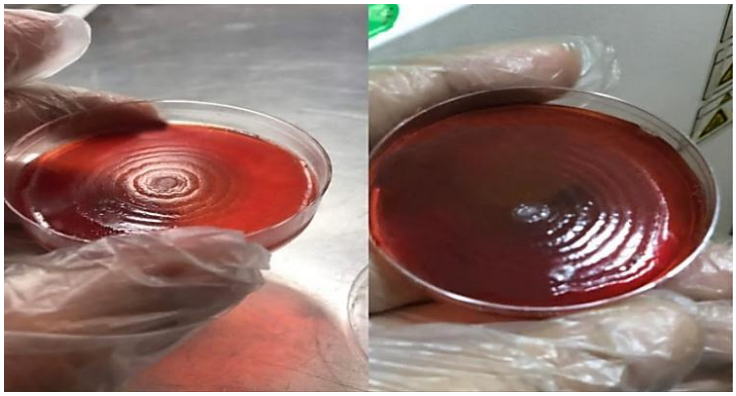

Fig. 1: Characteristic **s**warming motility (bull's-eye pattern) of *P. mirabilis*

bioMérieux Customer: Microbiology Chart Report Printed November 8, 2021 12:56:17 AM CST

Patient Name: 40, . Patient ID: ERHDF
Location: Physician:
Lab ID: 160 Isolate Number: 1

Organism Quantity:
Selected Organism : Proteus mirabilis

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	4.93 hours	**Status:** Final
Selected Organism	91% Probability	**Proteus mirabilis**	
	Bionumber:	0417100341462231	
ID Analysis Messages			

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	(+)	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Fig. 2: *P. mirabilis* identification using Vitek-2 compact system

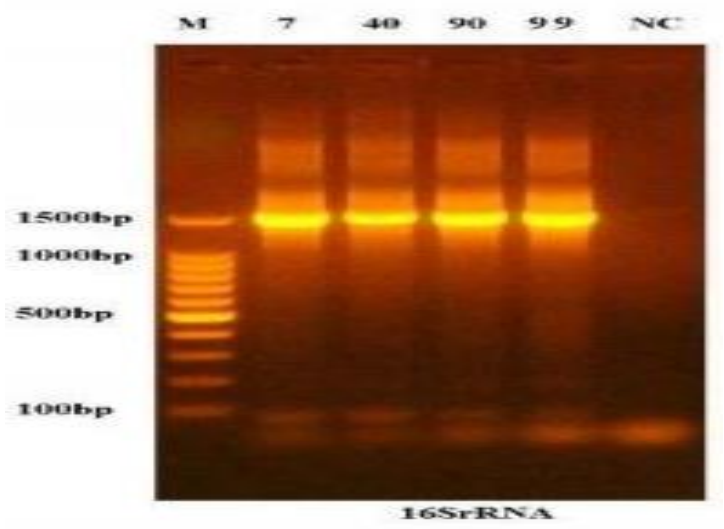

Fig. 3: Molecular confirmation of *P. mirabilis* by PCR.
M: 1.5kbp ladder marker, Lanes 7,40,90,99: Band showing amplification of *16srRNA* gene.

Antibiotic susceptibility test

The susceptibility of *P. mirabilis* isolates (n=35) was tested against 16 antibiotics using the Vitek-2 compact system and the results for each isolate interpreted based on the output by the system **(**Fig. 4). Total results for all isolates showed *P. mirabilis* to be resistant to (minocycline (71.40%), followed by ticarcillin (68.57%), trimethoprim/sulfamethoxazole (65.70%), ticarcillin/clavulanic acid (57.10%), and gentamicin (54.28%). Among the bacterial isolates only seven (20%) of the isolates were considered as extensively-drug resistant (XDR). *P. mirablis* showed highest sensitivity to Meropenem (2.85%), followed by amikacin (5.70%), piperacillin/tazobactam (8.57%), imipenem (27.10%), ciprofloxacin (31.40%), and piperacillin (34.20%) (Fig. 5). Two of the isolates (isolate nos.90 and 99) that were extensively drug resistant isolates were selected for further studies.

Effect of painkillers and anti-inflammatory drugs on bacterial growth

Out of these seven (XDR) isolates two of the most resistant isolates were selected to test the effect of drugs Nefopam, Paracetamol, Piroxicam, Dexamethasone and Olfen by agar well diffusion method. Results showed that all the drugs tested, in their varying concentrations, had no effect or change on growth of the two isolates compared to the control, and presented the same results (Fig. 6).

Organism Quantity:
Selected Organism : Proteus mirabilis
Source: **Collected:**
Comments:

Susceptibility Information | **Analysis Time:** 10.93 hours | **Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ticarcillin	64*	*R	Imipenem	8	R
Ticarcillin/Clavulanic Acid	<= 8	*R	Meropenem	1	S
Piperacillin	64	I	Amikacin	>= 64	R
Piperacillin/Tazobactam	<= 4	S	Gentamicin	>= 16	R
+Cefalexin		R	Tobramycin	>= 16	R
Ceftazidime	>= 64	R	Ciprofloxacin	>= 4	R
Cefepime	>= 64	R	Minocycline	>= 16	R
Aztreonam	32	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

*= AES modified **= User modified

AES Findings
Confidence: Consistent with correction

Fig. 4: Antibiotic susceptibility test for *P. mirabilis* using Vitek-2 system

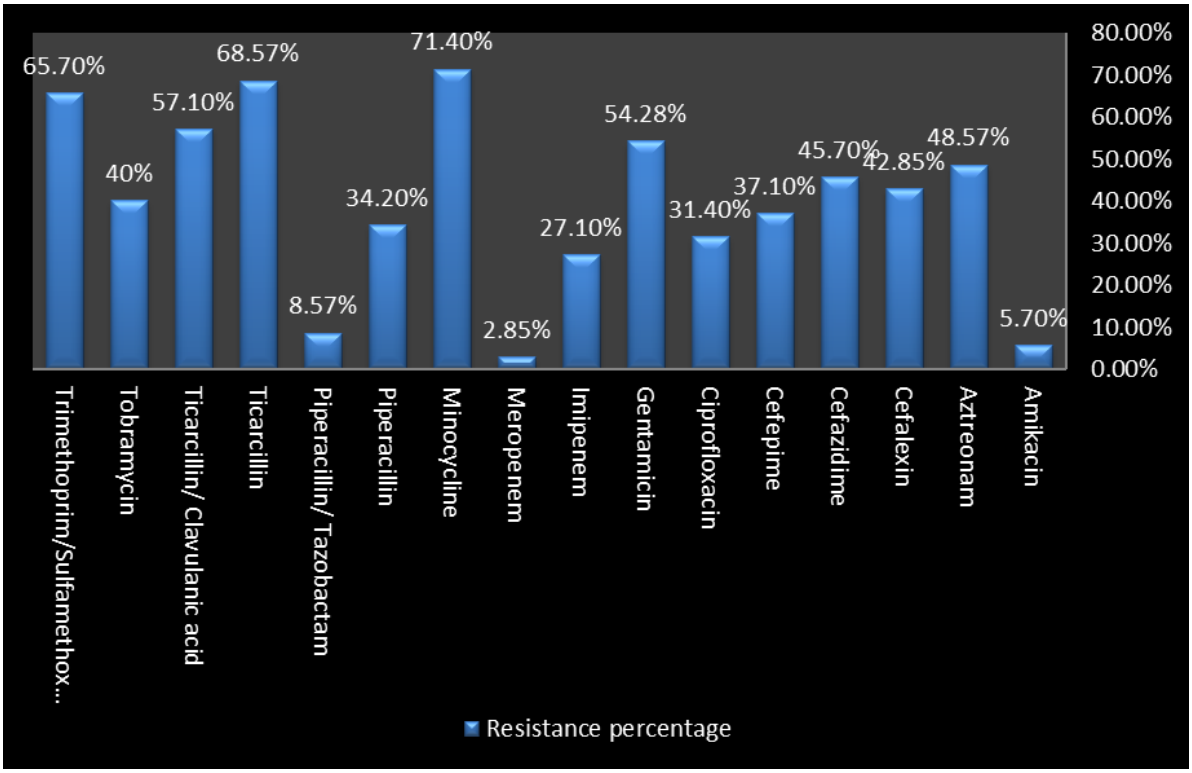

Fig. 5: Antibiotics resistance percentage for *P. mirabilis* isolates

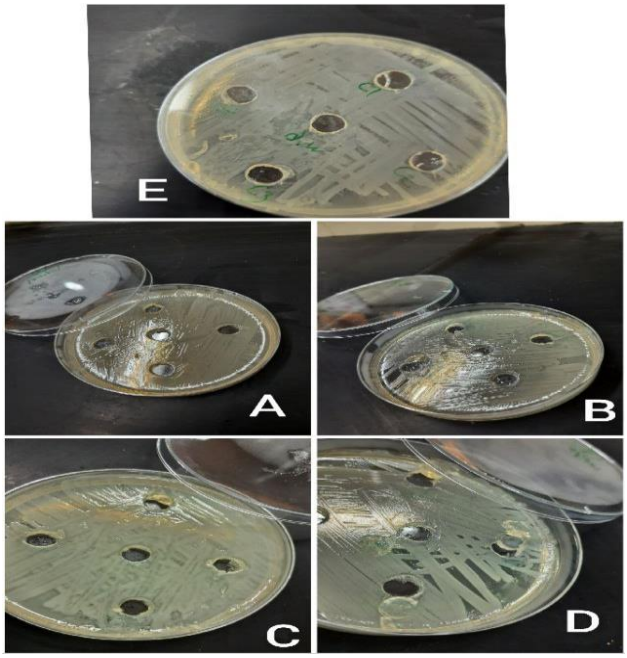

Fig. 6: Agar gel diffusion test for *P.mirablis* growth in the presence of
A: Paracetamol, B: Olfen, C: Piroxicam, D: Nefopam, E: Dexamethasone

Combined effect of drugs and antibiotics on the growth and antibiotic susceptibility of *P. mirabilis*

The growth of the tested isolates (isolate nos.90 and 99) was not affected by the different painkiller and anti-inflammatory drugs used (Fig. 6). However, in combination with antibiotics these drugs showed varying effect on growth. The zones of inhibition of the combined drug and antibiotic in relation to control are presented in Table 3. As shown in Fig.7 and Table 3 *P. mirabilis* growth was inhibited when the antibiotics were combined with Olfen (75 mg/2ml) and Paracetamol (500 mg/5ml). However, the antibiotics in combination with drugs of varying concentrations showed either synergistic or antagonistic effects.

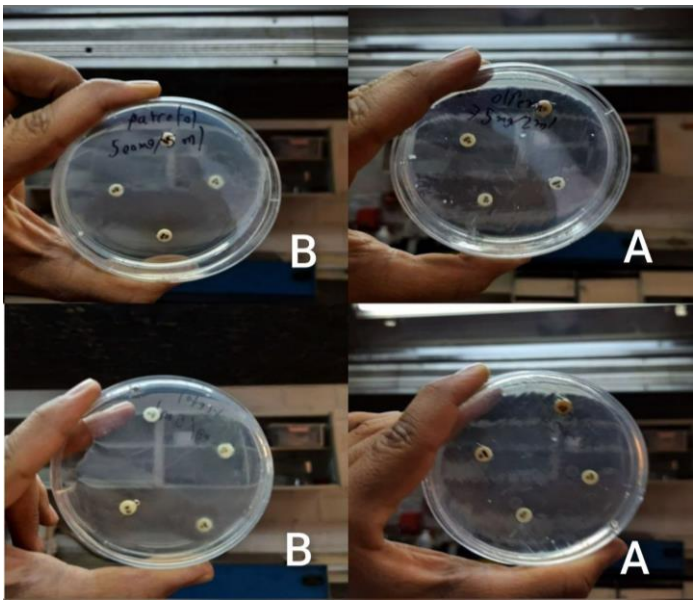

Fig. 7: Antibiotic susceptibility test for *P. mirabilis* treated with
A. Olfen (75 mg/2ml) and B. Paracetamol (500 mg/5ml)

Table 3: Inhibition zone diameter (in mm) for combined effect of drugs with antibiotics

Antibiotics	**Control**	**Drugs**	**Concentrations**			
			C1	**C2**	**C3**	**C4**
Meropenem	4.6	Dexamethasone	5.8	5.3	5.2	5.1
		Paracetamol	NG	7.1	5.4	4.9
		Nefopam	5.7	5.5	5.1	5
		Olfen	NG	5.8	5.3	5.8
		Piroxicam	2.4	2.3	4.8	4.7
		Dexamethasone	4.7	4.8	4.8	4.8
		Paracetamol	NG	6.2	5.2	4.3

Imipenem	4	Nefopam	5.2	4.5	3.9	4.2
		Olfen	NG	5.2	4.6	5.3
		Piroxicam	3.7	3.9	4.3	4.3
Cefotaxime	1.1	Dexamethasone	2.5	1.3	1.7	3.5
		Paracetamol	NG	2	3.2	1.8
		Nefopam	1	3.7	1	3
		Olfen	NG	0.8	1.3	1.7
		Piroxicam	2.8	3.2	4.2	3.6
Ampicillin/cloxacillin	0.5	Dexamethasone	1	0.8	0.8	1.2
		Paracetamol	NG	0.5	0.5	0.5
		Nefopam	0.9	0.5	0.8	0.8
		Olfen	NG	0.5	1.3	0.9
		Piroxicam	1.2	1.1	1.5	0.5
Trimethoprim	2.5	Dexamethasone	1.9	2	2.7	4.7
		Paracetamol	NG	1.7	4.3	2.5
		Nefopam	2.9	2.1	2	3.3
		Olfen	NG	0.8	3.8	4.8
		Piroxicam	5	5	4.8	2.7
Gentamicin	1.5	Dexamethasone	2.3	2.2	2	2.4
		Paracetamol	NG	2.3	2.5	1.6
		Nefopam	1.6	2.4	2	2
		Olfen	NG	1	2.6	2.2
		Piroxicam	2	2.1	3	2.1
Tetracycline	2.4	Dexamethasone	0.7	0,6	0.5	0.5
		Paracetamol	NG	0.9	2.8	2.6
		Nefopam	3.1	0.9	2.7	2.6
		Olfen	NG	0.5	1.8	1.6
		Piroxicam	1.4	1.2	0.5	0.5
Ciprofloxacin	2.7	Dexamethasone	4.8	4.7	4.1	4.5
		Paracetamol	NG	3.9	4.5	3.4
		Nefopam	3.2	4.7	2.7	3.6
		Olfen	NG	1.4	2.9	4.9
		Piroxicam	3.3	2.9	4.6	4.7

Control: Zone of inhibition for bacteria when tested with drugs alone

DISCUSSION

The prevalence of *P. mirabilis* isolated from urine samples in this study, was found to be higher than the prevalence reported by Treska *et al*., (13) and Dalia *et al.,* (14). This increased prevalence for *P. mirabilis* could be attributed to hygiene, lack of contamination and increased multi drug resistance to antibiotics. *P*. mirabilis has the ability to confer resistance to next generation antibiotics through horizontal gene transfer (HGT), thus causing serious health problems. *P. mirablis* in this study, showed highest resistance to minocycline (71.4%). Minocycline is a semisynthetic tetracycline-derived antibiotic currently used systemically to treat a wide range of infections caused by Gram-negative and Gram-positive bacteria. Minocycline is primarily bacteriostatic, with a mechanism of action similar to other tetracycline antibiotics, i.e., inhibition of bacterial protein biosynthesis via binding to the 30S ribosomal subunit and inhibiting the ligation of the aminoacyl-tRNA (15). A study by Serry *et al*.,(16) reported *P. mirabilis* isolates to be completely resistant to tetracycline (100%). Resistance percentage for gentamicin was 54.28% in this study, and was in line with a previous study (17). Gentamicin is an aminoglycoside and a powerful broad-spectrum antimicrobial, which are inhibitors of protein synthesis in prokaryotes (17). In contrast to the results of an earlier study (17), which revealed that imipenem was the most effective drug against *P. mirabilis*, this investigation only found a resistance percentage of 27.1%. The antibiotic imipenem belonging to the carbapenem group can penetrate the bacterial cell wall, inactivate the intracellular autolytic inhibitor enzymes, leading to the killing of the bacterial cell (18). Similarly, the lowest resistance percentage (2.85%) was seen for meropenem which also belongs to the class carbapenem which agrees with the results by Serry *et al.,* (16) who also reported least rate of resistance to meropenem. Despite the synergic effect and constant pattern of Olfen (75mg/2ml) and Paracetamol (500 mg/5ml) other concentrations of drugs shown no specific pattern each had shown different independent effect (synergic or antagonistic effect) might be depending on chemical composition, mode of action ,interaction with each antibiotic and concentration of each drug which resulted in increasing of bacterial sensitivity to antibiotics (synergic effect) or decreasing bacterial sensitivity to antibiotics (antagonistic effect). The drugs Nefopam, Paracetemol, Piroxicam, Dexamethasone, and Olfen, had no direct effect on bacterial growth at the phenotypic level when used alone, but the antibiotic susceptibility was slightly changed when combined

with each of them; the activity of antibiotics slightly increased were different, and each concentration of those drugs had a different effect on bacterial sensitivity towards antibiotics that was both synergistic or antagonistic. Our findings run counter to the study by Zimmermann and Curtis (19), who reported that diclofenac (commercially known as olfen) can inhibit the growth of *Proteus* with minimal inhibitory concentration (MIC) of 0.20mg/ml. In contrast, a study by Abbas *et al.*, (20) showed that diclofenac had no significant effect or change on bacterial growth compared to control (20). Bacteria have diverse intrinsic resistance mechanisms, such as reduced uptake, altering a drug target, drug inactivation, and drug efflux, which may contribute to the disparities in drug activity and resistance (21, 22) observed in this study.

CONCLUSION

Drugs dexamethasone, paracetamol, nefopam and olfen tested had no direct effect on bacterial growth of *P. mirabilis* alone but when used with antibiotics few of the isolates showed synergistic effect in certain concentrations and increased the sensitivity of *P. mirabilis* towards antibiotics such as olfen (75mg/2ml) and Paracetemol (500mg/5ml), while other drugs and other concentrations showed different effects that was either synergistic or antagonistic.

CONFLICTS OF INTEREST

None.

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