Original Article

Genotypic and Cartwheel Phenotypic Detection of AcrAB-TolC, AcrAD-TolC, and AcrEF-TolC Pumps in *Proteus mirabilis*

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Abstract

Background: Opportunistic pathogen *Proteus mirabilis* is resistant to multiple antibiotic classes and frequently causes infections. A significant threat to global public health exists in the emergence and spread of novel antimicrobial resistance genes. Sensitivity was recently found to be decreased by RND efflux pump (EP) genes. Using the Cartwheel Ethidium Bromide (EtBr) method, efflux pump activity in bacteria can be measured. **Objective:** The purpose of this study was to detect RND pumps (AcrAB-TolC, AcrAD-TolC, and AcrEF-TolC) genotypically and phenotypically among *P. mirabilis* isolated from urinary tract infections (UTI). **Materials and Methods:** Standard biochemical tests and PCR using the *atpD* gene as species-specific primers were used to confirm the diagnosis of suspected *P. mirabilis* isolates. The disc diffusion method was used to test antibiotic susceptibility. The cartwheel EtBr method was used to screen pump activity, whereas PCR was used to detect the RND genes. **Results:** Out of 515 urine specimens collected from patients with UTI, only 70 isolates were confirmed by PCR as *P. mirabilis*. Nearly all isolates were multidrug resistant (MDR) with Multiple antibiotic resistance (MAR) index values ranging from 0.15 to 0.6. The phenotypic detection of pump by EtBr cartwheel method revealed that the efflux activity was categorized as 64.3% positive, 24.3% intermediate, and 11.6% negative. The frequency of *acrA, acrB, acrD, acrE, acrF*, and *tolC* genes were 51.4%, 61.4%, 88.6%, 94.3%, 88.6%, and 51.4%, respectively. **Conclusion:** All MDR isolates of *P. mirabilis* harbor at least a single RND pump with valuable activity pushing an alarm and thinking about anti-efflux strategies for treatment future.

Keywords: Cartwheel, efflux pump, ethidium bromide, MAR index, *P. mirabilis*, RND genes

Introduction

Proteus mirabilis is a common opportunistic pathogen causing severe illness in humans and has been described as an etiological agent in UTI,[1] usually blamable for recording serious bacterial infections in hospitals.[2] Antimicrobial medicines have proven essential weapons in the fight against infection because they are currently the most widely prescribed treatment drugs.[3] MAR is often brought on by the manifestation of plasmids that hold one or more resistance genes in bacteria. The MAR index is a useful, reliable, and affordable approach for locating the sources of antibiotic-resistant organisms.[4] When it exceeds 0.2, that points to the high-risk source of contamination, where antibiotics are recurrently used.[5]

One of the most common and versatile types of antibiotic resistance mechanisms is EP, which can be divided into five families: RND (resistance-nodulation-division), MFS (major facilitator superfamily), ABC (ATP-binding cassette), SMR (small multidrug resistance), and MATE (multidrug and toxic compound extrusion), The RND pumps are unique to Gram-negative bacteria, whereas the other members are shared by both groups.[6]

The AcrAB-TolC, AcrAD-TolC, and AcrEF-TolC EPS are RND members of transporters in the majority of Enterobacteriaceae.[7] They are tripartite proteinaceous transporter frameworks that permit direct antibiotic extrusion from the cytosol or periplasm to the external

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of bacteria and have been linked to clinically significant antibiotic resistance and MDR bacteria. They entail a periplasmic adaptor protein, an inner membrane transporter, and an outer membrane channel.[6,8] Tetracycline, chloramphenicol, macrolides, and fluoroquinolones were amongst the substrates for this EPS. Also, it has been linked with decreased tigecycline susceptibility in a variety of bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *P. mirabilis*, and *Enterobacter cloacae*.[9,10] Due to gram-negative bacteria having an RND EP and a dual membrane structure, they are inherently less sensitive to antimicrobials than gram-positive bacteria.[11]

Cartwheel EtBr, a recently created straightforward, rapid, and affordable approach, is used to show EP activity in bacteria. It allows comparison of many and different isolates based on their capacity to extrude EtBr.[12] The dye' application in this assay is based on the knowledge that fluorescent molecules, when used in sub-lethal doses, enter cells passively through diffusion but can only be expelled by active EP. Therefore, an estimation of the relative intracellular concentration of the dye at any point in time can be directly correlated to the activity of the EPS.[13]

EtBr emits very weak fluorescence in aqueous solution or media, but the intensity rises with an increase in their concentration in the cytoplasm or periplasm of viable cells, mostly due to its inter-chelating with cellular components like DNA. As a result, cells with less efflux activity will have higher intracellular dye concentrations, resulting in stronger fluorescence, and vice versa.[12] Fluorescence is absent in MDR bacteria, which suggests that EPS is functioning.[14]

This study aimed to investigate the prevalence of MAR index, and detection of *P. mirabilis* EPS activity by PCR technique and cartwheel EtBr method. This method is frequently used to examine the expression and function of EPS in bacteria and can aid in the discovery of new antibiotic targets.

Materials and Methods

Bacterial isolates

In this investigation, a total of 70 strains of *P. mirabilis* were isolated from 515 urine specimens of UTI patients who attended Al Hillah Teaching, Margan, Alamam Al Sadeq hospitals, and private clinic labs in Babylon City for the period between January and June 2022. Midstream urine was collected in a disinfected screwcap container.

Bacterial identification

All specimens are directly inoculated on the MacConkey agar, and the non-lactose fermented isolates were identified by a series of biochemical tests and inoculated onto the UTI chromogenic agar. For the swarming phenomenon test, suspected isolates were cultured on blood agar plates and then incubated at 37°C for 24 h.[15,16]

DNA extraction

The Favoregen Gnomically DNA Extractions kit was used to separate gnomically identified DNA from suspected *P. mirabilis* isolates following the manufacturer's protocol.

PCR technique

All strains were subjected to molecular characterization by detecting specific species *atpD* genes and PCR detection of *acrA, acrB, acrD, acrE, acrF,* and *tolC* genes using specific primer pairs that are itemized in Table 1. Amplification of DNA was carried out in a final volume of 25 µL, "consisting of upstream primer (10 pmol) and downstream primer (10 pmol) 1 µL, DNA template (50–500 ng) 4 µL, nuclease-free water (ddH2O) 6.5 µL, and Accustart TM Taq PCR SuperMix 12.5 µL (Promega). The PCR products (5 µL) were evaluated in an 1.5% (w/v) agarose gel by electrophoresis with 1×TBE buffer and

Table 1: Details of primers used in this study

Gene	Primer sequence (5′- 3′)	Prodect size (bp)	Annealing (°C)	Ref.
atpD	F: GGTGCGGGTGTTGGTAAAAC	570	55ºC	this study
	R: TGAATCCAGTGGGTCAACCG			
acrA	F: ATCACCTTTCGCACTGTCGT	256	58.3ºC	Ali and Al-Dahmoshi[17]
	R:CGACAAACAGGCCCAACAAG			
acrB	F:CATAAACACGCCCTGGTCCT	432	60.3ºC	
	R:GCTACCCGTAAGTCGATGGG			
TolC	F: CGATCGTGATGCTGCCTTTG	596	58.3ºC	
	R: GGTTGCGTTTTTCGGCTTCT			
acrD	F:GCCGTGCAGCAAGTACAAAA	424	58.3ºC	
	R:CTGGTGTTTGCAGCAGTGAC			
acrE	F:CGCTGCAATTCTCCGATGTG	376	60.3ºC	
	R:GCAGTATCTGCGGGGGTATC			
acrF	F: ATCCTCGCCGCTTTTGGTTA	626	57.2ºC	
	R: AACACTTTTTGCGTCCGCTC			

visualized by staining using red safe dye. The product size was located by contrast with a 100 bp DNA ladder.[18] UV transilluminator was positioned to notice the DNA bands, and then the gel was photographed with a digital camera.

Antimicrobial susceptibility testing

was done using the Kirby-Bauer disc diffusion method, following the guidelines of the Clinical Laboratory Standard Institute (CLSI),[19] against 20 antibiotics. The MAR index (calculated as a/b, where "a" represents the number of antibiotics the isolate was resistant to and "b" represents the total number of antibiotics the isolate was tested against) was calculated for each bacterial isolate. In general, an MAR index value >0.2 is observed when the isolate is exposed to high-risk sources of antibiotics (several antibiotics are used); in contrast, an MAR index value ≤ 0.2 is observed when antibiotics are seldom or never used.[5]

Cartwheel EtBr method

The efflux of EtBr was tested by the method described by Suresh *et al.* and Martins *et al.*,[8,13] with a slight modification. Concisely, Mueller-Hinton agar plates containing 0 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, and 20 µg/mL of dye were prepared from a stock solution of EtBr (5 mg/mL) on the same day of the experiment and protected from light. Then, the plates were divided into sectors by radial lines. Overnight cultures of the bacterial isolates were adjusted to 0.5 of a McFarland standard and swabbed on EtBr agar plates starting from the plate center towards its edges. They were wrapped in aluminum foil to protect them from the light and incubated at 37°C for 18 h. The plates were examined using the UV-light trans-illuminator and photographed. The minimum concentration of EtBr producing fluorescence in the bacterial isolates was recorded. Isolates that fluoresced at the lowest EtBr concentration did not have active efflux pumps, whereas those that did not fluoresced did.[20,21]

Ethical approval

This study was directed in agreement by means of the ethical values that have origin in the Declaration of Helsinki and attained from the general directorate of health and Laboratory of the Hospitals and Scientific Committee from the College of Sciences/University of Babylon, Hilla City, Iraq. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject data, and the permission formula were reviewed and approved by a local ethics committee according to project no.: m 220101 (including the number and the date on 4-1-2022) to get this approval.

Results

P. mirabilis was identified from UTI patients' urine in the current investigation in 70 strains from 515 urine samples. The *atpD* specific species gene and the PCR technique were used to complete the molecular identification, which produced 100% confirmation of the strains. All strains, as seen in Figure 1, generated bands with a comparable size (570 bp), which corresponded to the product size of the primers used for identification.

The spectra of drug resistance developed in those isolates were scored based on their MAR index values through 20 antibiotic agents used up for resistance testing, depending on CLSI.[19] The obtained wide-spectrum MAR index values range from 0.15 to 0.6. A total of 70 *P. mirabilis* isolates were screened phenotypically for the presence of efflux pumps via the cartwheel-EtBr method. The isolates efflux activity was categorized according to the recorded EtBr minimum concentrations in each strain as follows: 64.3% (45/70) positive efflux activity isolates that emitted fluorescence at 15 and 20 µg/mL EtBr concentrations and isolates that did not show any fluorescence even at high concentrations; 24.3% (17/70) intermediate efflux activity isolates that emitted fluorescence at 10 µg/mL

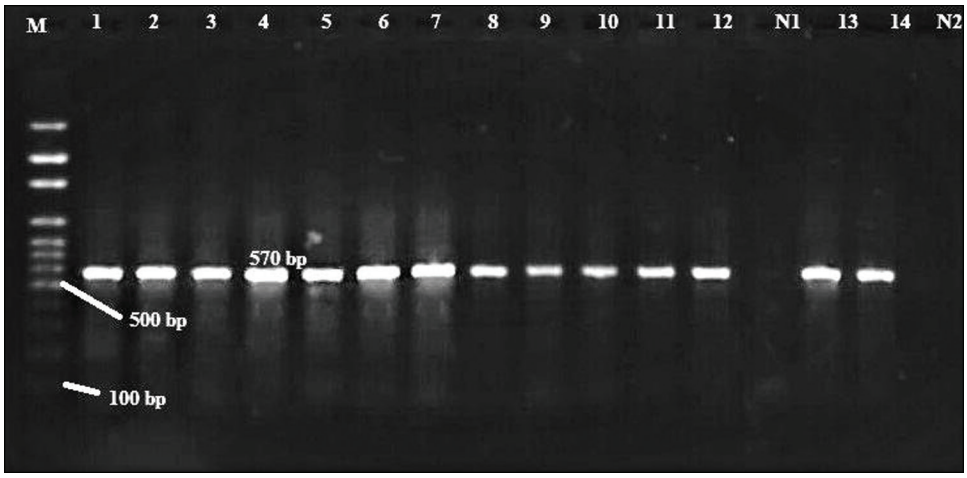

Figure 1: Results of *P. mirabilis* isolates' *atpD* gene am ˌplifications, separated on a l.5% a ˌgarose ˌgel, TBE lx at 80 V for 60 min, and electrophoresis stained with red safe. DNA 100-bp marker. N1 and N2 were negative control (*E. coli and P. vulgaris*)

Table 2. Results of Cartwheel-EtBr method and MAR index values

EtBr-CW	No. (%)	Mar index mean± std. deviation	*P* Value ($P \leq 0.05$)
Positive	45 (64.3)	.3667±.11531	.001*
Intermediate	17 (24.3)	.3000 ±.07906	
Negative	8 (11.4)	.2188 ±.07039	
Total	70 (100)	.3336 ±.11380	

*Significant difference under $P \leq 0.05$

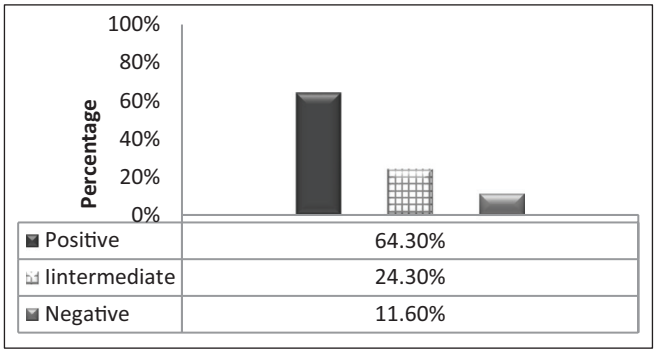

Figure 2: Percentage of Efflux pumps in *P. mirabilis* isolates detection by EtBr cartwheel method

EtBr concentration; and 11.6% (8/70) negative efflux activity isolates that emitted fluorescence at 5 µg/ ml EtBr concentration [Table 2 and Figures 2 and 3]. Out of the 45 positive isolates, P10, P16, P18, P26, P30, P47, P54, and P57 do not fluoresce even at the highest EtBr concentration used in this assay. Positive efflux pump isolates had MAR index values of 0.2 to 0.6, whereas intermediate efflux activity isolates had MAR index values of 0.2–0.4, and negative isolates had MAR index values of 0.15-2, except P34 and P42, which had MAR indices of 0.35 and 0.3, respectively [Table 2]. From this result, it appears that there is a significant correlation between the presence of the pumps and the MAR index at the level of $P \leq 0.05$.

This research was planned with the priority of RND efflux pump genes in mind. The prevalence of *acrA, acrB, acrD, acrE, acrF,* and *tolC* genes were 51.4%, 61.4%, 88.6%, 94.3%, 88.6%, and 51.4%, respectively. *P. mirabilis* strains produced the same band size (256bp, 432bp, 596bp, 424bp, 376bp, 626 bp, correspondingly), which was the product size of the primers used for this identification [Figures 4 and 5].

RND efflux genes displayed a variety of patterns, Fourteen isolates with the *acrA*/*acrB*/*acrD*/*acrE*/*acrF*/*tolC* pattern

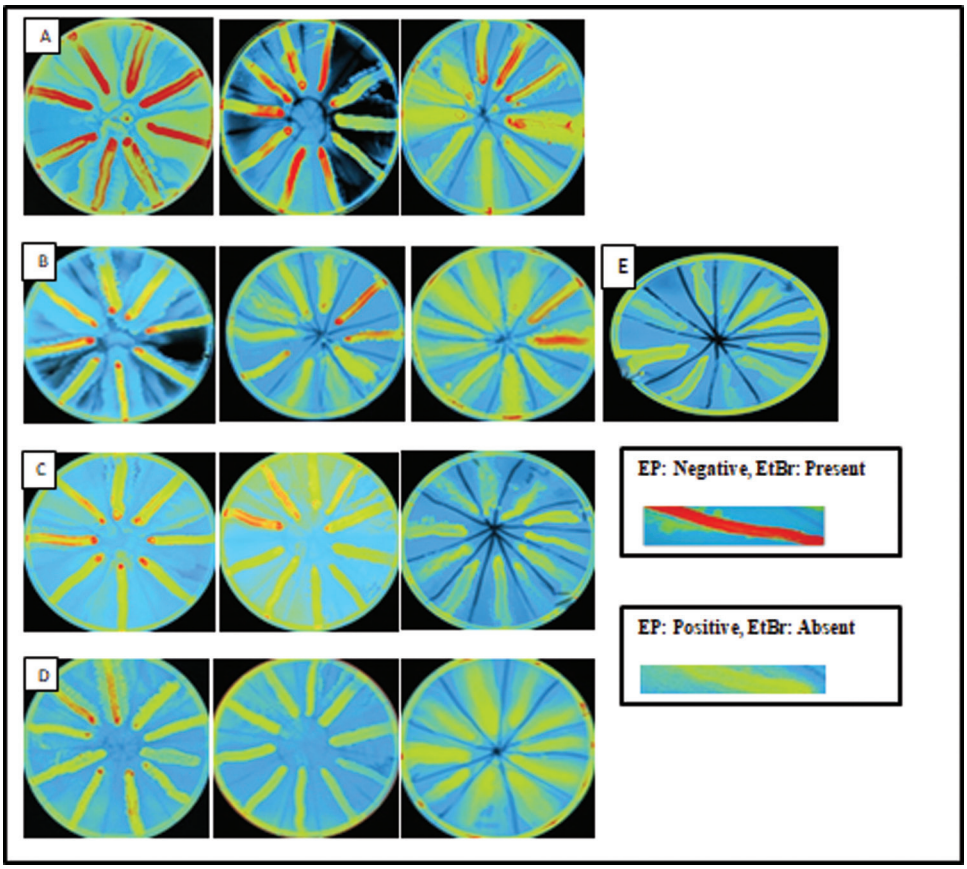

Figure 3: Determination of RND efflux pump activity by cartwheel method EtBr. Mueller Hinton plates containing varying concentrations of ethidium bromide, A: 5 µg/mL, B: 10 µg/mL, C: 15 µg/mL, D: 20 µg/mL, and E: 0 µg/mL (control)

accounted for the majority of them (20%), as evident in Table 3.

Table 4 shows the assembly between RND pump genes and EtBr cartwheel results. Out of fourteen strains with *acrA*/*acrB*/*acrD*/*acrE*/*acrF*/*tolC* pattern (positive to all genes used in this study), eleven isolates (P12, P25, P45, P47, P52, P54, P56, P60, P65, P69 and P70) were positive to EtBr cartwheel test, whereas the remaining three isolates (P27, P55 and P64) were intermediate. Other patterns, based on the number of genes that appeared in isolates, oscillate between positive, intermediate, and negative.

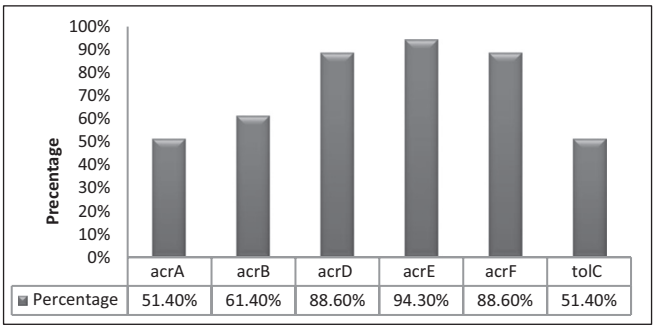

Figure 4: Percentage of RND genes appearance in *P. mirabilis*

Discussion

The current investigation set up that the *atpD* gene, which was created specifically for this study, had a potent selective ability to discriminate isolated *P. mirabilis* from other *Proteus* spp. The *atpD* gene, which encodes for the ATP synthase -subunit for the production of ATP from ADP, is one of the best-preserved genes in *Proteus* species, and it is more conservative when compared to *16S rRNA* gene.[22,23] Qu *et al.*[24] identified *P. mirabilis* using the *atpD* gene. The three studies mentioned above have been reported to be a highly selective force for distinguishing *P. mirabilis* from other *Proteus* spp.

The MAR index is a practical, trustworthy manner for identifying the origins of antibiotic-resistant microbes.[4] This index is an epidemiological tool used for risk analysis of the environment due to bacterial contamination, and it is now used to determine whether the isolate originated from an environment where several antibiotics were used or not. The majority of the isolates in this study had an

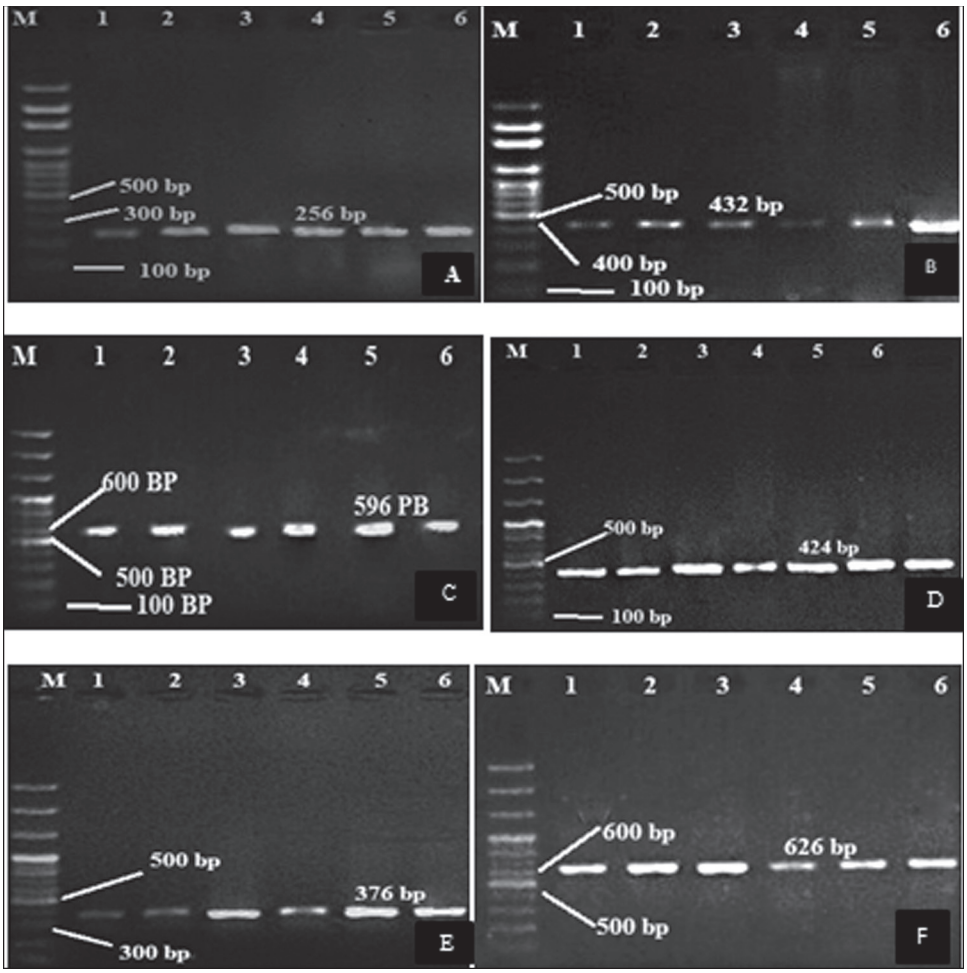

Figure 5: Results of *P. mirabilis* isolates' RND genes (A:*acrA,* B:*acrB,* C:*TolC,* D:*acrD,* E:*acrE and* F:*acrF*) am ,plifications were separated on a l.5% a ,garose ,gel, TBE lx at 80 volts for 60 min, and electrophoresis stained with red safe. DNA 100-bp marker

Table 3: RND efflux genes pattern in the *P. mirabilis* isolates

Isolate No.	Gene pattern	No.(%)
P1, P68	*acrA/acrD/acrE/acrF/tolC*	2/70 (2.9)
P2,P3,P5,P7,P22,P28,P29,P31,P35	acrD/acrE/acrF/tolC	9/70 (12.9)
P4,P9,P13,P14,P18,P20,P21,P26,P37,P43,P53,P59	*acrA/acrB/acrD/acrE/acrF*	12/70 (17.1)
P6,P23,P30,P32,P33,P40,P42,P58	*acrD/acrE/acrF*	8/70 (11.4)
P8,P15	*acrA/acrB/acrD/acrE/ tolC*	2/70(2.9)
P10,P62,P66	*acrA/acrD/acrE/acrF*	3/70 (4.3)
P11,P17	*acrA/acrB/acrD/acre*	2/70 (2.9)
P12,25,P27,P45,P47,P52,P54,P55,P56,P60,P64,P65, P69,P70	*acrA/acrB/acrD/acrE/acrF/ tolC*	14/70 (20)
P16,P24,P39,P49,P50	*acrB/acrD/acrE/acrF*	5/70 (7.1)
P19	*acrB/acrF*	1/70 (1.4)
P36,P41,P51	*acrE/acrF/tolC*	3/70 (4.3)
P38,P46,P48,P57,P67	*acrB/acrD/acrE/acrF/tolC*	5/70 (7.1)
P44	*acrE/tolC*	1/70 (1.4)
P60	*AcrB*	1/70 (1.4)
P63	*acrA/acrB*	1/70(1.4)

P, *P. mirabilis* isolates numbers

MAR score of more than 0.2, indicating that they had been extensively exposed to a high risk of contamination since they frequently use antibiotics. The current outcomes are in agreement with the findings of Meena *et al.*,[3] in which 40 MDR *E.coli* were tested, they found that the highest value of the MAR index was 0.7, whereas the lowest value was 0.3.

The cartwheel EtBr Agar Method is a technique used to measure the activity of EP in bacteria by using EtBr fluorescent dye, which is pumped out of the bacterial cell by the EPS. The principle of this test is the passage of this dye across the cytoplasmic membrane and its subsequent intracellular accumulation inside the bacterial cell. According to Sibin *et al.*,[14] 22% of Enterobacteriaceae had efflux activity, the present result was contrasted with that of Patil *et al.*,[25] who found that 49.47% of positive EP organisms, were *K. pneumoniae* followed by *E. coli* and *P. mirabilis*, 6 of which failed to fluoresce even at the highest concentration of EtBr used. Notably, it was discovered that 32.3% of the *P. mirabilis* isolates displayed EP activity.[26]

Our results are almost in agreement with those published by Al-Fayyadh *et al.*[27] and AL Marjani *et al.*,[28] showed that 76.6% of *E. coli* and % 76 of *P. aeruginosa* isolates showed positive results for EP activity. According to a study conducted in India by Thakur *et al.*,[29] 60% of the MDR *E. coli* isolates tested positive for the ethidium bromide fluorescence test, whereas only 40% tested negative.

This study is in agreement with a finding by Hameed,[30] which established that 85% of *P. aeruginosa* isolates were positive, 10% were intermediate, and 5% were negative, and with a study by Al-Saadi[31] which found that 70% of *E. coli* isolates were positive. In a study conducted in Egypt by Attallah *et al.*[32] on *S. aureus,* it was found that 10.4% were negative, 43.8% were intermediate, and 45.8% were positive. Ugwuanyi *et al.*[20] found that only 59% of the *Pseudomonas* isolates phenotypically showed efflux pump activity. ADded research[21] revealed that 66.6% of *S. Typhimurium* isolates showed evidence of pronounced efflux activity. The presence of EP activity, however, is not always associated with antibiotic resistance. Aside from antibiotic resistance, the pumps are physiologically involved in the extrusion of toxic metabolites, dyes, and chemicals that are toxic to the cells.

The findings of other studies, including one by Abdel-Karim *et al.*[33] in which 72 *S. aureus* isolates were subjected to the qualitative assessment of efflux by the Cartwheel method; 25.0% were positive, 40.3% were intermediate, and 34.7% were negative, did not corroborate the findings of this study. Numerous studies have highlighted the contribution of EP to multi-antibiotic resistance. The mechanism of action of EP differs from other mechanisms of bacterial resistance, such as the production of beta-lactamase enzymes, in that is, working on more than one group of antibiotics, that is, a single EP can expel a wide range of antibiotics. However, The difference between studies may be due to the type and number of isolates, the concentration of the dye, and the bacterial suspension used.

Eight of the 45 positive isolates do not fluoresce even at the highest EtBr concentration used in this assay. This result could be attributed to an augmented expression of the efflux pump, resulting in an increased outflow of EtBr from the bacteria, and subsequent decreased fluorescence.[34] The higher the concentration of EtBr required to produce fluorescence of the bacterial mass, the greater the efflux capacity of the bacterial cells,[12] and isolates that fluoresced at the lowermost concentration

Table 4: Prevalence *of acrA, acrB, acrD, acre, acrF,* and *tolC* RND efflux genes in *P. mirabilis* with EtBr-cart wheel results

Isolate NO.	*acrA*	*acrB*	*acrD*	*acrE*	*acrF*	*tolC*	EtBr-CW	Isolate NO.	*acrA*	*acrB*	*acrD*	*acrE*	*acrF*	*tolC*	EtBr-CW
P1	+	-	+	+	+	+	P†	P36	-	-	-	+	+	+	P
P2	-	-	+	+	+	+	P	P37	+	+	+	+	+	-	P
P3	-	-	+	+	+	+	P	P38	-	+	+	+	+	+	P
P4	+	+	+	+	+	-	I††	P39	-	+	+	+	+	-	I
P5	-	-	+	+	+	+	P	P40	-	-	+	+	+	-	I
P6	-	-	+	+	+	-	I	P41	-	-	-	+	+	+	I
P7	-	-	+	+	+	+	I	P42	-	-	+	+	+	-	N
P8	+	+	+	+	-	+	N‡	P43	+	+	+	+	+	-	I
P9	+	+	+	+	+	-	P	P44	-	-	-	+	-	+	N
P10	+	-	+	+	+	-	P	P45	+	+	+	+	+	+	P
P11	+	+	+	+	-	-	P	P46	-	+	+	+	+	+	P
P12	+	+	+	+	+	+	P	P47	+	+	+	+	+	+	P
P13	+	+	+	+	+	-	N	P48	-	+	+	+	+	+	I
P14	+	+	+	+	+	-	P	P49	-	+	+	+	+	-	P
P15	+	+	+	+	-	+	I	P50	-	+	+	+	+	-	I
P16	-	+	+	+	+	-	P	P51	-	-	-	+	+	+	N
P17	+	+	+	+	-	-	P	P52	+	+	+	+	+	+	P
P18	+	+	+	+	+	-	P	P53	+	+	+	+	+	-	P
P19	-	+	-	-	+	-	P	P54	+	+	+	+	+	+	P
P20	+	+	+	+	+	-	P	P55	+	+	+	+	+	+	I
P21	+	+	+	+	+	-	I	P56	+	+	+	+	+	+	P
P22	-	-	+	+	+	+	P	P57	-	+	+	+	+	+	P
P23	-	-	+	+	+	-	P	P58	-	-	+	+	+	-	P
P24	-	+	+	+	+	-	P	P59	+	+	+	+	+	-	I
P25	+	+	+	+	+	+	P	P60	+	+	+	+	+	+	P
P26	+	+	+	+	+	-	P	P61	-	+	-	-	-	-	I
P27	+	+	+	+	+	+	I	P62	+	-	+	+	+	-	P
P28	-	-	+	+	+	+	N	P63	+	+	-	-	-	-	P
P29	-	-	+	+	+	+	I	P64	+	+	+	+	+	+	I
P30	-	-	+	+	+	-	P	P65	+	+	+	+	+	+	P
P31	-	-	+	+	+	+	P	P66	+	-	+	+	+	-	P
P32	-	-	+	+	+	-	N	P67	-	+	+	+	+	+	P
P33	-	-	+	+	+	-	P	P68	+	-	+	+	+	+	P
P34	-	-	-	-	-	-	N	P69	+	+	+	+	+	+	P
P35	-	-	+	+	+	+	P	P70	+	+	+	+	+	+	P

P, *P. mirabilis* isolate number; +, gene present; -, gene absent, P †, positive to EtBr-CW, I††, intermediate to EtBr-CW, N‡, negative to EtBr-CW

of EtBr were noted as lacking active efflux pumps, and vice versa[20] This means that bacteria cannot excrete and pump the dye that has accumulated, implying that gene expression is low, that is, few pumps, and the dye is concentrated in the cells.

When the EtBr cartwheel method results were compared to the MAR index, it was discovered that positive EP isolates had an MAR index of 0.2 to 0.6, intermediate efflux activity isolates had a MAR index of 0.2–0.4, and negative EP isolates had a MAR index of 0.15–2, with the exception of P34 and P42, which had MAR indices of 0.35 and 0.3, respectively. These findings were parallel to those informed by Meena *et al.*,[3] who found that when the MAR index was low, the pumps were few or not found, whereas when the MAR index was high, the pumps were numerous. The current findings indicate a direct association between the number of antibiotics effective against bacteria resistant to them and the phenotypic expression of pumps.

The high prevalence of EP in this study indicates that activity-based antibiotic resistance is one of the mechanisms of drug resistance prevalent in MDR isolates.[21] This may be due to the overexpression of EP or the down-regulation of porins.

Eleven of fourteen strains with the *acrA*/*acrB*/*acrD*/*acrE*/*acrF*/*tolC* pattern were positive for the EtBr cartwheel test, whereas three isolates were intermediate. Other patterns vibrate between positive, intermediate, and negative based on the number of genes that seem in them. There was no complete efflux, that is, no 100% decrease. As a result, it

was shown that active EP performance, along with some other mechanism such as a decrease in the number of porins, to prevent antibiotic entry into the bacterial cell, may be responsible for this multidrug phenotype. It is thought to be caused by either an active EP that excretes EtBr or a decrease in the number of porins that prevent this dye from entering the cell.[3,13]

P. mirabilis isolate is a potentially infectious pathogen that, despite being isolated from a wound, can cause kidney infection and its related complications. It also has numerous antibiotic resistance mechanisms, including biofilm formation, swarming mobility, efflux systems, and enzymatic detoxification.[35] The current finding proved that *P. mirabilis* had AcrAB-TolC, AcrAD-TolC, and AcrEF-TolC efflux systems.

AcrAB's functional activity can be supplemented by another efflux system, AcrEF, in *Salmonella enterica* antibiotic mutants, minor rises in *acrA, tolC*, and *acrF* gene expression levels were experiential, signifying that AcrAB, tolC, and acrEF are not up-regulated.[36] The tripartite pump AcrAB-TolC is primarily in charge of efflux-mediated multidrug resistance in *E. coli*, and consist of AcrA periplasmic protein that links the outer membrane-bound TolC to the transporter AcrB, an inner membrane protein that forms a homo-trimer and has extensive periplasmic domains that are supposed to be answerable for drug binding and transport.[37] In Gram-negative bacteria, overexpression of this efflux pump is an inherent mechanism of multidrug resistance.[38]

AcrA and AcrB's homologs, respectively, are AcrE and AcrD/AcrF. AcrE can interact with AcrD, which does not hold its private periplasmic adaptor protein (PAP), and establish that the residues formerly identified in AcrB binding are also involved in AcrD binding. Bacterial susceptibility to AcrD substrates was dramatically reduced when AcrD and AcrE were co-expressed, showing that AcrE offers an alternative PAP to AcrD and can cooperate to create a functional complex.[39]

Although AcrA is the homologous PAP for AcrB, it has been shown that AcrA is necessary for the AcrD to function as a tripartite efflux system since AcrD is missing a PAP-encoding gene.[40]

Conclusion

All MDR isolates of *P. mirabilis* harbor at least a single RND efflux pump with valuable efflux activity pushing an alarm and thinking about anti-efflux strategies for treatment future.

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Conflicts of interest

There are no conflicts of interest.

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