Microbes and Infectious Diseases 2023; 4(1): 182-193

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Coexistence of integrons class 1 and 2 with emergence of class 3 among *Proteus mirabilis* clinical isolates from Alexandria, Egypt

Amel Elsheredy* *, *Eman Faisal, Eglal El Sherbini, Nancy Attia

Department of Microbiology, Medical Research Institute, Alexandria University,Alexandria, Egypt.

ARTICLE INFO

Article history:
Received 16 September 2022
Received in revised form 29 September 2022
Accepted 2 October 2022

Keywords:
Proteus mirabilis
Antimicrobial resistance
MDR
XDR
Integrons

ABSTRACT

Background: The aim of the study was to investigate the antimicrobial resistance and prevalence of integrons in 100 *Proteus mirabilis* (*P. mirabilis*) clinical isolates from Egypt. **Methods:** Vitek-2 Compact system was used for bacterial identification, and antimicrobial susceptibility was performed using the Kirby-Bauer method. Primers for the integrase gene (*intI1, intI2, and intI3*) were used to screen for integrons using polymerase chain reaction (PCR). Additionally, the variable regions of class 1 and 2 integrons were amplified and sequenced. **Results:** Urine was the most common specimen (75%) followed by pus (21%). The highest antibiotic resistance among isolates was to ampicillin (61%), followed by trimethoprim-sulfamethoxazole (50%), with 34% of strains being multidrug-resistant (MDR). Class 1 was the most prevalent integron detected in 80%, with 8 different gene cassettes, the most prevalent being (*AadA and dfrA17*). Class 2 was detected in 66% of isolates, and class 3 integron in 21% of isolates. To the best of our knowledge, this is the first report of integron 3 in *P. mirabilis* clinical isolates. **Conclusions:** In summary, antimicrobial resistance and integrons are prevalent among *P. mirabilis* isolates in Egypt which poses a great threat to the treatment of MDR bacterial infections therefore, special measures should be taken to prevent spread of integrons and associated resistance genes.

Introduction

Proteus mirabilis (P. mirabilis) is an important opportunistic pathogen that can cause various infections, such as urinary tract infections (UTIs), particularly among patients with urinary tract abnormalities or indwelling catheters, wound infections, bacteremia, pneumonia and other infections in both community and healthcare-associated settings [1,2]. The pathogenicity of *P. mirabilis* is related to its ability to expresses several virulence factors, including biofilm formation, swarming motility, urease activity, and production of enzymes and cytotoxins for bacterial adherence, colonization, and tissue invasion [3-5].

Normally, *P. mirabilis* has intrinsic resistance to polymyxin, nitrofurantoin, tetracycline and tigecycline [6]. Moreover, additional rapid acquisition and dissemination of antimicrobial resistance genes has been increasing. Infection by multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. mirabilis* isolates has increased worldwide in the past few years which represents a threat that challenge the treatment of these bacterial infections [7-9].

DOI: 10.21608/MID.2022.163262.1385
* *Corresponding author:* Amel Elsheredy
E-mail address: amel.elsheredy@alexu.edu.eg

In Egypt, *P. mirabilis* is a common cause of urinary tract infection (UTI) with increasing emergence and spread of MDR *P. mirabilis* isolates [9-11]. Extensive overuse and misuse of antibiotics especially in developing countries has resulted in enhanced selection pressures, leading to the evolution of antibiotic-resistant strains [9]. Understanding the molecular mechanisms through which resistance genes are acquired and propagated might aid in the development of novel antibacterial strategies. Horizontal transmission of mobile genetic elements, such as plasmids, transposons, and integrons containing resistance genes play a significant role in the dissemination of antibiotic resistance among bacterial species [12].

Integrons are genetic elements that can efficiently capture and express exogenous gene cassettes. They are well-known for their involvement in spreading of antibiotic resistance genes, especially among Gram-negative bacteria [13,14]. The integrase gene (*intI)*, the gene for the recombination site (*attI*), and the promoter (*Pc*), which encourages the expression of integrated gene, are all crucial components of an integron [15,16].

Until present, five classes of integrons have been identified based on nucleotide sequence analysis of the integrase gene, with the first three (class1, 2, and 3) being clinically significant, as they are involved in the continuing accumulation of resistance gene cassettes [15]. Integrons of class 1 are the most prevalent type and have been frequently reported in a lot of clinical bacterial isolates therefore, they have been thoroughly investigated [17,18]. Two conserved segments, 3′ conserved segment (3′ CS) and 5′ conserved segment (5′ CS), along with internal gene cassettes encoding antimicrobial resistance genes, make up the structure of class 1 integrons [15,16].

Integrons in class 2 are less frequent and diverse than those in class 1. They are linked to the Tn7 family of non-replicative transposons. Class 3 integron, which has been reported as rare and identified only in a limited number of bacterial isolates, is less known than class 1 and 2 and their 3'conserved segment has yet to be characterized [19,20].

Researchers often target two locations of integrons; the integrase enzyme gene for detecting integrons and identifying integron classes and the variable area, which is positioned between two conserved sections in the integron. Because the length these variable areas is determined by the number of its gene cassettes, PCR products come in a variety of sizes [16,17].

Studies are being conducted in many regions of the world to investigate the prevalence of various integron classes and their relevance to the emergence of antibiotic resistance in different bacteria [21-24]. Previous research highly connected the existence of Integrons, with resistance to many antimicrobial drugs especially in Gram negative bacteria [21-24]. Accordingly, identifying, and characterizing integrons appears to be critical for analyzing the epidemiology of antibiotic resistance genes, particularly in places where data is limited. As a result of growing emergence of drug resistant *P. mirabilis* isolates in our region and the scare available data to elucidate the mechanism of this issue, therefore, in this study we investigated the prevalence of class 1, 2 and 3 integrons, their gene cassette contents, and its relationship with antibiotic resistance pattern among *P. mirabilis* collected from different clinical specimens in Egypt.

Materials and methods

Bacterial isolates

This study was carried out during a period of 12 months (from June 2020 to June 2021). During this period, we prospectively collected a total of 100 isolates of *P. mirabilis*, from various types of clinical specimens from the microbiology laboratory of medical research institute, Alexandria University, Egypt. The isolates were collected from non-repetitive clinical specimens submitted to the laboratory for routine culture and sensitivity. Standard biochemical tests were used to identify isolates, and then all *P. mirabilis* suspected isolates were confirmed by VITEK 2 automated ID System (bioMerieux, France). For further molecular investigations, identified isolates were stored on Luria Bertani broth mixed with 20% glycerol and kept at –80°C.

Antimicrobial susceptibility testing

The susceptibility of isolates was determined using disc diffusion technique according to Clinical laboratory standards institute (CLSI) recommendations [25]. The following antibiotics were assessed: ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), ampicillin-sulbactam (10/10 μg), piperacillin-tazobactam(100/10 μg), cefepime(30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), aztreonam (30 μg), imipenem (10 μg), meropenem (10 μg), ertapenem

(10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and trimethoprim- sulphamethoxazole (1.25/23.75 µg). Additionally, ofloxacin (5 µg), and norfloxacin (10 µg) were assessed in urine samples and tobramycin (10 µg) in pus swabs. Multidrug-resistant (MDR) and XDR isolates were defined, according to the guidelines of the European Centre for Disease Prevention and Control [26].

Polymerase chain reaction for screening class 1,2 and 3 integrons

Bacterial DNA was extracted from fresh overnight *P. mirabilis* cultures by boiling method [27]. Briefly few colonies were emulsified in sterile distilled water and incubated for 15 minutes in a boiling water bath, then rapidly cooled on ice for 5 minutes before centrifugation at 14,000 rpm for 15 minutes. The resulting clear supernatant was diluted by 10 folds and utilized as a DNA template for PCR amplification. Using a NanoDrop 2000 (Thermo Fisher Scientific, Inc), the concentration and purity of the isolated DNA was assessed. PCR was performed using primers listed in **table (1)** to hybridize conserved areas of encoded integrase genes *intI,II, and III*. PCR reaction mixture was 25 µl containing 12.5 µl Dream Taq Hotstart Green master mix (Thermo Fisher), 10 pmol of each primer, and 5 µl DNA extract. A negative control was made by mixing the identical ingredients with water instead of the DNA. The amplification consisted of initial denaturation at 95°C for 2 minutes followed by 30 cycles of 30 seconds denaturation at 95°C, 15 seconds annealing and 30 seconds extension at 72°C. Amplified products were separated by electrophoresis in 1.5% agarose gel.

Identifying the variable regions of class 1 and 2 integrons

Integron 1 and 2 positive isolates were further tested for characterization of class 1 and 2 integrons variable regions and their resistance-encoding gene cassettes using two pairs of generalized primers **(Table 1)**. Purified PCR products were sequenced by BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The resulting sequences were analyzed using the BLAST (Basic Local Alignment Search Tool) service. (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Blast results in the Comprehensive Antibiotic Resistance Database (CARD) database https://card.mcmaster.ca/analyze/blast.

Statistical analysis

Data were statistically analyzed using SPSS program version 25.0. Qualitative data were described using number and percent. Significance of the obtained results was judged at the 5% level. Chi-square, Fisher's Exact and Monte Carlo tests were used to compare between different groups.

Results

Bacterial isolates and antimicrobial resistance

The majority of *P. mirabilis* isolates in the present study were from urine (75%), followed by pus (21%), then blood and broncho-alveolar lavage (2% each). As shown in **figure (1)**; 69% of isolates were resistant to 1 or more of the tested antibiotics. Highest resistance was against ampicillin (61%), and trimethoprim-sulfamethoxazole (50%). Meropenem, ertapenem, and imipenem showed the highest activity against isolates and only 6%, 9%, and 10% of isolates were resistant to them, respectively. A total of 34 isolates were MDR, while 9 isolates were XDR.

Prevalence of class 1, 2, and 3 integrons

Among the 100 *P. mirabilis* isolates included in the PCR assay for detection of the three classes of integrons; 93 of the isolates were positive for integrons including *IntI I, II and III* while 7 isolates were negative for integrons. The frequency of the three classes of integrons among the integron positive isolates is shown in **table (2).** Class 1 integron was the most prevalent integron detected in 80%, followed by class 2 in 66%, and finally class 3 in 21% of isolates **(Figure 2).** Also, 55.9% of integron positive isolates harbored two different classes of integrons together and in 11.8% of them the 3 classes of integrons coexisted together.

Analysis of the gene cassettes embedded in the class 1 and 2 integrons

Variable region in the integron 1 was successfully amplified in 66 isolates revealing 8 different DNA fragments based on PCR amplicons size (200, 250,600, 750, 1,000, 1,500-, 2,000-, and 2,500-bp) **(Figure 3).** These amplified regions were sequenced, revealing several combinations of 8 gene cassettes expressing various types of resistance determinants **(Table 3).** The most prevalent gene cassettes were those conferring resistance to aminoglycosides (*aadA and aadB*), trimethoprim (*dfrA, dfrA12, and dfrA17*), beta-lactamase (*blaSHV and blaVIM*). Fourteen isolates harbored integrons without cassettes. Class 2 integrons carried two different cassette arrays: one of 200 bp including *blaCMY and blaTEM* beta-lactamases, and 250 bp

including *blaRUB* beta-lactamase and *ErmO -srmA* methyltransferase conferring resistance to spiramycin. The partial sequences of class 1 and 2 integrons from this study were deposited in GenBank with accession numbers [ON778443–ON778456].

Antimicrobial resistance profiles of integron-positive and integron-negative *P. mirabilis* isolates

Comparison between the antibiotic resistance of the isolates according to the existence of integrons is shown in **table (4).** Isolates harboring integron 1 were more likely to be resistant to almost all the tested antibiotics. Whereas, for integron 2 positive isolates this association was revealed in a fewer number of antibiotics including some of beta lactams and trimethoprim-sulfamethoxazole. On the other hand, there was no significant association between the presence of class 3 integrons and resistance to different antibiotics. As shown in **table (5)**; 97.1% and 100 % respectively of MDR and XDR strains harbored integrons either as one class or more. Moreover, these resistance patterns were most prevalent in strains harbored two classes of integrons together especially class 1 and 2 integrons representing 61.8% and 55.6% in MDR and XDR respectively.

Table 1. List of primers used for the detection of the target genes.

Target Gene or Region	Primer	Ta	Sequence (5′→3′)	Amplicon size (bp)	Reference
IntI 1	IntI1F IntI1R	56 ºC	ACGAGCGCAAGGTTTCGGT GAAAGGTCTGGTCATACATG	565	[28]
IntI 2	IntI2F IntI2R	58 ºC	GTGCAACGCATTTTGCAGG CAACGGAGTCATGCAGATG	403	[28]
IntI 3	IntI3F IntI3R	58 ºC	CATTTGTGTTGTGGACGGC GACAGATACGTGTTTGGCAA	717	[28]
Class 1 integron variable region	5'-CS 3'-CS	54 ºC	GGCATACAAGCAGCAAGC AAGCAGACTTGACCTGAT	Variable	[29]
Class 2 integron variable region	Ti-F Ti-B	50 ºC	ACCTTTTTGTCGCATATCCGTG CTAACGCTTGAGTTAAGCC	Variable	[28]

Ta: Annealing temperature

Table 2. Distribution of the 93 *P. mirabilis* isolates positive for integrons according to the coexistence of different classes of integrons.

Integrons	Positive isolates	
	No.	%
One class of integrons only	**30**	**32.3**
IntI 1	19	20.4
IntI 2	9	9.7
IntI 3	2	2.2
Two classes of integrons together	**52**	**55.9**
IntI 1+ IntI 2	44	47.3
IntI 1+ IntI 3	6	6.5
IntI 2+ IntI 3	2	2.2
Three classes of integrons together IntI 1+IntI 2+ IntI 3	**11**	**11.8**
Total positive for integrons	**93**	**100**

Table 3. Characterization of gene cassette arrays according to the amplicon size of class 1 and 2 integron variable regions in the *P. mirabilis* isolates.

Integron class	Amplicon size(bp)	Gene Cassette array	No. of isolates (%)
IntI 1 (N=66)	200	*blaSHV aadA*	3 (4.5%)
	250	*ND*	14 (21.2%)
	600	*aadA , dfrA aacA*	10 (15.1%)
	750	*dfrA17 aadB,aadA*	6 (9.1%)
	1000	*aadA*	9 (13.6%)
	1500	*aadA, dfrA17*	18 (27.3%)
	2000	*aadA, blaVIM*	4 (6.1%)
	2500	*aadA, dfrA12*	2 (3.1%)
IntI 2 (N=59)	200	*blaCMY, blaTEM*	20 (33.9%)
	200	*ND*	10 (16.9%)
	250	*blaRUB, ErmO-srmA*	29 (49.2%)

ND: Not determined

Table 4. Antibiotic resistance pattern of *P. mirabilis* isolates according to integrons 1, 2, and 3 positivity.

Antibiotic	No. (%) of antibiotic resistant isolates (n=100)	No. (%) of antimicrobial resistant isolates according to integrons								
		IntI 1			*IntI 2*			*IntI 3*		
		Positive (n=80)	Negative (n=20)	P_1 value	Positive (n=66)	Negative (n=34)	P_2 value	Positive (n=21)	Negative (n=79)	P_3 value
Ampicillin	61 (61.0%)	59 (73.8%)	2 (10.0%)	0.000*	42 (63.6%)	19 (55.9%)	0.451	11 (52.4%)	50 (63.3%)	0.362
Cefotaxime	35 (35.0%)	34 (42.5%)	1 (5.0%)	0.002*	28 (42.4%)	7 (20.6%)	0.030*	7 (33.3%)	28 (35.4%)	0.857
Ceftriaxone	35 (35.0%)	34 (42.5%)	1 (5.0%)	0.002*	28 (42.4%)	7 (20.6%)	0.030*	7 (33.3%)	28 (35.4%)	0.857
Ceftazidime	33 (33.0%)	32 (40.0%)	1 (5.0%)	0.003*	26 (39.4%)	7 (20.6%)	0.058	6 (28.6%)	27 (34.2%)	0.627
Cefepime	26 (26.0%)	25 (31.3%)	1 (5.0%)	0.017*	22 (33.3 %)	4 (11.8%)	0.020*	5 (23.8%)	21 (26.6%)	0.797
Aztreonam	33 (33.0%)	32 (40.0%)	1 (5.0%)	0.003*	28 (42.4%)	5 (14.7%)	0.005*	7 (33.3%)	26 (32.9%)	0.971
Ampicillin-sulbactam	25 (25.0%)	24 (30.0%)	1 (5.0%)	0.021*	21 (31.8%)	4 (11.8%)	0.028*	6 (28.6%)	19 (24.1%)	0.671
Piperacillin/tazobactam	10 (10.0%)	9 (11.3%)	1 (5.0%)	0.682 FE	8 (12.1%)	2 (5.9%)	0.487 FE	0 (0.0%)	10 (12.7%)	0.115 FE
Amoxicillin-clavulanate	30 (30.0%)	29 (36.3%)	1 (5.0%)	0.006*	26 (39.4%)	4 (11.8%)	0.004*	7 (33.3%)	23 (29.1%)	0.708
Imipenem	10 (10.0%)	10 (12.5%)	0 (0.0%)	0.205 FE	9 (13.6%)	1 (2.9%)	0.158 FE	3 (14.3%)	7 (8.9%)	0.434 FE
Meropenem	6 (6.0%)	6 (7.5%)	0 (0.0%)	0.597 FE	5 (7.6%)	1 (2.9%)	0.661 FE	2 (9.5%)	4 (5.1%)	0.603 FE
Ertapenem	9 (9.0%)	9 (11.3%)	0 (0.0%)	0.198 FE	6 (9.1%)	3 (8.8%)	1.00 FE	2 (9.5%)	7 (8.9%)	1.00 FE
Gentamicin	34 (34.0%)	33 (41.3%)	1 (5.0%)	0.002*	25 (37.9%)	9 (26.5%)	0.254	7 (33.3%)	27 (34.2%)	0.942
Amikacin	30 (30.0%)	29 (36.3%)	1 (5.0%)	0.006*	21 (31.8%)	9 (26.5%)	0.580	7 (33.3%)	23 (29.1%)	0.708
Tobramycin (N=21)	7 (33.3%)	7 (8.8%)	0 (0.0%)	1.00 FE	5 (7.5%)	2 (5.9%)	1.00 FE	0 (0.0%)	7 (8.9%)	0.521 FE
Ciprofloxacin	35 (35.0%)	33 (41.3%)	2 (10.0%)	0.009*	27 (40.9 %)	8 (23.5%)	0.084	6 (28.6%)	29 (36.7%)	0.487

Levofloxacin	32 (32.0%)	30 (37.5%)	2 (10.0%)	0.018*	25 (37.9%)	7 (20.6%)	0.079	6 (28.6%)	26 (32.9%)	0.705
Norfloxacin (n=75)	17 (22.7%)	17 (29.8%)	0 (0.0%)	0.008* FE	13 (19.7 %)	4 (11.8%)	0.223	5 (23.8%)	12 (15.2%)	0.514 FE
Ofloxacin (n=75)	19 (25.3%)	19 (23.8%)	0 (0.0%)	0.004* FE	15 22.7 %)	4 (11.8%)	0.116	5 (23.8%)	14 (17.7%)	0.753 FE
Trimethoprim-sulfamethoxazole	50 (50.0%)	49 (61.3%)	1 (5.0%)	0.000*	40 (60.6 %)	10 (29.4%)	0.003*	11 (52.4%)	39 (49.4%)	0.806

FE**:** Fisher Exact
p_1*:* p value for comparing between antibiotic resistance in *IntI 1*positive and in *IntI 1* negative strains
p_2: p value for comparing between antibiotic resistance in *IntI 2* positive and in *IntI 2* negative strains
p_3: p value for comparing between antibiotic resistance in *IntI 3* positive and in *IntI 3* negative strains
*: Statistically significant at $p \leq 0.05$

Table 5. Correlation between patterns of antibiotic resistance and presence of integrons among the 100 *P. mirabilis* isolates.

Integrons	**Pattern of antibiotic resistance**						***p*1**	***p*2**	***p*3**
	Non-MDR (n=57)		**MDR (n=34)**		**XDR (n=9)**				
	No.	**%**	**No.**	**%**	**No.**	**%**			
Negative for integrons	**6**	**10.5**	**1**	**2.9**	**0**	**0.0**			
One class of integrons only	**24**	**42.1**	**5**	**14.7**	**1**	**11.1**	**0.007***	**0.137**	**FE p=1.000**
IntI 1	13	22.8	5	14.7	1	11.1			
IntI 2	9	15.8	0	0.0	0	0.0			
IntI 3	2	3.5	0	0.0	0	0.0			
Two classes of integrons together	**23**	**40.4**	**24**	**70.6**	**5**	**55.6**	**0.005***	**FE p=0.478**	**FE p=0.442**
IntI 1 & 2	18	31.6	21	61.8	5	55.6			
IntI 1 & 3	3	5.3	3	8.8	0	0.0			
IntI 2 & 3	2	3.5	0	0.0	0	0.0			
Three classes of integrons together *IntI 1, 2 & 3*	**4**	**7.0**	**4**	**11.8**	**3**	**33.3**	**FE p=0.466**	**FE p=0.048***	**FE p=0.147**

FE**: Fisher Exact**
p_1: p value for comparing between Non-MDR and MDR
p_2: p value for comparing between Non-MDR and XDR
p_3: p value for comparing between MDR and XDR
*: Statistically significant at $p \leq 0.05$

Figure 1. Frequency of antibiotic resistance within the 100 *P. mirabilis* isolates, R isolates include (intermediate + resistant).

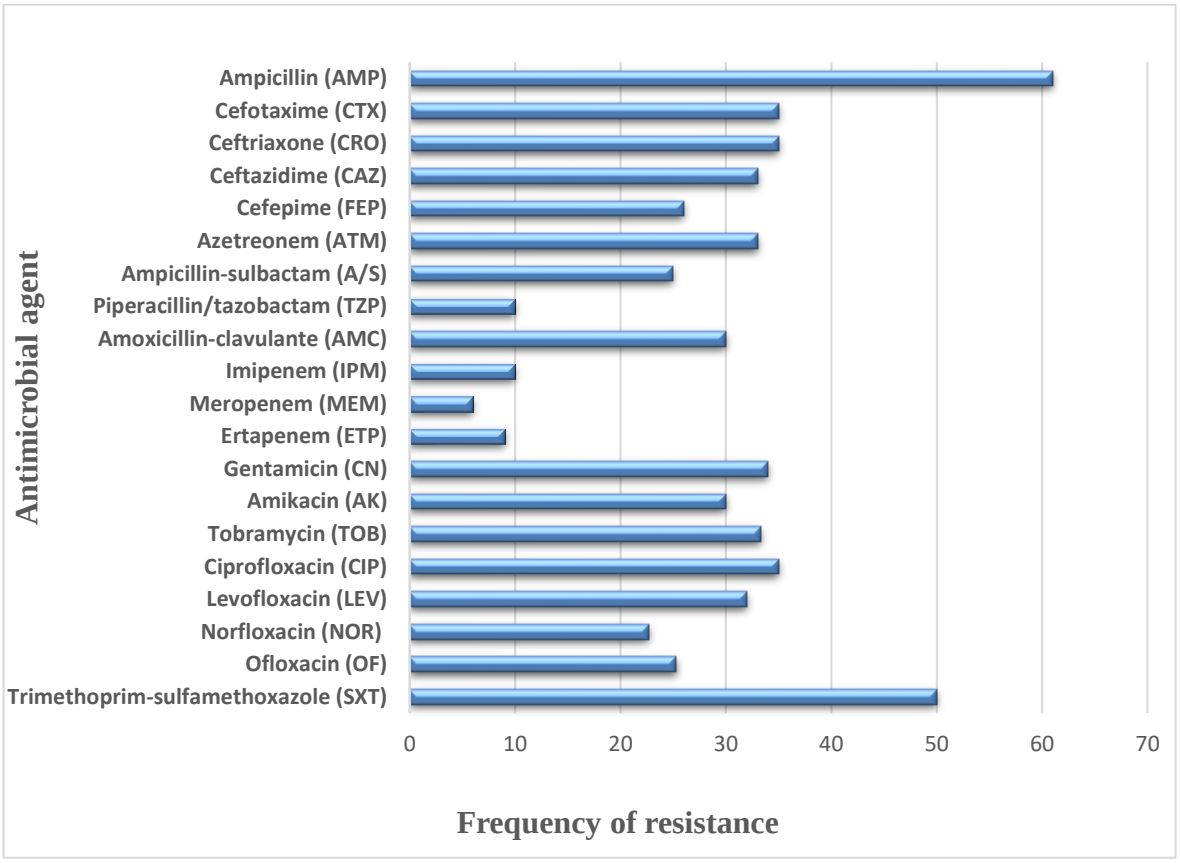


Figure 2. Ethidium bromide-stained agarose gel electrophoresis for detection of integron amplicon products. A) class 1 integron amplicon products (565 bp) represented by lanes 1, 2, 4, 5, 6, 7, 8 and 9. Lane 3 represents the DNA ladder (100 bp), while lane 10 represents the negative control. B) class 2 integron amplicon products (403 bp) represented by lanes 1, 2, 4, 6, 8 and 9. Lane 3 represents the DNA ladder (100 bp), while lane 10 represents the negative control. C) class 3 integron amplicon products (717 bp) represented by lanes 2, 4, 5, 6, 7 and 9. Lane 3 represents the DNA ladder (100 bp), while lane 1 represents the negative control.

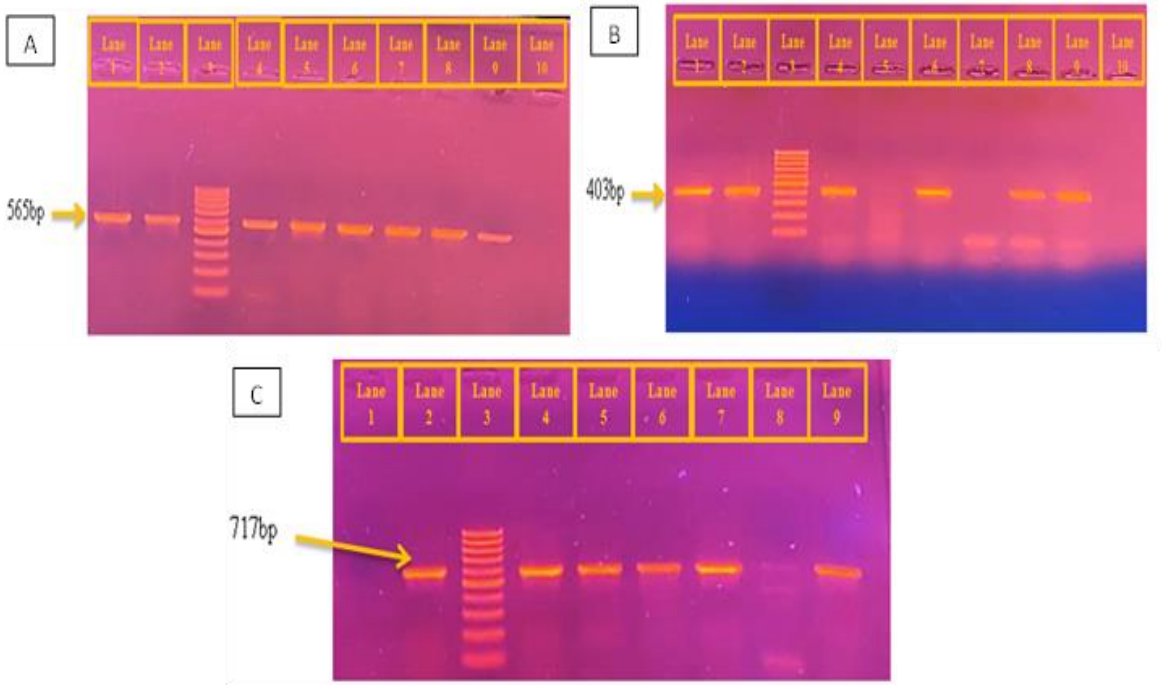
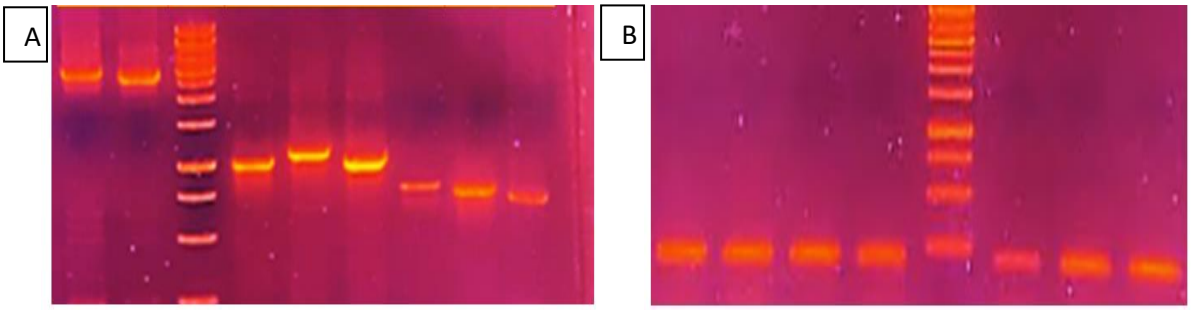


Figure 3. Ethidium bromide-stained agarose gel electrophoresis for detection of: A) IntI 1 IVRS amplicon products. Lane 3 represents the DNA ladder (1000 bp). Lane 1 and 2 show band size 2500bp, while lanes 4, 5 and 6 showed bands around 1000 bp in s size. Lanes 7, 8 and 9 showed bands of 750 bp in size. B) IntI 2 IVRS amplicon products. Lane 5 represents the DNA ladder (1000 bp). Lane 1, 2, 3, 4, 6, 7 and 8 show band size 250 bp.



Discussion

The emergence and dissemination of (MDR) *P. mirabilis* isolates is becoming increasingly reported [7-9]. There are different genetic mechanisms associated with the multidrug resistance phenotype of *P. mirabilis*, one of these mechanisms is the acquisition of antimicrobial resistance genes from other resistant pathogens through mobile genetic elements including integrons posing a serious threat that restricts the available therapeutic options [14]. This study was designed to determine the prevalence of integrons, their gene cassette contents, and its relationship with antibiotic resistance among *P. mirabilis* clinical isolates. Most of *P. mirabilis* strains were isolated from urine samples which is in accordance with different studies which stated that *P. mirabilis* strains are responsible mainly for complicated urinary tract infections [1,5,18]. In contrast, **Pal et al.** [30] and **Alabi et al.** [31] reported that most of their *P. mirabilis* were from pus. A total of 34% of our isolates were MDR, while 9% were XDR. The percentage of MDR in *P. mirabilis* reported varied among studies. Although a higher percentage of MDR was stated in **Mirzaei et al.** [21] and **Alabi et al.** [31] (82.5% and 55.6% respectively), **Oliveira et al.** [32] and **Mirzaei et al.** [33] reported a lower multidrug resistance percentage of 7.1% and 14.5% respectively. These discrepancies might be related to variances in in the sources of samples and regional differences in the abundance of bacterial strains and variable standards and controls for antibiotic prescription and use.

The majority of the examined isolates were resistant to ampicillin (61%) followed by trimethoprim-sulfamethoxazole (50%). Similarly, a previous recent study in Egypt indicated 64.5% resistance to ampicillin, but a lower resistance rate (19.4%) to trimethoprim-sulfamethoxazole [11]. Our results are in accordance with previous studies from different parts of the world that also reported high resistance rate to these two antibiotics [5, 31-33]. This might be explained by the extensive misuse of these drugs in the treatment of UTIs. On the contrary, the highest sensitivity of our isolates was against carbapenems as only 6%, 9%, and 10% of isolates were resistant to meropenem, ertapenem, and imipenem respectively. These results are comparable to other studies that reported low resistance rate to carbapenems [30,33]. However, other studies have reported a higher resistance rate to carbapenems like **Tabatabaei et al.** [34]. A low resistance (10%) was revealed among isolates against piperacillin-tazobactam, this rate was somewhat higher than reported in other research as in **Xiao et al.** [35] **Pal et al.** [30] and **Oliveira et al.** [32] as none of their *P. mirabilis* isolates were resistant to piperacillin-tazobactam. Resistance to aminoglycosides (gentamicin and amikacin) was observed in 34% and 30% respectively. This represents a higher rate of resistance as compared to previous studies that revealed a more susceptibility of their *P. mirabilis* isolates to aminoglycosides [5,31,32].

Integrons have been identified by their strong link with the spread of antibiotic resistance genes and the emergence of resistance phenotypes within microbial populations. In Egypt there are limited reports about the prevalence of integrons in Gram-negative bacteria, however data about integrons prevalence among *P. mirabilis* isolates is scare [23,36,37].

In the current study, class 1 integron was the dominant type detected in 80% of isolates, and

among these 80 integron-positive isolates, gene cassettes were amplified in 66 isolates (82.5%), with 8 different gene cassettes in different combinations and the most prevalent gene cassette array detected in 18 isolates was encoding for aminoglycoside and trimethoprim resistance (*AadA, dfrA17*). These results are comparable to the results reported by **Wei et al.** [19] which indicated that 63% of *Proteus* isolates harbored class 1 integrons, and variable regions were successfully amplified in 72.9% of them with 8 different gene cassette arrays also mainly encoding for aminoglycoside and trimethoprim resistance. In a different study, **Lu W et al.** [20] reported predominance of class 1 integrons in 46% *P. mirabilis* isolates with variable regions successfully amplified in 94.2% of them including six different gene cassette arrays. From Egypt in **Malek et al.** [23] study from 6 *P. mirabilis* isolates, class 1 integrons was detected only in 3 isolates with *ereA2-dfrA5* gene cassette detected in 2 of them.

Many studies had reported class 2 integrons as the second most ubiquitous class of integrons among clinical bacteria [19,20]. In the current study, class 2 integrons were detected in 66% of *P. mirabilis* isolates, and the variable regions were successfully amplified in 89.5% (59/66) of them revealing four types of gene cassette encoding mainly for beta-lactamase and spiramycin resistance. The high proportion of *P. mirabilis*-carrying class 2 integrons in this study was consistent with other reports; like **Wei et al.** [19] and **Lu et al.** [20] who revealed class 2 integrons in 66% and 40.7% of *P. mirabilis* isolates respectively and the variable regions were successfully amplified in 100% and 81.9% of them respectively revealing different gene cassette arrays. No amplification products of the variable region were detected in 17.5% and 10.6% of integron 1 and 2 positive isolates respectively. This might be attributed to variation in the primer binding site or the extensive size of gene cassette.

The identification of class 3 integrons has been limited within a few microorganisms with a low rate. Nevertheless, in many previous studies class 3 integrons could not be detected among *P. mirabilis* strains like in **Lu et al.** [20]. among the 150 strains of *P. mirabilis*, class 3 integrons were not detected. Additionally, **Alabi et al.** [31] and **Mirzaei et al.** [33] reported that none of their *P. mirabilis* isolates harbored *IntI 3*. It worth mentioning that, in the current study, class 3 integrons were successfully detected in 21% of the *P. mirabilis* isolates. To the best of our knowledge, this is the first report from Egypt detecting *P. mirabilis* clinical isolates carrying class 3 integrase genes suggesting possible horizontal transfer from other bacterial species. Unfortunately, integron 3 embedded gene cassette could not be further investigated due to the lack of data for characterization of integron 3 and therefore, its variable region was not amplified.

Concerning the coexistence of more than one type of integrons, 67.7% of integron positive isolates in the current study harbored more than one class of integrons. Both integrons 1 and 2 were found in 47.3% of integron positive isolates and 11.8% of them carried the three classes of integrons simultaneously. These results agree with **Wei et al.** [19]. as in 56.2% of their isolates class 1 and 2 integrons existed together. On the other hand, it was a higher percent as compared to results reported by **Lu et al.** [20] and **Mirzaei et al.** [33] who found only 26% and 15% of their *P. mirabilis* isolates respectively harbored both 1 and 2 integrons together. This co-occurrence of multiple integrons types, implying that they are placed at various locations on the isolate's chromosome and plasmids.

Comparing the results of integrons with antibiotic resistance pattern, it appears that resistance to antibiotics was significantly higher among *P. mirabilis* isolates carrying different integrons. Presence of mainly class 1 integrons and, to a lesser extent, class 2 integrons were significantly associated to antibiotics resistance. Moreover, 97.1% and 100 % respectively of MDR and XDR isolates harbored integrons either as one class or combined especially class 1 and 2 integrons. In agreement with our results, **Alabi et al.** [31] reported that all their MDR *P. mirabilis* isolates carried integrase genes, either *IntI 1*, *IntI 2*, or both. Also, in **Malek et al.** [23] presence of class 1 integrons was significantly associated with MDR in Enterobacteriaceae.

On the contrary class 3 integrons carriage lacked this significant association with resistance to different antimicrobial drugs. Additionally, the two isolates that harbored only *IntI 3* were non-MDR. These results imply that the role of class 3 integrons in spread of antimicrobial resistance is less than class 1 and 2 integrons and its role in dissemination of resistance genes needs to be further investigated.

Conclusions

In conclusion, this study revealed that different classes of integrons carrying antibiotic resistance gene cassettes are widely disseminated among *P mirabilis* clinical isolates, which poses a significant hazard for MDR propagation. Also, for the first time in Egypt, we report the discovery of class 3 integrons in *P. mirabilis* strains which establishes a baseline of data that may be used for future monitoring and assessment of the role of this integron class in *P. mirabilis* antibiotic resistance. This growing dominance of resistant strains emphasizes the significance of restricting antibiotic usage to be based only on susceptibility testing and implementing effective infection control strategies to limit the spread of MDR microorganisms.

Ethical consideration

This study was approved by the Ethics Committee of the medical research institute, Alexandria University. Bacterial isolates were collected from clinical samples send to the microbiology laboratory for routine culture and sensitivity without the requirement for any patient-related data and complete subject anonymity therefore, no informed patient consent was required.

Conflict of interest

All authors declare no conflict of interests.

Financial disclosure

Non to disclose.

Authors' contributions

This work was carried out in collaboration between all authors. Elsheredy A and El Sherbini E performed study conception and design. Elsheredy A, Faisal E, and Attia N managed all practical experiments. Elsheredy A and Faisal E managed Analysis of data and wrote the manuscript. El Sherbini E and Attia N performed critical revision of the manuscript. All authors read and approved the final manuscript.

References

1- **Schaffer JN, Pearson MM.** Proteus mirabilis and urinary tract infections. Microbiol Spectr 2015;3(5): 10.1128.

2- **O'Hara CM, Brenner FW, Miller JM.** Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clin Microbiol Rev 2000;13(4):534-546.

3- **Armbruster CE, Mobley HLT, Pearson MM.** Pathogenesis of Proteus mirabilis Infection. EcoSal Plus 2018;8(1): 10.1128/ecosalplus.ESP-0009-2017.

4- **Wasfi R, Hamed SM, Amer MA, Fahmy LI.** Proteus mirabilis Biofilm: Development and Therapeutic Strategies. Front Cell Infect Microbiol 2020; 10:414.

5- **Hussein EI, Al-Batayneh K, Masadeh MM, Dahadhah F, Al Zoubi MS, Aljabali AA, et al.** Assessment of Pathogenic Potential, Virulent Genes Profile, and Antibiotic Susceptibility of Proteus mirabilis from Urinary Tract Infection. Int J Microbiol 2020; 2020:1231807.

6- **Stock I.** Natural antibiotic susceptibility of Proteus spp., with special reference to P. mirabilis and P. penneri strains. J Chemother 2003;15(1):12-26.

7- **Ferri M, Ranucci E, Romagnoli P, Giaccone V.** Antimicrobial resistance: a global emerging threat to public health systems. Crit Rev Food Sci Nutr 2017; 57:2857–2876.

8- **Ventola CL.** The antibiotic resistance crisis: part 1: causes and threats. P T 2015;40(4):277-283.

9- **Khalifa HO, Soliman AM, Ahmed AM, Shimamoto T, Nariya H, Matsumoto T, et al.** High Prevalence of Antimicrobial Resistance in Gram-Negative Bacteria Isolated from Clinical Settings in Egypt: Recalling for Judicious Use of Conventional Antimicrobials in Developing Nations. Microb Drug Resist 2019;25(3):371-385.

10- **Helmy M, Wasfi R.** Phenotypic and molecular characterization of plasmid mediate AmpC -lactamases among Escherichia coli, Klebsiella spp., and Proteus mirabilis isolated from urinary tract infections in Egyptian hospitals. J Biomed Biotechnol 2014; 4:12–20

11- **Elekhnawy E, Sonbol F, Abdelaziz A, Elbanna T.** An investigation of the impact of

triclosan adaptation on Proteus mirabilis clinical isolates from an Egyptian university hospital. Braz J Microbiol 2021;52(2):927-937.

12-**Girlich D, Bonnin RA, Dortet L, Naas T.** Genetics of Acquired Antibiotic Resistance Genes in Proteus spp. Front Microbiol. 2020; 11:256.

13-**Leverstein-van Hall MA, M Blok HE, T Donders AR, Paauw A, Fluit AC, Verhoef J.** Multidrug resistance among Enterobacteriaceae is strongly associated with the presence of integrons and is independent of species or isolate origin. J Infect Dis 2003;187(2):251-259.

14-**Rowe-Magnus DA, Mazel D.** The role of integrons in antibiotic resistance gene capture. Int J Med Microbiol 2002;292(2):115-125.

15-**Gillings MR.** Integrons: past, present, and future. Microbiol Mol Biol Rev 2014;78(2):257-277.

16-**Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, et al.** Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob 2015; 14:45.

17-**Kaushik M, Kumar S, Kapoor RK, Virdi JS, Gulati P.** Integrons in Enterobacteriaceae: diversity, distribution and epidemiology. Int J Antimicrob Agents 2018;51(2):167-176.

18-**Chen CM, Lai CH, Wu HJ, Wu LT.** Genetic characteristic of class 1 integrons in proteus mirabilis isolates from urine samples. Biomedicine (Taipei) 2017;7(2):9.

19-**Wei Q, Hu Q, Li S, Lu H, Chen G, Shen B, et al.** A novel functional class 2 integron in clinical Proteus mirabilis isolates. J Antimicrob Chemother 2014;69(4):973-976.

20-**Lu W, Qiu Q, Chen K, Zhao R, Li Q, Wu Q.** Distribution and Molecular Characterization of Functional Class 2 Integrons in Clinical Proteus mirabilis Isolates. Infect Drug Resist 2022; 15:465-474.

21-**Mirzaei A, Nasr Esfahani B, Raz A, Ghanadian M, Moghim S**. From the Urinary Catheter to the Prevalence of Three Classes of Integrons, β-Lactamase Genes, and Differences in Antimicrobial Susceptibility of Proteus mirabilis and Clonal Relatedness with Rep-PCR. Biomed Res Int 2021;2021:9952769.

22-**Mokracka J, Gruszczyńska B, Kaznowski A.** Integrons, β-lactamase and qnr genes in multidrug resistant clinical isolates of Proteus mirabilis and P. vulgaris. APMIS 2012;120(12):950-958.

23-**Malek MM, Amer FA, Allam AA, El-Sokkary RH, Gheith T, Arafa MA**. Occurrence of classes I and II integrons in Enterobacteriaceae collected from Zagazig University Hospitals, Egypt. Front Microbiol 2015;6:601.

24-**Xu L, Deng S, Wen W, Tang Y, Chen L, Li Y, et al.** Molecular typing, and integron and associated gene cassette analyses in Acinetobacter baumannii strains isolated from clinical samples. Exp Ther Med 2020;20(3):1943-1952.

25-**Clinical and Laboratory Standards Institute (CLSI)**. Performance standards for antimicrobial susceptibility testing, (30th.). CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.

26-**European Centre for Disease Prevention and Control (ECDC):** 2017. Antimicrobial Resistance Surveillance in Europe 2014. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). In.: ECDC Stockholm, Sweden.

27-**Ahmed OB, Dablool AS.** Quality improvement of DNA extracted by boiling

method in Gram negative bacteria. Int. J. Bioassays 2017; 6: 5347–9.

28-Su J, Shi L, Yang L, Xiao Z, Li X, Yamasaki S. Analysis of integrons in clinical isolates of Escherichia coli in China during the last six years. FEMS Microbiol Lett 2006;254(1):75-80.

29-Zhang H, Shi L, Li L, Guo S, Zhang X, Yamasaki S, et al. Identification and characterization of class 1 integron resistance gene cassettes among Salmonella strains isolated from healthy humans in China. Microbiol Immunol 2004;48(9):639-645.

30-Pal N, Hooja S, Sharma R, Maheshwari RK. Phenotypic detection and antibiogram of β-lactamase-producing proteus species in a tertiary care hospital, India. Ann Med Health Sci Res 2016;6:267-73.

31-Alabi OS, Mendonça N, Adeleke OE, da Silva GJ. Molecular screening of antibiotic-resistant determinants among multidrug-resistant clinical isolates of Proteus mirabilis from SouthWest Nigeria. Afr Health Sci 2017;17(2):356-365.

32-Danilo de Oliveira W, Lopes Barboza MG, Faustino G, Yamanaka Inagaki WT, Sanches MS, Takayama Kobayashi RK, et al. Virulence, resistance and clonality of Proteus mirabilis isolated from patients with community-acquired urinary tract infection (CA-UTI) in Brazil. Microb Pathog 2021;152:104642.

33-Mirzaei A, Habibi M, Bouzari S, Asadi Karam MR. Characterization of Antibiotic-Susceptibility Patterns, Virulence Factor Profiles and Clonal Relatedness in Proteus mirabilis Isolates from Patients with Urinary Tract Infection in Iran. Infect Drug Resist 2019;12:3967-3979.

34-Tabatabaei A, Ahmadi K, Shabestari AN, Khosravi N, Badamchi A. Virulence genes and antimicrobial resistance pattern in Proteus mirabilis strains isolated from patients attended with urinary infections to Tertiary Hospitals, in Iran. African health sciences 2021;21(4):1677-1684.

35-Xiao L, Wang X, Kong N, Cao M, Zhang L, Wei Q, Liu W. Polymorphisms of Gene Cassette Promoters of the Class 1 Integron in Clinical Proteus Isolates. Front Microbiol 2019;10:790.

36-Abdel-Rhman SH, Elbargisy RM, Rizk DE. Characterization of Integrons and Quinolone Resistance in Clinical Escherichia coli Isolates in Mansoura City, Egypt. Int J Microbiol 2021;2021:6468942.

37-Rizk DE, El-Mahdy AM. Emergence of class 1 to 3 integrons among members of Enterobacteriaceae in Egypt. Microb Pathog 2017;112:50-56.

Elsheredy A, Faisal E, El Sherbini E, Attia N. Coexistence of integrons class 1 and 2 with emergence of class 3 among *Proteus mirabilis* clinical isolates from Alexandria, Egypt. Microbes Infect Dis 2023; 4(1): 182-193.