Prevalence and characterization of plasmid-mediated quinolone resistance genes in *Proteus* species isolated from different patients

Dalia G. Ali[1]; Gamal F. M. Gad[1]; Osman A. O. Ismail[2]; Hala R. Ahmed[1*]; Reham A. Ibrahem[1]

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Minia University, Minia, 61519, Egypt;
[2]Department of General Surgery, Faculty of Medicine, Minia University, Minia, 61519, Egypt

[*]Corresponding author E-mail: halaradyahmed@yahoo.com

Abstract

Proteus spp. are widely distributed opportunistic pathogens that can cause various human infections. A total of 361 clinical specimens were obtained from patients who were attending to different hospitals in El-Minia governorate, Egypt. Approximately 23 % of the samples belong to *Proteus* spp. isolates which were obtained from various clinical sources. After biochemical identification, 42.1 % of isolates were found to belong to *Proteus vulgaris* and 57.8 % to *P. mirabilis*. The urine samples collected from catheterized patients represented 32.6 % of all the clinical specimens, and the majority of the recorded isolates were *Proteus* spp. The antibacterial sensitivity of the *Proteus* spp. was examined using 16 different antibiotics from various families. The most effective antibiotics were Amikacin; Levofloxacin, and Meropenem, recording 68.6 %, 66.2 %, and 62.2 % of the isolates sensitivity to each of these antibiotics, respectively. Using the *ureR*-based PCR, 48 % of the isolates were identified as *P. mirabilis*. Moreover, the *Qnr* genes (*i.e.*, *qnrA*, *qnrB*, *qnrS*, *qnrD*, and *qnrC*) and the *aac (6')-Ib-cr* gene had been identified in 40 % of *P. mirabilis* isolates. The aims of the study were to investigate the prevalence of *Proteus* spp. in El-Minia, Egypt; determine the antibacterial susceptibility pattern of these isolates, and characterize the PMQR genes in *Proteus* spp. Quinolone resistance in *P. mirabilis* isolates might have been brought on by mechanisms other than *qnr* and *aac (6')-Ib* genes. Finally, since *Proteus* spp. are widespread in the environment; healthcare facilities must uphold stringent sanitation standards to reduce the incidence of the nosocomial infections.

Keywords: *Proteus* spp., Quinolone, PMQR, PCR

1. Introduction

In 1885, Hauser identified the genus *Proteus* for the first time (Sadiq and Abd Alhadee, 2009). *Proteus* spp. are Gram-negative rods that are members of the *Enterobacteriaceae* family (Knirel *et al*., 2011). These species are parts of the humans and animals' intestinal tract normal bacterial flora, and due to fecal pollution; these species are prevalent in the soil and water (O'Hara *et al*., 2000).

Currently, the identified species of the genus *Proteus*, include *P. mirabilis*; *P. vulgaris*, *P. penneri*, *P. cibarius*, *P. terrae*, *P. hauseri*, and three unnamed genospecies 4, 5, and 6 (Girlich *et al*., 2020). All species of this genus; with the exception of *P. cibarius* and *P. terrae*, have been isolated from clinical specimens sampled from humans (Hamilton *et al*., 2018). However, *P. mirabilis* and *P. vulgaris* account for the vast majority of the clinical isolates (Ioannou and Vougiouklakis, 2020).

After *Escherichia coli* and *Klebsiella pneumonia*; *P. mirabilis* is the third most prevalent cause of urinary tract infections (UTI) and complicating UTIs in those patients undergoing long-term catheterization (Filipiak *et al*., 2020). It can also cause infections of the eye; ear, nose, and skin, as well as infections of the wounds; respiratory system, and osteomyelitis (Schaffer and Pearson, 2017).

Proteus' infectious nature is typically correlated with their antibiotic resistance, which helps their survival in various healthcare facilities. *Proteus* has a number of virulence features, including the ability to adhere to surfaces; penetration into the host's body, and dissemination throughout the body. Swimming; swarming, and twitching are three kinds of movement recognized in the genus Proteus (Hola *et al*., 2012). The swarming phenomenon is the most characteristic feature that distinguishes Proteus rods from the other members of its family (Rózalski *et al*., 1997). It has

many other virulence factors, including adhesion by five common fimbriae type; flagella, toxins, several enzymes [*i.e*., hemolysin, *Proteus* toxic agglutinin (Pta), and urease], quorum sensing, and immune evasion (Hayder *et al*., 2020). The ability of *Proteus* to form a biofilm is considered as an important virulence factor, which helps this genus in its survival and antibacterial resistance (El-Kazzaz, 2021). *UreR* is the only known regulatory gene observed in *P. mirabilis*, which can only be detected in urea-inducible gene clusters (Zhang *et al*., 2013).

The resistance of *P. mirabilis* to the routinely used treatments is increasing by time (Allawi and Motaweq, 2019). Two mechanisms of quinolone resistance can be found: Chromosomal-mediated resistance and plasmid-mediated resistance. The chromosomal-mediated resistance includes mutations in the quinolones' primary target molecules "Quinolone resistance determining region (*QRDR*)" in DNA gyrase and topoisomerase IV. The *GyrA* and *GyrB* encode for the tetrameric enzyme DNA gyrase, which is composed of two subunits. The *ParC* and *ParE* encode for the topoisomerase IV enzyme also (Pathirana *et al*., 2018).

Three mechanisms are involved in the plasmid-mediated quinolone resistance (*PMQR*): *Qnr* genes, which include the *qnrA*; *qnrB*, *qnrC*, *qnrD*, and *qnrS* genes that mediate target protection, by interacting with DNA gyrase and topoisomerase IV. Moreover, they confer resistance to the quinolones by inhibiting quinolone entry into the enzyme-DNA cleavage complexes (Mirzaei *et al*., 2019; Yassine *et al*., 2019). The second mechanism is mediated by a type of an aminoglycoside-modifying enzyme *aac(6')Ib*; called *aac(6')Ib-cr*, which acts by inactivating the fluoroquinolones *via* an unprotected amino nitrogen on the piperazine ring. Finally, *PMQR* resistance that is mediated through plasmid-mediated quinolone efflux

pumps, such as those encoded by the *QepA* and *OqxAB* genes (Vila *et al*., 2011).

Because of the significance of *Proteus* species as opportunistic pathogens that can cause a range of human diseases, the objectives of this study were isolation and detection of *Proteus* spp. from different clinical sources; molecular characterization of *P. mirabili*s using polymerase chain reaction (PCR) by detection of the urease enzyme gene; *ureR*, which is regarded as a diagnostic feature of these bacteria, and studying the effect of different antibacterial agents against these tested *Proteus* isolates.

2. Materials and methods

2.1. Bacterial isolation

From November 2017 to July 2018; approximately 361 clinical urine samples were collected from patients undergoing treatments at El-Minia governorate hospitals, including El-Minia General Hospital; El-Minia University Main Hospital, and El-Minia University Hospital for Kidney and Urology. The specimens were collected from urinary tract infection patients (70 specimens); catheterized patients (49 specimen), otitis media (78 specimen), wound and burn infections (153 specimens), and swabs from sore throat (11 specimens). For initial bacterial isolation and diagnosis, all specimens were cultured individually on different culture media, such as Macconkey agar. Subsequent identification was based on the cultural features and performing different biochemical assays, in reference to Kamel and Al-Yasseen, (2009).

2.2. Identification of *Proteus* isolates

Proteus spp. had been identified using different biochemical assays, such as Indole test. The bacterial isolates were inoculated individually in Sulfide-indole-motility (SIM) medium, incubated for 24-48 h at 37 °C, and then 0.5 ml of Kovac's reagent was added. The tubes were gently shaken and allowed to stand for a few minutes. The formation of a cherry red ring layer on the medium's surface indicated that the

isolate belong to *P. vulgaris*, whereas *P. mirabilis* isolates yielded negative results (Ahmed, 2015).

2.3. Antibacterial susceptibility assay

According to the Clinical and Laboratory Standard Institute (CLSI. 2018), approximately 16 different antibiotics drugs that were widely found in the Egyptian markets were employed in the antibacterial susceptibility testing for all the Proteus isolates using the Kirby-Baurer disc diffusion method (Bauer, 1966). These used antibiotics included ampicillin (25 μg); amoxicillin / clavulanic (30 μg), cefepime (10μg), cefoxitin (30 μg), ceftriaxone (30 μg), gentamicin (30 μg), amikacin (10μg), ciprofloxacin (10 μg), levofloxacin (5 μg), imipenem (10 μg), meropenem (10 μg), azetronam (30 μg), trimethoprim/sulphamethxazole (25 μg), and tetracycline (30 μg). Only the urine recovered isolates were tested for nitrofurantoin (300 μg) resistance. Meanwhile, all isolates were tested for chloramphenicol (30 μg) resistance except for the urine isolates. The diameter of each antibacterial disc's inhibition zone (mm) was measured using a calibrated ruler. Results were interpreted according to the tables of performance standards for antimicrobial discs susceptibility tests that were outlined by CLSI. (Mirzaei *et al*., 2019).

2.4. Molecular studies

Identification of *P. mirabilis* was carried out by amplification of *ureR* gene, and characterization of the PMQR genes in the Proteus spp. was performed using PCR-based technique.

2.4.1. Bacterial DNA extraction

The bacterial DNA was extracted using the boiling procedure. A loopful of bacteria that was cultivated on nutrient agar (NA) was inoculated into 500 μl of sterile dist. water, and mixed for 30 s using a vortex. After 10 min. of boiling at 100°C, the resulting suspension was chilled for an additional 10 min, and centrifuged (8000 ×g) for 5 min. The supernatant was

collected and used as a template DNA (Cattoir *et al*., 2007).

2.4.2. Detection of *P. mirabilis* isolates using PCR technique

In this study, in order to identify *P. mirabilis* as being the most recovered *Proteus* isolates, *ureR*-based PCR was used, which amplifies a specific region of the *ureR* gene found in *P. mirabilis*. The forward and reverse primers; *ureRF1*: 5′-GGTGAGATTTGTATTAATGG- 3′, and *ureRR1*: 5′-ATAATCTGGAAGATGACGAG- 3′; respectively, were used to amplify the 225 bp DNA product of *P. mirabilis*. The employed reaction conditions were as following; 4 min. of denaturation at 94 °C, 40 sec of denaturation at 94 °C for 30 cycles, 1 min. of annealing at 58 °C, 20 sec of extension at 72 °C, and 10 min. of extension at 72 °C. To determine the size of the bacterial DNA, the PCR products were

electrophoresed using a 1% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light (Zhang *et al*., 2013).

2.4.3. Detection of the PMQR genes by multiplex PCR assay

To detect the *QNR* genes (*i.e*., *qnrA*; *qnrB*, *qnrS*, *qnrD*, and *qnrC*) and *aac (6')-Ib-cr* gene, which confer Proteus' resistance to the quinolones, multiplex PCR based technique was used. More than one target sequence was amplified using more than one pair of primers in a single reaction tube, which was carried out employing the primers provided in Table (1). The used processing conditions were; Initial denaturation for 2 min. at 95 °C, followed by 35 cycles at 95 °C for 50 sec; at 53 °C for 40 sec, and at 72 °C for 80 sec, and a final extension at 72 °C for 5 min. (Dasgupta *et al*., 2015; Park *et al*., 2006).

Table 1: Oligonucleotide primers used in the multiplex PCR assay

Gene	Primer	Sequence (5'-3')	Size (bp)	References
qnrA	QnrAm- F	5′-AGAGGATTTCTCACGCCAGG- 3′	580	(Pathirana *et al*., 2018)
	QnrAm- R	5′-TGCCAGGCACAGATCTTGAC- 3′		
qnrS	Qnrsm- F	5′-GCAAGTTCATTGAACAGGGT- 3′	428	
	Qnrsm- R	5′-TCTAAACCGTCGAGTTCGGCG- 3′		
qnrB	QnrB- F	5′-GATCGTGAAAGCCAGAAAGG- 3′	496	
	QnrB- R	5′-ACGATGCCTGGTAGTTGTCC- 3′		
qnrC	qnrC- F	5′-GGGTTGTACATTTATTGAATC- 3′	447	
	qnrC- R	5′-TCCACTTTACGAGGTTCT- 3′		
qnrD	qnrD- F	5′-CGAGATCAATTTACGGGGAATA- 3′	582	
	qnrD- R	5′-AACAAGCTGAAGCGCCTG- 3′		
aac(6')Ib	aac(6′)-Ib- F	5′-TTGCGATGCTCTATGAGTGGCTA- 3′	482	(Park *et al*., 2006)
	aac(6′)-Ib-R	5′-CTCGAATGCCTGGCGTGTTT- 3′		

1969

2.5. Statistical analysis

The analysis of data was carried out using the IBM SPSS version 25 statistical package software. Data were expressed as number and percentage for qualitative data. Analyses were done to compare categorical variables using Fisher's exact test. Kappa test was used to assess the agreement between two variables. *p*-value less than 0.05 was considered statistically significant.

3. Results
3.1. Bacterial isolation

Approximately 83 isolates were found to be belonging to *Proteus* spp. as a result of growth of these isolates on MacConkey agar and recording positive results in the different biochemical assays. Out of the 361 samples collected from patients with various infections, 16 isolates were isolated from catheterized patients' urine samples; 18 isolates from urine samples collected from hospitalized patients with urinary tract infections, 27 isolates from infected wounds specimens, and 22 isolates were recovered from otitis media, as illustrated in Table (2).

3.2. Identification of *Proteus* isolates

Using an Indole test, about 48 *P. mirabilis* isolates and 35 *P. vulgaris* isolates were identified. The *P. mirabilis* isolates were recovered from urine samples collected from urinary tract infected hospitalized patients; urine samples from catheterized patients (9), infected wounds (12), and from otitis media (16). On the other hand, *P. vulgaris* isolates were obtained from urine samples from urinary tract infected hospitalized patients (7), urine samples from catheterized patients (7), infected wounds (15), and from otitis media(6), as presented in Table (3).

3.3. Detection of antibacterial susceptibility of the *Proteus* isolates

About sixteen different types of widely used antibiotics were tested for their susceptibility patterns against *Proteus* isolates using a disk diffusion assay, as shown in Table (4). Amikacin and levofloxacin, followed by meropenem expressed the highest antibacterial effectiveness against the *Proteus* isolates with recorded percentages of sensitivity of; 68.6 %, 66.2 %, and 62.2 %, respectively. However, the *Proteus* isolates displayed high resistance against cefoxitin (98.7 %); ampicillin (98.7 %), amoxicillin/ clavulanic (93.9 %), and trimethoprim/sulphamethxazole (90.3 %). On the other hand, the least levels of resistance were observed against ciprofloxacin (46.9 %); azetronam (46.9 %), imipenem (46.9 %), and gentamicin (42.1%).

3.4. Molecular studies

3.4.1. Confirmation of the identity of *P. mirabilis* isolates using PCR

Using *ureR*-based PCR method; 40/ 83 isolates were confirmed as *P. mirabilis* (48.0 %). The PCR product band was detected at 225 bp, as shown in Fig. (1).

3.4.2. PMQR characterization via multiplex PCR assay

Using multiplex PCR, about 16/ 40 (40 %) *Proteus* spp. were observed to harbor some of the *Qnr* genes and *aac(6')Ib* gene, as illustrated in Table (5). The *qnrA* gene was detected in 6 (15 %) of *P. mirabilis* isolates at 580 bp, as shown in Fig. (2). The *qnrB* gene was observed in 6 (15 %) isolates at 496 bp (Fig. 3). Meanwhile, four (10 %) isolates were recorded to harbor the *qnrD* gene that was detected at 582 bp (Fig. 4), and 2 (5%) isolates had the *qnrS* gene at 428 bp (Fig. 5). However, the *qnrC* gene could not be detected in any isolate. About 4 isolates (10 %) were recorded to have the *aac(6')Ib* gene, which was observed at 482 bp, as shown in Fig. (6).

Table 2: Prevalence of *Proteus* isolates recovered from different patients with different types of infections

Type of specimen	No. of collected specimens	No. of *Proteus* isolates	% of prevalence
Urinary tract infections	70	18	25.7
Catheterized patients	49	16	32.6
Wound infections	153	27	17.6
Otitis media	78	22	28.2
Throat	11	0	0
Total	361	83	22.9

Where; The percentage (%) of *Proteus* isolates were correlated to the number of samples collected from each type of infection

Table 3: Prevalence of *P. mirabilis* and *P. vulgaris* isolated from different patients in relation to type of infection

Types of infection	*P. mirabilis*		*P. vulgaris*	
	No.	% of prevalence	No.	% of prevalence
Urinary tract infections	11	61.1	7	38.8
Catheterized patients	9	56.2	7	43.7
Wound infections	12	44.4	15	55.5
Otitis media	16	72.7	6	27.2
Total	48	57.8	35	42.1

Where; The percentage (%) of *P. mirabilis* and *P. vulgaris* were correlated to the number of *Proteus* isolates recovered from each type of infection

Table 4: Antimicrobial susceptibility patterns of *Proteus* isolates

	Antibiotic	No. of sensitive *Proteus* isolates	%	No. of resistant *Proteus* isolates	%
1	Ampicillin	1	1.2	82	98.7
2	Amoxicillin/ Clavulinic acid	5	6	78	93.9
3	Gentamicin	48	57.8	35	42.1
4	Amikacin	57	68.6	26	31.3
5	Cefepime	27	32.5	56	67.4
6	Cefoxitin	1	1.2	82	98.7
7	Ceftriaxone	23	27.7	60	72.2
8	Ciprofloxacin	44	53	39	46.9
9	Levofloxacin	55	66.2	28	33.7
10	Imipenem	44	53	39	46.9

11	Meropenem	52	62.6	31	37.3
12	Trimethoprim/Sulphamethxazole	8	9.6	75	90.3
13	Azetronam	44	53	39	46.9
14	Tetracycline	13	16.6	70	84.3
15	Chloramphenicol for OM and IW isolates only (n=47)	5	10.6	42	89.3
16	Nitrofurantoin for UTI and CAT isolates only (n=34)	7	20.5	27	79.4

Where; The percentage (%) of resistant or sensitive *Proteus* isolates was correlated to the total number of recovered *Proteus* isolates. OM: Otitis media; WI: wound infection; UTI: urinary tract infection; CAI: catheter associated infection

Table 5: Prevalence of *Qnr* genes and *aac(6')Ib* gene in relation to the total number of PCR confirmed *P. mirabilis* isolates

qnr genes	No. of *P. mirabilis* isolates (n=40)	% of prevalence
qnrA gene	6	15
qnrB gene	6	15
qnrC gene	0	0
qnrD gene	4	10
qnrS gene	2	5
aac(6')Ib gene	4	10

Where; The percentage (%) of *P. mirabilis* isolates that had *qnr* and *aac(6')Ib* genes were correlated to the total number of confirmed *P. mirabilis* isolates
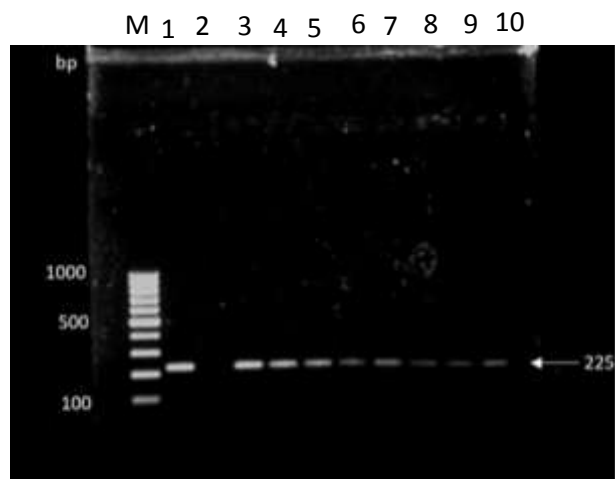

Fig. 1: PCR amplifications of *ureR* gene. The PCR products appeared at 225 bp. PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide, compared to 1 Kbp DNA ladder and visualized under ultraviolet light. Where; M: 1 Kbp DNA marker; 1,3,4,5,6,7,8,9: positive samples; 2,10: negative samples
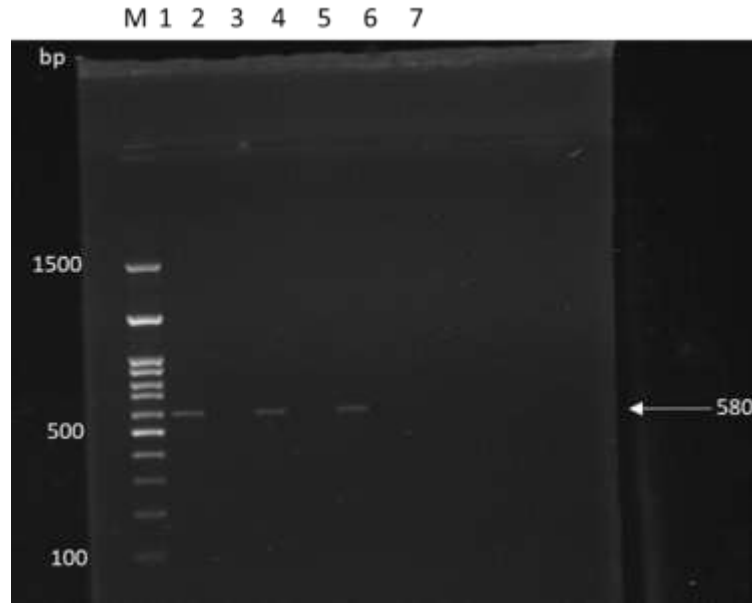
Fig. 2: PCR amplifications of the *qnrA* gene. The PCR products appeared at 580 bp. PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide, compared to 1.5 Kbp DNA ladder and visualized under ultraviolet light. Where: M: 1.5 Kbp DNA marker; 1,3,5: positive samples; 2,4,6,7: negative samples
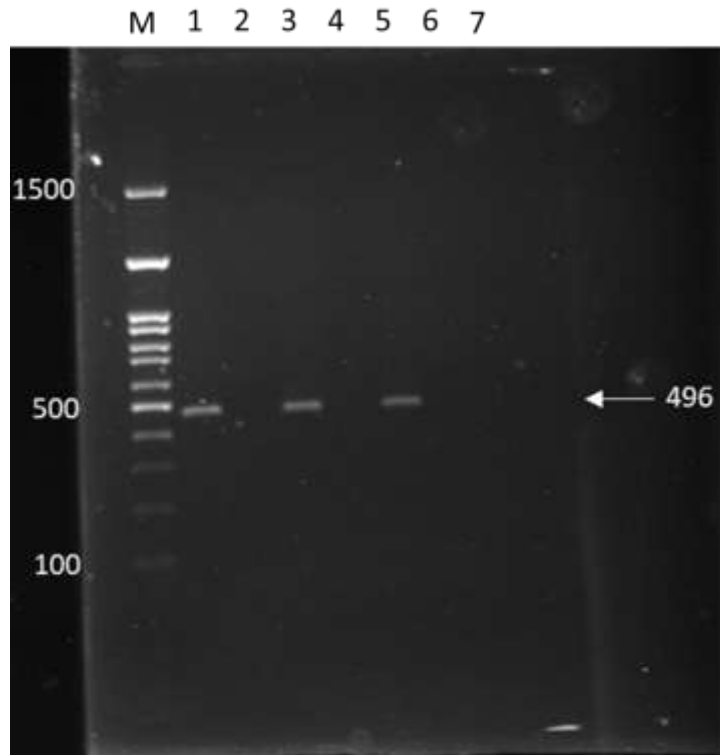

Fig. 3: PCR amplifications of the *qnrB* gene. The PCR products appeared at 496 bp. PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide, compared to 1.5 Kbp DNA ladder and visualized under ultraviolet light Where; M: 1.5 Kbp DNA marker; 1,3,5: positive samples; 2,4,6,7: negative samples
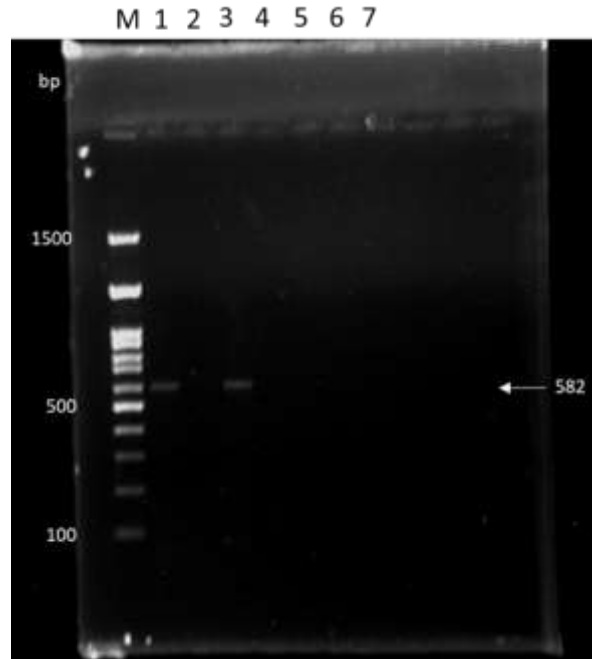
Fig. 4: PCR amplifications of *qnrD* gene. The PCR product appear at 582bp**.** PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide, compared to 1.5 Kbp DNA ladder and visualized under ultraviolet light Where: M: 1.5 Kbp DNA marker; 1,3: positive samples; 2,4,5,6,7: negative samples
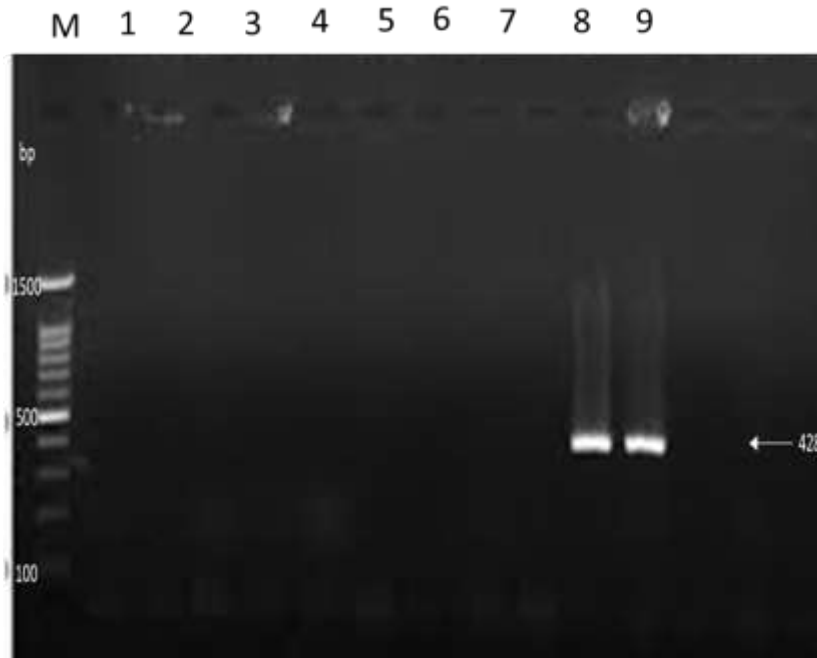

Fig. 5: PCR amplifications of *qnrS* gene. The PCR products appeared at 428 bp. PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide, compared to 1.5 Kbp DNA ladder and visualized under ultraviolet light. Where: M: 1.5 Kbp DNA marker; 8,9: positive samples; 1,2,3,4,5,6,7: negative samples
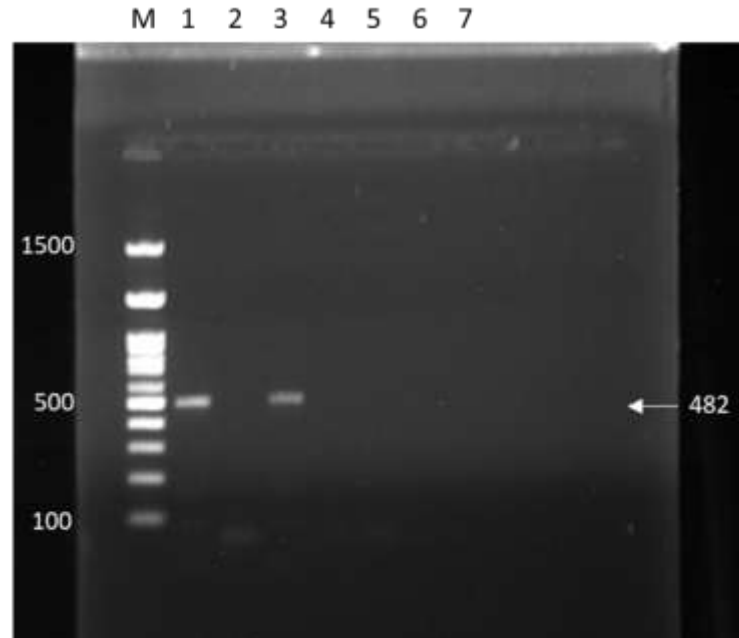
Fig. 6: PCR amplifications of *aac(6')Ib* gene. The PCR products appeared at 482 bp. PCR products were electrophoresed on a 1% agarose gel stained with ethidium bromide, compared to 1.5 Kbp DNA ladder and visualized under ultraviolet light Where: M: 1.5 Kbp DNA marker; 1,3: positive samples; 2,4,5,6,7: negative samples

3.5. Relationship between antibacterial sensitivity and the occurrence of *PMQR* genes

The relation between the antibacterial patterns of ciprofloxacin (10 μg), levofloxacin (5 μg), and the presence of *PMQR* genes and *aac(6')Ib* gene, is demonstrated in Table (6). The results showed that only 4/6 isolates that had *qnrA* gene were both ciprofloxacin and levofloxacin resistant, 4/6 isolates that bear the *qnrB* gene were ciprofloxacin resistant, and 2/6 were levofloxacin resistant. All isolates that expressed the *qnrD* gene were sensitive to both ciprofloxacin and levofloxacin; however, all isolates that harbor the *qnrS* gene were sensitive to both ciprofloxacin and levofloxacin. Meanwhile, all isolates that had *aac(6')Ib* gene were resistant to both of ciprofloxacin and levofloxacin. There was a significant correlation between the occurrence of *aac(6')Ib* gene and levofloxacin resistance (*p* value < 0.05).

4. Discussion

Overall, in both of the community and hospital environments; *Proteus* spp. are regarded as significant infectious pathogenic bacteria. Approximately 361 clinical samples were collected in this study to investigate the presence of *Proteus* spp. *Proteus* bacteria were detected in 23 % of the tested samples. This result is compatible with that observed by Parajuli *et al*., (2021), while a similar study reported by Bahashwan and El Shafey, (2013) revealed that only 3 % of all isolated bacteria were identified as *Proteus* spp.

The various kinds of virulence factors of *Proteus* bacteria have a significant role in its invasiveness and therefore the increased percentage of its isolates. In addition, the improper use of antibacterial agents increases the infections caused by *Proteus* spp., the use of infected urinary catheters and\or other

1975

Table 6: The relation between the antibacterial resistance against ciprofloxacin (10 μg) and levofloxacin (5 μg), and the presence of *PMQR* genes and *aac(6')Ib* gene

Genes	Presence	Ciprofloxacin		*p* value	Levofloxacin		*p* value
		Sensitive N=19	Resistant N=21		Sensitive N=23	Resistant N=17	
qnrA gene	-	17(89.5%)	17(81%)	0.664	21(91.3%)	13(76.5%)	0.373
	+	2(10.5%)	4(19%)		2(8.7%)	4(23.5%)	
qnrB gene	-	17(89.5%)	17(81%)	0.664	19(82.6%)	15(88.2%)	1
	+	2(10.5%)	4(19%)		4(17.4%)	2(11.8%)	
qnrC gene	-	19(100%)	21(100%)	1	23(100%)	17(100%)	1
	+	0(0%)	0(0%)		0(0%)	0(0%)	
qnrD gene	-	15(78.9%)	21(100%)	0.042[*]	19(82.6%)	17(100%)	0.123
	+	4(21.1%)	0(0%)		4(17.4%)	0(0%)	
qnrS gene	-	17(89.5%)	21(100%)	0.219	21(91.3%)	17(100%)	0.449
	+	2(10.5%)	0(0%)		2(8.7%)	0(0%)	
aac(6')Ib gene	-	19(100%)	17(81%)	0.108	23(100%)	13(76.5%)	0.026[*]
	+	0(0%)	4(19%)		0(0%)	4(23.5%)	
Number of +ve genes	None	11(57.9%)	13(61.9%)	0.677	13(56.5%)	11(64.7%)	0.183
	One	6(31.6%)	4(19%)		8(34.8%)	2(11.8%)	
	Two	2(10.5%)	4(19%)		2(8.7%)	4(23.5%)	

Where; * Represent the significant statistical differences that were detected using Fisher's exact test (*p* value < 0.05)

indwelling equipment in an unsanitary environment in some hospitals, may also have a role in this problem.

P. mirabilis and *P. vulgaris* were the two *Proteus* spp. isolated in the present study. *P. mirabilis* accounted for 57.8 % of the isolates while *P. vulgaris* accounted for only 42.1 %. These results are consistent with those presented by Ahmed, (2015) study, which reported that the rate of isolation for *P. mirabilis* was (66.6 %), while for *P. vulgaris* it was 33.3 %. Our study revealed that samples of urine collected from catheterized patients have the highest prevalence rate of *Proteus* spp. (32.6 %), followed by otitis media (28.2 %). In a previous study carried out in Baghdad at 2015, *Proteus* spp. isolated from UTI represented 60 %; those isolated from wounds accounted for 23.3%, and 16.6 % were isolated from burns (Ahmed, 2015). In contrast to this result, a study performed in Ghana at 2010 revealed that wound isolates were the most common (64.5 %), followed by ear swabs

(Patrick *et al*., 2010). Another study conducted in India reported that 80.2 % of the *Proteus* spp. were isolated from pus, while 8.9 % were recovered from urine (Pal *et al*., 2016).

The widespread antibacterial resistance of *Proteus* spp. isolated from various clinical samples has been reported in several previous studies reported by Kumburu *et al*., (2017); Allawi and Motaweq, (2019). In the current study, about 82 *Proteus* isolates (98.7 %) showed resistance to Ampicillin, where the widespread usage of antibiotics in the treatment of numerous infectious disorders may account for this result. Meanwhile, the combination of amoxicillin and clavulanic acid was effective for only 5 isolates, recording 92.7 % of resistance. Similar results were observed in several previous studies reported by Sanches *et al*., (2019); Thabit *et al*., (2020); Parajuli *et al*., (2021). In the current study, resistance of *Proteus* spp. to the other cephalosporins has been studied, where cefoxitin; ceftriaxone, and cefepime showed noticeable

1976

resistance by 98.7 %, 72.2 %, 67.4 %, respectively. A similar study conducted by Kumburu *et al*., (2017) reported that *Proteus* isolates had 53.6 % resistance to ceftriaxone. Meanwhile, at 2019 in Saudi Arabia, the recorded sensitivity pattern of *Proteus* isolates were 78.2 % , 21.8 %, 23.2 % for cefoxitin; ceftriaxone and cefepime, respectively (Bandy and Tantry, 2021). The resistance to carbapenem antibiotics (*i.e*., Imipenem and Meropenem) was also observed in this study, where meropenem was more effective than imipenem, as 52 *Proteus* isolates were sensitive to meropenem (62.6 %), while only 44 *Proteus* isolates were sensitive to imipenem (53 %). Similarly, in India at 2008, the susceptibility of *Proteus* isolates to imipenem and merpenem antibiotics was 96.8 % and 100 %, respectively (Sonavane *et al*., 2008). In Iran at 2010, the *Proteus* isolates susceptibility to imipenem was 100 % (Khorshidi and Sharif, 2010). In another study conducted in Egypt by Bahashwan and El Shafey, (2013), imipenem had the highest sensitivity percentage (91 %) against *Proteus* isolates. While in Sudan at 2020, the *Proteus* isolates showed complete sensitivity to imipeneme (100 %) and 94.4 % sensitivity to meropenem (Hamid *et al*., 2020). Studying the antibacterial resistance to aminoglycosides (*i.e*., Gentamicin and Amikacin) showed that amikacin was more effective than gentamicin, as 57 *Proteus* isolates were sensitive to amikacin (68.6 %) and 48 isolates only were sensitive to gentamicin (57.8 %), in consistent with the findings of the previous study reported by Kadhim, (2017), where the *Proteus* isolates demonstrated different sensitivities of 62.7 % and 58.8 % to amikacin and gentamicin, respectively. In a later study, Han *et al*., (2020) recorded that the resistance to amikacin and gentamicin was 20 % and 46 %, respectively. Currently, the recorded quinolone resistance of *Proteus* spp. (*i.e*., Ciprofloxacin and Levofloxacin) was 46.9 % and 33.7%, respectively. In a similar study carried out in India by Rao *et al*., (2014), the observed antibacterial resistance to ciprofloxacin and levofloxacin was 37 %, 13 %, respectively. While in

Tanzania, the ciprofloxacin resistance was 39.3 % (Kumburu *et al*., 2017). Another similar study conducted by Uzoamaka *et al*., (2017) showed that the *Proteus* isolates had sensitivity percentages of 68.4 %, 78.9 % to ciprofloxacin and levofloxacin; respectively. In a previous study, *Proteus* isolates showed high antibacterial resistance to tetracycline (84.3 %) (Nemati, 2013). Currently, we observed that the chloramphenicol resistance was (87.2 %), while in another study carried out in Tanzania; it was 67.9 % (Kumburu *et al*., 2017). In this study, the resistance to nitrofurantoin was 73.5 %, in agreement with the previous study reported by Sonavane *et al*., (2008) that only 22.4 % of the *Proteus* isolates were susceptible to nitrofurantoin.

Using *ureR*-based PCR, the results of the present study revealed that 48.0 % of *Proteus* isolates were confirmed as *P. mirabilis*. Several similar studies used *ureR*-based molecular method for identification of *P. mirabilis* (Alatrash and Al-yasseen, 2017; Kamil and Jarjes, 2021). Additionally, the existences of *PMQR* and *aac(6')Ib* genes were examined, which can encourage the development of higher levels of quinolone resistance in the Gram-negative bacteria. Currently, only 16 *P. mirabilis* isolates showed the existence of *PMQR* and *aac(6')-Ib* genes by 35 % and 10 %, respectively Among all the recovered isolates, *qnrA* and *qnrB* genes were the most common (15 %), followed by *qnrD* and *aac(6')Ib* (10 %), and *qnrS* (5 %). However, in this study no *Proteus* isolate was recorded to harbor the *qnrC* gene. However in Canada, only 14 % of *Proteus* isolates had been reported to have *qnrB* gene, while 26 % were harboring *aac(6')-Ib* gene (Han *et al*., 2020). All Proteus isolates that had *qnrB* and *aac(6')-Ib* genes were resistant at least to either norfloxacin or ciprofloxacin. However, another study reported by Mirzaei *et al*., (2019) that 4.5 % and 0.9 % of *Proteus* isolates had *aac(6')-Ib-cr* and *qnrA* genes, respectively. Kotb *et al*., (2019) study showed that *qnrB* gene had been detected in 62.9 % of the resistant isolates, which represented the most

1977

common gene among the quinolone resistant *Proteus* isolates. The present study showed that *Proteus* spp. had higher ciprofloxacin resistance than that of levofloxacin. The relationship between the presence of *qnr* genes, *aac(6')-Ib* gene and the resistance of ciprofloxacin and levofloxacin was analyzed. The obtained results recorded a significant correlation between levofloxacin resistance and the existence of *aac(6')-Ib* gene ($p < 0.05$).

Conclusion

Since *Proteus* spp. are so common in the environment; strict sanitation standards must be maintained within the healthcare facilities to decrease the rate of nosocomial infections. Moreover, the use of catheters should be avoided whenever possible, and if it is necessary; it must be applied under clean precautions and for restricted intermittent periods. Furthermore, the improper use of antibiotics is a contributing factor in the *Proteus* spp. growing resistance to the most studied antibiotics. Therefore, understanding the local bacterial etiology and susceptibility patterns is necessary to identify any potential changes that may happen. In *P. mirabilis* isolates, the quinolone resistance may have been caused by other mechanisms in addition to *qnr* genes and the *aac(6')-Ib* gene; hence, more future researches are required to examine these possible resistance mechanisms.

Acknowledgement

Conflict of interest

No conflict of interests exists between the authors of this study.

Funding source

Ethical approval

This study was conducted in accordance with the Ethical standards. Before beginning this work, the Faculty of Pharmacy, Minia University Ethical Committee gave its approval (Reference number: 230504).

5.   References

Ahmed, D.A. (2015). Prevalence of *Proteus* spp. in some hospitals in Baghdad City. Iraqi Journal of Science. 56(1): 665-672.

Alatrash, A.K.M. and Al-yasseen, A. (2017). Detection of *UreR* and *UreC* among *Proteus mirabilis*. Asian Journal of Pharmaceutical and Clinical Research. 10(8): 386-389. https://doi.org/10.22159/ajpcr.2017.v10i8.18987

Allawi, F.A. and Motaweq, Z.Y. (2019). Phenotypic and molecular correlation between biofilm production and antibiotic resistance of *Proteus mirabilis* isolated from different clinical sources/IRAQ. Turkish Journal of Physiotherapy and Rehabilitation. 32(3): 3-9.

Bahashwan, S.A. and El Shafey, H.M. (2013). Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. European Scientific Journal. 9(27): 4-7. https://doi.org/10.19044/esj.2013.v9n27p%25p

Bandy, A. and Tantry, B. (2021). ESBL Activity, MDR, and Carbapenem Resistance among Predominant Enterobacterales Isolated in 2019. Antibiotics. 10(6): 744-749. https://doi.org/10.3390/antibiotics10060744

Bauer, A. (1966). Antibiotic susceptibility testing by a standardized single diffusion method. American

Journal of Clinical Pathology. 45(3): 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Cattoir, V.; Poirel, L.; Rotimi, V.; Soussy, C.J. and Nordmann, P. (2007). Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. Journal of antimicrobial chemotherapy. 60(2): 394-397. https://doi.org/10.1093/jac/dkm204

CLSI. (2018). Clinical and Laboratory Standards Institute. https://file.qums.ac.ir/repository/mmrc/CLSI-2018-M100-S28.pdf

Dasgupta, N.; Laskar, N.B.; Choudhury, N.A. and Bhattacharjee, A. (2015). Carriage and transferability of quinolone resistant determinants harboured within environmental isolates of *Enterobacteriaceae*. Archives of Clinical Microbiology. 6(4): 1-5.

El-Kazzaz, S.S. (2021). Virulence Factors Associated with Quinolone Resistance in *Proteus* Species Isolated from Patients with Urinary Tract Infection. Egyptian Journal of Medical Microbiology. 30(1): 115-123. https://doi.org/10.51429/EJMM30115

Filipiak, A.; Chrapek, M.; Literacka, E.; Wawszczak, M.; Głuszek, S.; Majchrzak, M. and Adamus-Białek, W. (2020). Pathogenic factors correlate with antimicrobial resistance among clinical *Proteus mirabilis* strains. Frontiers in Microbiology. 11(3): 37-43. https://doi.org/10.3389/fmicb.2020.579389

Girlich, D.; Bonnin, R.A.; Dortet, L. and Naas, T. (2020). Genetics of acquired antibiotic resistance genes in *Proteus* spp. Frontiers in Microbiology. 11(1): 256-259 https://doi.org/10.3389/fmicb.2020.00256

Hamid, M.H.; Arbab, A.H. and Yousef, B.A. (2020). Bacteriological profile and antibiotic susceptibility of diabetic Foot infections at Ribat University hospital: a retrospective study from Sudan. Journal of Diabetes and Metabolic Disorders. 19(3): 1397-1406. https://doi.org/10.1007/s40200-020-00660-8

Hamilton, A.L.; Kamm, M.A.; Ng, S.C. and Morrison, M. (2018). *Proteus* spp. as putative gastrointestinal pathogens. Clinical Microbiology Reviews. 31(3): e00085-00017. https://doi.org/10.1128/CMR.00085-17

Han, P.; Luo, L.; Shahid, M.; Li, M.; Wang, S.; Nobrega, D.B. et al. (2020). Prevalence, Genetic Diversity and Antimicrobial Resistance of *Proteus mirabilis* Isolated from Dogs Hospitalized in Beijing. Pakistan Veterinary Journal. 40(1):37-42 http://dx.doi.org/10.29261/pakvetj/2019.101

Hayder, T.; Abusaiba, H.; Alasedi, K. and Aljanaby, A. (2020). *Proteus mirabilis* virulence factors: Review. International Journal of Pharmaceutical Research. 13(3): 2145-2149. http://dx.doi.org/10.31838/ijpr/2021.13.01.169

Hola, V.; Peroutkova, T. and Ruzicka, F. (2012). Virulence factors in *Proteus* bacteria from biofilm communities of catheter-associated urinary tract infections. FEMS Immunology and Medical Microbiology. 65(2): 343-349. http://dx.doi.org/10.1111/j.1574-695X.2012.00976.x.

Ioannou, P. and Vougiouklakis, G. (2020). Infective endocarditis by *Proteus* species: a systematic review. Germs. 10(3): 229-235. https://doi.org/10.18683/germs.2020.1209

Kadhim, A.S. (2017). Antimicrobial resistance patterns and extended spectrum beta-lactamases producing by *Proteus mirabilis* isolated from different sources. Al-Mustansiriyah Journal of

1979

Science. 28(1): 47-54. https://doi.org/10.23851/mjs.v28i1.311

Kamel, A. and Al-Yasseen, A.K. (2009). Phenotypic and Genotypic Characterization of Some Virulence Factors among *Proteus mirabilis* Isolated from Clinical Samples in Al-Najaf Al-Ashraf/Iraq. Journal of Global Pharma Technology. 11(5): 471-478.

Kamil, T.D. and Jarjes, S.F. (2021). Molecular Characterization of *Proteus* spp. from Patients Admitted to Hospitals in Erbil City. Polytechnic Journal. 11(2): 95-99. https://doi.org/10.25156/ptj.v11n2y2021.pp95-99

Khorshidi, A. and Sharif, A. (2010). Imipenem resistance among Gram-negative and Gram-positive bacteria in hospitalized patients. Iranian journal of public health. 39(2): 110-117.

Knirel, Y.A.; Perepelov, A.V.; Kondakova, A.N.; Senchenkova, S.Y.N.; Sidorczyk, Z.; Rozalski, A. et al. (2011). Structure and serology of O-antigens as the basis for classification of *Proteus* strains. Innate Immunity. 17(1): 70-96. https://doi.org/10.1177/1753425909360668

Kotb, D.N.; Mahdy, W.K.; Mahmoud, M.S. and Khairy, R.M. (2019). Impact of co-existence of *PMQR* genes and *QRDR* mutations on fluoroquinolones resistance in *Enterobacteriaceae* strains isolated from community and hospital acquired UTIs. BMC Infectious Diseases. 19(1): 1-8. https://doi.org/10.1186/s12879-019-4606-y

Kumburu, H.H.; Sonda, T.; Mmbaga, B.T.; Alifrangis, M.; Lund, O.; Kibiki, G. et al. (2017). Patterns of infections, aetiological agents and antimicrobial resistance at a tertiary care hospital in northern Tanzania. Tropical Medicine and International Health. 22(4): 454-464. https://doi.org/10.1111/tmi.12836

Mirzaei, A.; Habibi, M.; Bouzari, S. and Asadi Karam, M.R. (2019). Characterization of antibiotic-susceptibility patterns, virulence factor profiles and clonal relatedness in *Proteus mirabilis* isolates from patients with urinary tract infection in Iran. Infection and drug resistance: 3(1): 3967-3979. https://doi.org/10.2147%2FIDR.S230303

Nemati, M. (2013). Antimicrobial resistance of *Proteus* isolates from poultry. European Journal of Experimental Biology. 3(6): 499-500.

O'Hara, C.M.; Brenner, F.W. and Miller, J.M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. Clinical Microbiology Reviews. 13(4): 534-546. https://doi.org/10.1128/cmr.13.4.534

Pal, N.; Sharma, R. and Maheshwari, R.K. (2016). Phenotypic Detection and Antibiogram of β-lactamase‑producing *Proteus* Species in a Tertiary Care Hospital, India. Annals of Medical and Health. 6(5): 267–273. https://doi.org/10.4103%2Famhsr.amhsr_413_15

Parajuli, H.; Shrestha, D.; Thapa, K.; Thapa, P.; Chaudhary, P.; Bhandari, D. et al. (2021). Surveillance of antimicrobial resistance of *Proteus* spp. in a tertiary hospital in Nepal. Asian Journal of Allied Health Sciences. 4(1): 4-9. https://doi.org/10.1111/j.1600-0463.2012.02923.x

Park, C.H.; Robicsek, A.; Jacoby, G.A.; Sahm, D. and Hooper, D.C. (2006). Prevalence in the United States of *aac (6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. Antimicrobial Agents and Chemotherapy. 50(11): 3953-3955. https://doi.org/10.1128/aac.00915-06

Pathirana, H.; Shin, G.W.; Wimalasena, S.; De Silva, B.; Hossain, S. and Heo, G.J. (2018). Prevalence and characterization of quinolone resistance genes in *Proteus* species isolated from pet

turtles. Journal of Exotic Pet Medicine. 27(4): 67-73. http://dx.doi.org/10.1053/j.jepm.2017.10.026

Patrick, K.F.; Solomon, N.A.; Adu-Sarkodie, Y. and Opoku-Okrah, C. (2010). Occurrence, species distribution and antibiotic resistance of *Proteus* isolates: A case study at the Komfo Anokye Teaching Hospital (KATH) in Ghana. International Journal of Pharma Sciences and Research. 1(9): 347:352

Rao, D.R.; Basu, R. and Biswas, D.R. (2014). Aerobic bacterial profile and antimicrobial susceptibility pattern of pus isolates in a South Indian Tertiary Care Hospital. Journal of Dental and Medical Sciences. 3(3): 59-62. https://doi.org/10.3855/jidc.10473

Rózalski, A.; Sidorczyk, Z. and Kotełko, K. (1997). Potential virulence factors of *Proteus* bacilli. Microbiology and Molecular Biology Reviews. 61(1): 65-89. https://doi.org/10.1128/mmbr.61.1.65-89.1997

Sadiq, O.S. and Abd Alhadee, L. (2009). Phenotypic and Genotypic Study of Sodium Azide Effect on *Proteus mirabilis* Swimming and Swarming Phenomenon. Journal of Global Pharma Technology. 10(6): 238-243.

Sanches, M.S.; Baptista, A.A.S.; de Souza, M.; Menck-Costa, M.F.; Koga, V.L.; Kobayashi, R.K.T. et al. (2019). Genotypic and phenotypic profiles of virulence factors and antimicrobial resistance of *Proteus mirabilis* isolated from chicken carcasses: potential zoonotic risk. Brazilian Journal of Microbiology. 50(3): 685-694. https://doi.org/10.1007%2Fs42770-019-00086-2

Schaffer, J.N. and Pearson, M.M. (2017). *Proteus mirabilis* and urinary tract infections. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management. 3(5): 383-433. https://doi.org/10.1128/microbiolspec.uti-0017-2013

Sonavane, A.; Mathur, M.; Turbadkar, D. and Baradkar, V. (2008). Antimicrobial susceptibility pattern in urinary bacterial isolates. Bombay Hospital Journal. 50(2): 240-244.

Thabit, A.G.; El-Sabour, A.; Nafie, A.M.A.; El-Mokhtar, M.A. and Biomy, Y.E. (2020). Detection of *Proteus* species in diabetic wounds and their antibiotic resistance profile analysis. Bulletin of Pharmaceutical Sciences. Assiut. 43(1): 1-10. https://doi.org/10.21608/bfsa.2020.93523

Uzoamaka, M.; Ngozi, O.; Johnbull, O.S. and Martin, O. (2017). Bacterial etiology of lower respiratory tract infections and their antimicrobial susceptibility. The American Journal of the Medical Sciences. 354(5): 471-475. https://doi.org/10.1016/j.amjms.2017.06.025

Vila, J.; Fàbrega, A.; Roca, I.; Hernández, A. and Luis Martínez, J. (2011). Efflux pumps as an important mechanism for quinolone resistance. Advances in Enzymology and Related Areas of Molecular Biology. 77(3): 167-174. https://doi.org/10.1002/9780470920541.ch5

Yassine, I.; Rafei, R.; Osman, M.; Mallat, H.; Dabboussi, F. and Hamze, M. (2019). Plasmid-mediated quinolone resistance: Mechanisms, detection, and epidemiology in the Arab countries. Infection, Genetics and Evolution. 76(3): 104020. https://doi.org/10.1016/j.meegid.2019.104020

Zhang, W.; Niu, Z.; Yin, K.; Liu, P. and Chen, L. (2013). Quick identification and quantification of *Proteus mirabilis* by polymerase chain reaction (PCR) assays. Annals of Microbiology. 63(2): 683-689. http://dx.doi.org/10.1007/s13213-012-0520-x

1981