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Antibiotic susceptibility of *P. mirabilis* isolated from clinical samples in Thi- Qar province

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***Abstract*— *Proteus* spp. Is a motile dimorphic Gram negative bacteria belonging to the order *Enterobacterales*. *P. mirabilis*, the most commonly isolated species from clinical samples. The present study aimed to investigate the resistance of *Proteus mirabilis* isolates to different antibiotics. The study included the collection of 487 samples from different clinical sources, including urine, burns, wounds, ear swabs and diabetic ulcers. Different ages were involved of both sexes. Samples were collected from patients attending Al-Nasiriya and Al- Hussain Teaching Hospitals and private laboratories in Thi-Qar province, Iraq, from 29th of November 2021 to 20th of Abril 2022. A total of the isolates were diagnosed by different laboratory tests. The prevalence of *P. mirabilis* was 8.6 % among all collected samples. Female infection rate was 52.4%, male infection rate was 47.6 %. The age group ≥ 44 years was most commonly infected with *P. mirabilis*. In addition most *P. mirabilis* isolates were obtained from UTIs 45%. The antibiotic susceptibility test results showed a high resistance for Cefepime 83.3%, Ceftriaxone 78.8%, Tobramycin 73.3%, Ampicillin 71.4%, Amoxicillin/clavulanic Acid 69%, and Cefotaxime 60%. However, ciprofloxacin was the highest effective antibiotic 54.7%, followed by Meropenem 50%, and Gentamicin 45%. Finally, amikacin had intermediate activity 19%, followed by Netilmicin 16.6%.**

Keywords*— *P. mirabilis*, Antibiotic resistance, β- lactams, Aminoglycosides

I. Introduction

Hauser originally referred to a shape-shifting bacterium he had obtained from putrefied meat as proteus in bacterial nomenclature in 1885(Hauser, 2013)[1]. *Proteus mirabilis*, the motile Gram-negative, dimorphic member of the *Enterobacteriaceae* family, has captivated scientists for many years due to its capacity to develop from short rods into long, multinucleate swarmer cells expressing thousands of flagella (Armbruster & Mobley, 2012)[2]. Members of the *Proteus* spp are common in the environment, including soil and water, where their presence is thought to be the result from fecal contamination. They are a normal component of the bacterial flora of both human and animal intestinal tracts. *P. mirabilis* is the most frequently isolated species from clinical samples, mostly from UTIs, but it can also be isolated from other infections such as the eye, ear, nose, skin, burn, meningoencephalitis, osteomyelitis, and wound infections (Girlich et al., 2020)[3]. A vast range of infections are caused by *P. mirabilis*, this species accounts for more than 3% of all nosocomial infections, and up to 44% of catheter-associated urinary tract infections, and its transmission is facilitated by an intrinsic translocation capacity utilizing peririchous flagella. Furthermore, *P. mirabilis* has a well-developed arsenal of exoenzymes such as urease, protease, and hemolysins, as well as a high biofilm-forming potential(Khayyat et al., 2021)[4]. Antibiotic resistance, or the ability of bacteria to withstand the effects of antibiotics for which they were once sensitive, poses a serious danger to the advancements made during the antibiotic era(Adedeji, 2016)[5]. like other Enterobacterales, Clinical strains of *P. mirabilis* have developed an increased resistance to antimicrobial drugs over the past few decades(Filipiak *et al*., 2020)[6].

II. Materials and Methods

Collection of Samples

During the period from 29th of November 2021 to 20th of Abril 2022, a total of 487 samples were collected from different clinical sources, including 316 urine, 63 smears of burns, 20 wound swab, 69 ear swab, and 19 diabetic ulcer swab from both genders and different ages from hospitals and private clinics in Thi-Qar province, Iraq. The samples were transported on Carry Blair swabs and cultured on Blood agar and MacConkey agar, incubated aerobically at 37 °C for 24 hours. The isolated bacteria were identified according to microscopic, morphologic, biochemical, and API 20E tests.

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Antibiotic Susceptibility Test

It was performed by Kirby-Bauer procedure on Muller Hinton agar (Neogene, UK). All isolates were tested against 11 antibiotics, and the results were interpreted according to clinical and Laboratory Standards Institute 2019. Amikacin 10 µg, Amoxicillin –Clavulanic Acid 30 µg, Cefepime 10µg Netilmicin 10µg, Gentamicin 5µg , Tobramycin 10 µg, Meropenem 10µg.

Statistical Analysis

The statistical analysis proceeded in all groups of study, descriptive statistics analyzed by using Chi-square P. value ≤ 0.01 was considered to be All analyses were performed with statistical Package for the social sciences SPSS for Windows version 23.0 SPSS Inc., Chicago, 111.

III. RESULTS

Out of 487 samples 45 9.24% were identified as *Proteus* spp. 42 93.75% were *P. mirabilis* while 3 6.25% belonged to *P. vulgaris*. The current study results showed that The isolation rate from the different clinical samples 19/316 isolates 6.0% from urine, 9/63 isolates 21.4% from the burn specimens, 3/20 isolates 14.3% from wounds , 7/69 isolates 10.1% from ear infections, and 4/19 isolates 22.1% from diabetic foot ulcers, as shown in Table 1.

The infection rate was 52.4% for females and 47.6% for males. As shown in TABLE II.

In the present study patients ‘ages were between 3-81 years. The mean age was 43.2 years. The current results showed that the highest isolation rate was in the age group > 44 47.6% followed by the 30 – 44 age group with 24.4%. While the lowest isolationn rate was 11.9% in the age group under 15 followed by the age group 15 – 29 with the isolation rates 19.0 % and 19% respectively, as shown in TABLE III.

All of the 42 *Proteus mirabilis* isolates were examined for antibiotic susceptibility tests against 11 antibiotics. As shown in TABLE IV. The results revealed that the maximum ratio of the resistance of *P. mirabilis*. isolates were recorded against for Cefepime 83.3%, Ceftriaxone 78.8%, Tobramycin 73.3%, Ampicillin 71.4%, amoxicillin/clavulanic Acid 69%, Cefotaxime 60%, and However, Ciprofloxacin was the highest effective antibiotic 54.7%, followed by Meropenem 50%, and Gentamicin 45%. Finally, amikacin had intermediate activity 19%, followed by Netilmicin 52.3%.

Clinical sample	**NO. of *P.miirabilis* isolates**	**Total NO. of samples**	**Percentage of *P. mirabilis* isolated from**	
			Total number of isolates	***Total number of samples***
urine	19	316	45.2%	6.0%
Burns	9	63	21.4%	14.3%
wounds	3	20	7.1%	15.0%
Ear swabs	7	69	16.7%	10.1%
Diabetic ulcers	4	19	9.5%	22.1%
total	42	487	100%	8.6%
Cal.X^2: 10.25	Tab.X^2:13.28		Df: 4	P-value: 0.01

TABLE I. Distribution of *P. mirabilis* according to the clinical sources

TABLE II. Distribution of *P .mirabilis* infections according to gender

Clinical sources	**males**	**%**	**Females**	**%**	**total**
urine	7	36.8	12	63.2	19
burns	5	55.6	4	44.4	9
wounds	2	66.7	1	33.3	3
Ear swabs	3	45.9	4	57.1	7
Diabetic ulcers	3	75	1	25	4
total	20	47.6	22	52.4	42
Cal.X^2 :2.81	**Tab.X^2: 13.27**		**Df:4**	**p-value: 0.01**	

TABLE III. Distribution of *P. mirabilis* infections according to age groups

Age Group (year)	NO.	%	
< 15	5	11.9	
15 – 29	8	19.0	
30 – 44	9	24.4	
> 44	20	47.6	
Total	42	100%	
Cal.X^2 : 12.28	Tab.X^2:11.34	Df:3	p-value: 0.01

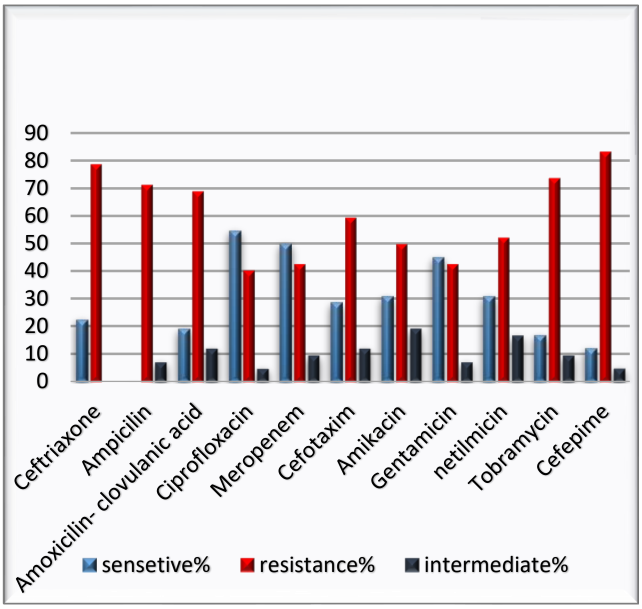

Fig. 1 Antibiotic susceptibility of *P. mirabilis* isolates

TABLE IV. Antibiotic susceptibility of *P. mirabilis* isolates against the different antibiotics

Activity / Antibiotics	Sensitive		Intermediate		Resistance	
	No.	***%***	***No.***	***%***	***No.***	***%***
Ceftriaxone	9	22.4	0	0.0	33	78.8
Ampicillin	9	21.4	3	7.1	30	71.4
Amoxicillin-clavulanic acid	8	19	5	11.9	29	69
Ciprofloxacin	23	54.7	2	4.7	17	40.5
Meropenem	21	50	3	9.52	18	42.8
Cefotaxime	12	28.6	5	11.9	25	59.5
Amikacin	13	30.9	8	19	21	50
Gentamicin	19	45	3	7.1	18	42.8
Netilmicin	13	30.9	7	16.6	22	52.3
Tobramycin	7	16.7	4	9.5	31	73.8
Cefepime	5	11.9	2	4.8	35	83.3
Total	139	30.2	42	10.7	279	59.1
CalX^2= 104.11	TabX^2= 37.57		Df = 20		P. Value < 0.01	

IV. Discussion

The present study showed no significant differences in the infection rate between different clinical samples. The isolation rate of *P. mirabilis* from urine specimens was similar to the results of another study by Rajkumar *et al.*, (2016)[7] from India 4%. While some other studies found higher rates of isolation such us Mahdi & Al-Deresawi,(2014)[8] and Jarjes, (2019)[9] from Iraq. Our finding for the isolation from ear swabs was similar to other local studies such as Mahdi & Al-Deresawi, (2014)[8] and H Ahmed, (2015)[10] whose results for the isolation from ear swabs were 10.4% and 12%, respectively. Isolation rate from wounds in the present study was 15% which agreed with another study from Nigeria by A. Mohammed *et al.*,(2013)[11] was 14.5%, Local studies by Al-Azawy *et al.*, (2015)[12] from Dyiala and Jarjes, (2019)[9] from Erbil, found a slightly higher rates for the isolation of *P. mirabilis* from wound specimens which were 36% and 24%, respectively. For burn Samples the isolation rate was 14.3% this result agreed with H Ahmed, (2015)[10] 8%, while Al-Azawy *et al.*, (2015)[12] and Jarjes, (2019)[9] Revealed higher rates of isolation 23.9% and 27.7% respectively. The isolation from diabetic foot ulcers recorded.

The highest prevalence for *P. mirabilis* in the present study 22.1%. this result was relatively similar to other studies such as studies by Gadepalli et al., (2006)[13] from India and Jaber & Almiyah, (2022)[14] from AL-Diwanyah city, whose revealed that the isolation rate was 32%. while the result by Al-Muhanna *et al.*, (2020)[15] from Maysan was 37%.

The present study showed no significant differences between males and females at getting the infection with *P. mirabilis* p value: 0.01. Our results agreed with Ahmed, (2015) [16] who found 56% females and 43% males were infected with *P. mirabilis* . Our results disagreed with other studies such as A study by Abdelkreem *et al.*,(2018)[17] from Sudan who found that 45% of *Proteus mirabilis* isolates were from females and 55% of the isolates from males, while Mirzaei *et al.*, (2019) [18] from Iran found that 71.8% of isolates were recovered from females and the remaining (28.2% were from males. de Oliveira *et al.*, (2021) [19] also revealed similar outcomes. 146 were isolated from females 79.7% and 37 (20.2% were from males.

The mean age of patients in the present study was 43.2 years. This result agreed with another study by Mirzaei *et al.*, (2019)[18] from Iran who found that the mean age of patients with *P. mirabilis* infections was 37.7 years. However, It disagreed with Xiao *et al.*, (2019)[20] from China found that patients had a mean age of 67.2 years.67.2 years. Most isolates of the present study were obtained from patients belonged to the ages older than 44. This might be because all isolates of diabetic foot ulcers were obtained from older patients. Most isolates of the present study were obtained from patients belonged to the ages older than 44. this might be because all isolates of diabetic foot ulcers were taken from older patients. Thabit *et al.*, (2020)[21] revealed that the majority of *Proteus* infections in diabetic wounds were detected in the age group >50-82 years. In addition, a lot of women > 44 at the menopause age may undergo hysterectomy which may lead to UTI with *P. mirabilis*. The age groups 15-29 and 30-44 also had high rates of infection because at these ages people generally more vulnerable to accidents like burns and wounds. de Oliveira et al., (2021)19 [19] showed that the majority of catheter associated UTI by *Proteus mirabilis* occurred in women 16–31 years followed by the age group 32–47 years. Zafar *et al.*, (2019)[22] found that The age group 16 – 30 had the highest rate of infection with wound's *P. mirabilis.*

The β-lactam antibiotics is a family of bactericidal drugs containing the β-lactam ring in their chemical structure. They are classified as penicillins, cephalosporins, carbapenems, penems also known as thiopenems, and monobactams. This classification depends on the chemical nature of the ring fused to the β-lactam pharmacophore unit, generating a noncoplanar bicyclic scaffold (Lima *et al.*, 2020). The outcomes of the current study showed a high resistance to the

Cefalosporins: Cefepime and Ceftriaxone 83.3% and 78.8%, respectively. These results for Ceftriaxone agreed with Algburi *et al*., (2020)[24] from Diyala province who showed that 90% of isolates were resistant. Rout *et al*., (2014) [25] also noted that 67.7% of the isolates was resistance to cefepime and 90.3% was the resistance to ceftriaxone. Jarjes *et al*., (2019)[9] found similar results for cefepime resistance as it was about 90%. on the other hand, this study disagreed with Mirzaei et al., (2019)[18] from Iran as he found that resistance for ceftriaxone was 10%. For cefotaxim the results was moderate resistance 59.5%. This result agreed with Rout *et al*., (2014)[25] who found that resistance to cefotaxim was 58%. but disagreed with Jabur *et al*.,(2013) and Al-Bassam & Al-Kazaz, (2013)[27] Whose results of resistance rates were 26.6% and 35%, respectively.

The study also revealed a high resistance to penicillin group (Ampicillin and Amoxicillin –clavulanic) Resistance to ampicillin was 71.4% this result corresponds with other studies such as Hussein *et al.,* (2020)[28], Al-Bassam & Al-Kazaz,(2013)[27] from Iraq and Bahashwan & El Shafey, (2013)[29] from KSA. as they found that resistance to ampicillin was 61.9%, 75% and 80%, respectively. But contradicted with other studies by Rafalskiy,(2020)[30] from Russia, and Cernohorská & Chvílová, (2011)[31] from Czech. Since there results were 45.7%, 23.0 %, respectively. Regarding to Amoxicillin –clavulanic acid, results showed that most isolates were resistant 69%. Similar results were noted by Rout *et al*.,(2014)[25] from Pakistan, Thabit *et al*.,(2020) [21] and Shaaban *et al*., (2020)[32] from Egypt whose results were 67.7%, 75%and 86%, respectively. The results of the carbapenem antibiotic meropenem showed moderate sensitivity 50% this result corresponded with the result of Gazel et al., (2021)[33] from turkey and Xiao *et al*.,(2019)[20] from china and Thabit et al.,(2020)[21] from Egypt who found that the susceptibility of *Proteus* isolates to meropenem were 48% 55.6% and 54%, respectively. these results contradict with Feglo & Opoku, (2014)[34] from Ghana who found that susceptibility was 100%.

Aminoglycoside AG antibiotics are used to treat many Gram-negative bacteria. Among various bacterial species, resistance to AGs arises through a variety of intrinsic and acquired mechanisms (Garneau-Tsodikova & Labby, 2016)[35]. Shikh-Bardsiri & Shakibaie, (2013)[36] from Iran found similar results for amikacin and gentamicin while Jabur *et al*.,(2013)[26] from Pakistan found a close results for gentamicin 50% but very different results for amikacin resistance 5%. Our findings also differ slightly with Rafalskiy,(2020)[30]. Since he found that resistance to gentamicin was 25.3% while for Netilmicin 38.1%. Rout *et al*., (2014)[25] also found different results for amikacin and gentamicin resistance 67.7% and 58% however his results for Netilmicin resistance agreed with ours 45.1%. On the other hand tobramycin registered the higher level of resistance between the tested aminoglycosides as 73%. This result agreed with Jarjes *et al*.,(2019)[9] as he revealed that 70% or less resistance pattern was identified to tobramycin but contradicted with Chen et al., (2018)[37] from China and Rostamzad et al., (2016)[38] from Iran whose result for tobramycin resistance was 9.4% and 48% respectively. Susceptibility to the fluoroquinolone antibiotic ciprofloxacin was 54.7% which coincide with other studies Thabit *et al*.,(2020)[21], Feglo & Opoku, (2014)[34] whose results were 49% and 60.23%, respectively. but differ slightly with Hussein *et al*.,(2020)[28] from Iraq who noted higher level of sensitivity 69.8% while Chen et al.,(2018)[37] and Gazel *et al.*, (2021)[33] from Turkey found lower levels 35% and 26%.

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