World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Prevalence pattern of multidrug resistant *Proteus mirabilis* recovered from wound of human and cats in Wasit governorate

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World Journal of Advanced Research and Reviews, 2023, 17(02), 453–459

Publication history: Received on 20 December 2022; revised on 01 Februay 2023; accepted on 04 February 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.17.2.0203

Abstract

The current work was conducted for isolation and identification of *Proteus mirabilis* from humans and cats. A total of one hundred and fifty samples were collected from hospitals of wasit city during the period from 23/7/2021 to 13/1/2022. These clinical samples included: (90) wound swabs from humans While the total number of samples amount to wound swabs in cats (60). These samples of humans were collected from Al- Zahra Teaching Hospital in wasit and the samples of cats is collected from different places. All isolates *Proteus mirabilis* were characterized according to the morphology and microscopic characteristics, along with the biochemical and confirmatory APi 20 E tests. These isolates were obtained from: humans (15) and cats (11).Then determination of antibiotics susceptibility pattern of recovered isolates. The human isolates showed resistant 100 % to Ampicillin, Penicillin, Trimethoprim-Sulfamethoxazole, Streptomycin, Chloramphenicol, Amoxicillin / clavulanic acid, Erythromycin, Tetracycline, Cefoxitin and Vancomycin. Sensitive (100%) for Gentamycin and Ofloxacin. otherwise the cat's isolates showed resistant (100%) to Ampicillin, Penicillin, Trimethoprim-Sulfamethoxazole, Streptomycin, Chloramphenicol, Amoxicillin / clavulanic acid, Erythromycin, Tetracycline, Ofloxacin, Cefoxitin and Vancomycin. Sensitive (100%) for Gentamycin only.

Keyword: *Proteus mirabilis*; Human; Cat; Api20E; Antibiotics

1. Introduction

The genus Proteus belong to the family Enterobacteriaceae (Pal et al., 2014). The Proteus are consist of spp including *Proteus mirabilis*, *P. vulgaris, P. penneri, P. hauseri, P. myxofaciens*, *P. alimentorum, P. cibarius, P. columbae, P. inconstans*, *P. morganii, P. terrae, and P. rettgeri* (O'Hara *et al*., 2000; Różalski *et al.*, 2007; Behrendt *et al*., 2015; Dai *et al*., 2018; Shaw and Clarke. 1955). *Proteus mirabilis* is a Gram-negative, rod-shaped, facultative, noncapsulated, non-spore forming, anaerobic, and motile bacterium(Pfaller. 1999) It is mostly found in natural environments and responsible for the infection of the pulmonary system, burns, skin, eyes, ears, nose, and the urinary tract, as well as gastroenteritis (Unachukwu *et al*., 2005; Ebringer and Rashid. 2009). *Proteus mirabilis* is the most common cause of nosocomial infections consider for 90% of all Proteus infections (Taiwo *et al*.,2002). A main problem in wound infections is the at all antimicrobial resistance in P. mirabilis (Taiwo *et al*.,2002 ; Anguzu and Olila. 2007 ; Okesola and Kehinde. 2008). *Proteus mirabilis* have heterologous β-lactamase genes that may become resistant to β-lactams (Livermore. 1995) Thereby, *Proteus mirabilis* may be resistant to broad-spectrum β-lactamase including penicillins and cephalosporins (Philippon *et al*., 2002). Urease enzyme is the one of the most important *Proteus mirabilis* utilizes in the pathogenesis of

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kidney and bladder stone formation there are have some genes such as ureA, ureB, ureC, ureD, ureE, ureF, ureG, and ureR on the ure operon are responsible for the production of the urease enzyme among which, ureC is a major contributor (Manos *et al.*, 2006)

Aim of the study

- Isolation and identification of *Proteus mirabilis* from wound.
- Study the extent of resistance of bacteria *Proteus mirabilis* to some antibiotics .
- Study of some factors associated with pathogenicity of Proteus such as urase production and phenylalanine deaminase.

2. Material and methods

2.1. Sample Collection

Overall 150 wounds samples. Were collected from humans the swab wounds were obtained from Al- Zahra Teaching Hospital in wasit. And also taken from cats the samples were collected from different places. The specimens were directly streaked onto MaCconkey and blood agar sand were incubated at 37Cº for 24 hours.

2.2. Identification of the Isolates

Isolates were identified depending on morphological and biochemical tests as compared with identification scheme described by (Holt *et al*., 1994), and according to API 20 E confirmatory test.

2.3. Antibiotic Sensitivity Test (Qualitative Disk Method)

Twelve antibiotic disks (Penicillin G, Ampicillin, Trimethoprim-Sulfamethoxazole, Ampicillin/Sulbactam, Streptomycin, Tetracycline, Doxycycline, Amoxicillin- Clavulanic acid, Erythromycin, Chloramphenicol, Clarithromycin, Cefixime, Gentamicin, Ceftriaxone, Enrofloxacin and Trimethoprim) were used to detect the sensitivity of isolates of *P. mirabilis* according to method described earlier (Bauer. 1966)

3. Results and discussion

3.1. Isolation and Characterization of Proteus spp.

Ninety samples wound swabs were collected from patients in wasit city hospitals. And sixty wound swabs from cats. as illustrated in table (1). Twenty-six local isolates were characterized depending on cultural and microscopic characteristic. Genus and species were characterized by using biochemical tests and API 20 E confirmatory test.

Table 1 Types of sample, number and Percentage of *Proteus mirabilis* isolated from human and cats' samples

Types of sample	Number of samples	Number of isolates	Percentage%
Human	90	15	16.66 %
Cats	60	11	18.33 %
Total	150	26	17.33%

3.2. Cultural Characteristics

The Proteus isolates were firstly identified as related to the genus Proteus by swarming phenomenon on blood agar and the bacteria on the macconkey agar appeared pale (Dharmadhikari and Peshwe. 2009).

3.3. Microscopically Characteristics

Microscopic examination of the bacteria appeared as gram negative and straight rods when it stained with gram stain (Holt *et al*., 1994).

3.4. Biochemical Characteristics

Table 2 Biochemical tests of the *P. mirabilis* isolates

No	Biochemical Test	*P.mirabilis*
1	Oxidase	-
2	Phenyl alanine Deaminase	+
3	Indole Production	-
4	Motility	+
5	Urease Production	+
6	catalase test	+
7	lactose Fermentation	-
8	Maltose Fermentation	-
9	H2S production	+

(-) a negative result, (+) a positive result.

Table 3 Api 20E technique of *Proteus mirabilis*

No.	Active ingredients	Symbol test	Results
	Ortho NitroPhenyl-Bd-Galactopyranside	ONPG	-
	L-arginine	ADH	-
	L-Lysine	LDC	-
	L-Ornithin	ODC	+
	Trisodium citrate	CIT	+
	Sodium thiosulfate	H_2S	+
	Urea	URE	+
	L-tryptophane	TDA	+
	L-tryptophane (indole production)	IND	-
	Sodiume pyruvate	VP	-
	Gelatin (bovine origin)	GEL	+
	D-Glucose	GLU	+
	D-Mannitol	MAN	-
	Inositol	INO	-
	D-Sorbitol	SOR	-
	L-Rhamnose	RHA	-
	D-Saccharose (sucrose)	SAC	-
	D-Melibiose	MEL	-
	Amygdaline	AMY	-
	L-Arabinose	ARA	-

Several biochemical tests were done to characterize Proteus isolates. All the isolates of *Proteus mirabilis* showed positive results to the biochemical tests, phenylalanine deaminase, urease and catalase test. These isolates were motile, and all these isolates were indole negative and H2S production. But all were oxidase test negative. Also Proteus isolates were unable to ferment lactose and maltose (Holt *et al*., 1994) as illustrated in Table (2).

For confirmation of the biochemical results, the API 20 E confirmatory were used for Enterobacteriaceae identification. The results revealed that the tested isolate were *P. mirabilis*. shows in the Table (4) and Figure (1)

Figure 1 Api 20 E technique for *Proteus mirabilis*

3.5. Antimicrobial susceptibility

The *Proteus mirabilis* antimicrobial susceptibility test isolates from humans shows that resistant to 100 % Ampicillin, Penicillin, Trimethoprim-Sulfamethoxazole, Streptomycin, Chloramphenicol, Amoxicillin/clavulanic acid, Erythromycin, Tetracycline, Cefoxitin and Vancomycin. Sensitive (100%) for Gentamycin and Ofloxacin the prevalence of MDR was 100%, the MARI was 0.83 as shown in Table (4).

Table 4 Susceptibility as antimicrobial versus isolates of *P. spp*. in human

No.	Antimicrobial type	Humans		
		S %	I %	R%
1.	Ampicillin (25)	0	0	100
2.	Penicillin G (10)	0	0	100
3.	Trimethoprim-Sulfamethoxazole (25)	0	0	100
4.	Streptomycin (25)	0	0	100
5.	Chloramphenicol (30)	0	0	100
6.	Clavulanic /Amoxicillin Acid (30)	0	0	100
7.	Erythromycin (10)	0	0	100
8.	Tetracycline (10)	0	0	100
9.	Gentamicin (10)	100	0	0
10.	Ofloxacin (5)	100	0	0
11.	Cefoxitin (25)	0	0	100
12.	Vancomycin (30)	0	0	100
	MARI	0.83		

S= sensitive, I= intermediate, and R= resistant, MAR index=mulita drug resistants', MARI = Multidrug Antibiotic resistance index.

The *Proteus mirabilis* antimicrobial susceptibility test isolates from cats that resistant (100%) to Ampicillin, Penicillin, Trimethoprim-Sulfamethoxazole, Streptomycin, Chloramphenicol, Amoxicillin / clavulanic acid, Erythromycin, Tetracycline, Ofloxacin, Cefoxitin and Vancomycin. Sensitive (100%) for Gentamycin 0nly. The prevalence of MDR was 100%; the MARI was also 0.91as shown in Table (5)

Table 5 Antimicrobial susceptibility against *Proteus mirabilis* Isolates in cat

No.	Antimicrobial type	S%	I%	R%
1.	Ampicillin (25)	0	0	100
2.	Penicillin G (10)	0	0	100
3.	Trimethoprim-Sulfamethoxazole (25)	0	0	100
4.	Streptomycin (25)	0	0	100
5.	Chloramphenicol (30)	0	0	100
6.	Clavulanic /Amoxicillin Acid (30)	0	0	100
7.	Erythromycin (10)	0	0	100
8.	Tetracycline (10)	0	0	100
9,	Gentamicin (10)	100	0	0
10.	Ofloxacin (5)	0	0	100
11.	Cefoxitin (25)	0	0	100
12.	Vancomycin (30)	0	0	100
MAR index		0.91		

S= sensitive, I= intermediate, and R= resistant, MAR index=mulita drug resistants', MARI = Multidrug Antibiotic resistance index.

Proteus is considered important main cause of hospital-acquired infections and a major contributor to the pathogenesis of wound infection. In the present study, 150 different wound samples were examined out of which for human 16.6% were positive for P. mirabilis while sample of cats 18.33%. *Proteus mirabilis* can cause both community and hospital-acquired infections, especially in immunocompromised patients. Based on the study by Brown (Brown. 1999). In our study recorded (16.6%) of P. mirabilis were isolated from of all positive samples of human wound and these samples are less than (Orrett. 1999; Reśliński *et al*., 2005), who recorded (19.64%) which was highly level when compared with findings of (Alatrash and Al-yasseen. 2017), who reported a prevalence rate of (19.3%) in human urine samples while according to and (Ali and Yousif. 2015) who reported an overall prevalence of (17.6%). In our current study we who found sample of cats 18.33% While (Hariharan *et al*., 2011) who reported (4.23%) in cats and this less than our percentage and another study show the percentage of isolation to the cats are (17.27%) by (Albert *et al*., 1992) Mohammed and other. (Mohammed *et al*., 2013) similarly reported in Nigeria that P. mirabilis was more frequently isolated from surgical wounds than burn and diabetic wounds. We identified P. mirabilis through biochemical tests such as Phenyl alanine Deaminase, catalase, oxidase, sugar fermentation, Indole Production, and urease and found results similar to the findings obtained by Al-Bassam and Al-Kazaz(Al-Bassam and Al-Kazaz. 2013). During our current study of *Proteus mirabilis*, it was laboratory diagnosed with several tests, including the API 20E test, and the test was to applied samples humans (Al-Jumaily, and Zgaer,.2016) and cats (Meloni *et al*., 2014). The control of wound infection has become more challenging due to the antibiotic resistance of infections. This study showed that P. mirabilis that isolate form human and cat was sensitive to Gentamycin and agreed with Zafar and other (Zafar *et al*., 2019) On the other hand resistant to Ampicillin, Penicillin, Trimethoprim-Sulfamethoxazole, Streptomycin,Chloramphenicol, Amoxicillin/ clavulanic acid, Erythromycin,Tetracycline, Ofloxacin, Cefoxitin and Vancomycin this study agreed with (Zafar *et al*., 2019; Mohammed and Hamzah. 2021) The isolates showed MDR to antimicrobial agents and the MAR index was more than 0.2 indicates that isolates are at risk, according to (Krumperman,1983; Magiorakos *et al*., 2012).

4. Conclusion

P. vermicola causes mainly wound inflomation in humans and cats. The high resistance of Proteus mirabilis would lead to a major problem in healthcare in human and cats. It should be considered the Proteus mirabilis that is pathogenic bacteria like other Proteus spp. Prevelance of Proteus mirabilis from human and cats wound. Its use in subsequent studies is considered very important in wound inflomation. Preservation of samples in suitable liquid nutrient media due to unsuitable conditions.

Compliance with ethical standards

Statement of ethical approval

The current work was conducted for isolation and identification of Proteus mirabilis from humans and cats.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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