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RESEARCH ARTICLE

Isolation, Identification, and Antibiotics Susceptibility Determination of *Proteus* Species Obtained from Various Clinical Specimens in Erbil City

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ABSTRACT

Fifty-two *Proteus* isolates, (47) *Proteus mirabilis*, (4) *Proteus vulgaris,* and (1) *Proteus hauseri* are being isolated from (200) clinical specimens taken from patients admitted to different hospitals in Erbil city/ Kurdistan region of Iraq. Specimens were of urine, wounds swabs, burn swabs, vaginal swabs, ear swabs, eye swabs, and sputum. All isolates were identified depending on cultural, morphological, biochemical characteristics, and confirmatory VITEK 2 system. Furthermore, VITEK 2 (antibiotic susceptibility testing) panel was used to determine the antibiotic susceptibility of *Proteus* isolates, and the results showed that all isolates were entirely resistant to tetracycline and tigecycline (100%), but sensitive to meropenem. Furthermore, the present study reported a case of rare *Proteus* species – *P. hauseri* – isolated from a patient with urinary tract infection in Erbil City which characterized by no swarming on blood agar.

Keywords: Antibiotic resistance; Erbil city; *Proteus hauseri*; Proteus species; VITEK 2

INTRODUCTION

The genus *Proteus* is a Gram-negative rod-shaped bacterium belongs to the Enterobacteriaceae family, where it is placed in the tribe Proteeae, together with the genera *Morganella* and *Providencia* (Rosalski et al., 2012). Since this genus was first described in 1885 by German microbiologist Gustav Hauser, *Proteus*, and in particular *Proteus vulgaris*, has undergone a number of major taxonomic revisions (O'Hara et al., 2000a).

In1982, Hickman et al. separated *P. vulgaris* into three biogroups on the basis of indole production. Biogroup one was indole negative and represented a new species, *Proteus penneri*, while biogroups two and three remained together as *P. vulgaris*. The studies of O'Hara et al. (2000b) confirmed the existence of four genomospecies within *P. vulgaris* biogroup 3, which were called *Proteus* genomospecies 3, 4, 5, and 6. These authors have proposed that genomospecies three be named *Proteus hauseri.*

Currently, the genus *Proteus* consists of five species: *Proteus mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri,* and *Proteus myxofaciens*, as well as three unnamed Proteus genomospecies (O'Hara et al., 2000b). *P. myxofaciens* is the only Proteus species without any significance in the pathogenicity of humans (Janda et al., 2006).

The most defining microbiologic characteristic of *Proteus* species is their swarming phenomenon, a multicellular differentiation process of short rods to elongated swarmer cells. It allows the population of bacteria to migrate on a solid surface. Swarming appears macroscopically as concentric rings of growth emanating from a single colony or inoculum (Jacobsen et al., 2008).

Microorganisms belonging to genus *Proteus* are widely distributed in the natural environment. They can be found in polluted water, in soil, and manure, where they play an important role in decomposing organic matter of animal origin. Besides, the saprophytic mode of life in the natural environment and in the intestines of humans and animals, *Proteus* species, under favorable conditions, are able to cause a variety of opportunistic nosocomial infections (Feglo et al., 2010). This pathogen has a diverse mode of transmission and hence can cause infection in different anatomical sites of the body (Nita et al., 2014), including those of the urinary tract (causes complicated UTIs with a higher frequency compared to other uropathogens and formation of urinary stones), respiratory tract, ear, nose, skin, burns, and wounds, it may also cause gastroenteritis (Jacobsen et al., 2008).

The constant increase in the antibiotic resistance of clinical bacterial strains has become an important clinical problem

(Adamus-Bialek et al., 2013). The evolution and spread of various mechanisms of antimicrobial resistance among common human pathogenic members of Enterobacteriaceae are of increasing concern and lead to narrowing of available therapeutic options (Boucher et al., 2009). However, the multidrug-resistant strains of *Proteus* species have also been reported worldwide (Singla et al., 2015). They have the ability to resist several different types of antibiotics and called multi antibiotic resistant (Dadheech et al., 2015).

Therefore, this study is concerned with isolation and identification (ID) of *Proteus* species from various clinical samples taken from patients admitted to main hospitals in the Erbil City/Kurdistan region of Iraq, as well as determination the susceptibility patterns of these isolates to different antibiotics.

MATERIALS AND METHODS

Samples Collection

Two hundred clinical specimens were collected aseptically from patients with symptomatic infections admitted to different hospitals in Erbil City (Erbil Teaching Hospital, West Erbil Emergency Hospital, CMC Hospital, and PAR Hospital) during the period from October 1, 2018, to April 1, 2019. Specimens were of urine samples (123), wounds swabs (25), burn swabs (18), vaginal swabs (6), ear swabs (8), eye swabs (15), and sputum (5). The specimens were directly inoculated in Tryptone Soya Broth and streaked onto MacConkey agar and blood agar plates and incubated aerobically at 37°C for 24 h.

Identification of the Isolates

Isolates were identified depending on cultural, morphological, biochemical characteristics (Betty et al., 2007), and confirmatory VITEK 2 system using (ID) GN cards (bioM´erieux Inc. USA).

Antibiotic Susceptibility Testing (AST)

AST-N326 panels (bioM´erieux Inc. USA) were used to determine the antibiotic susceptibility.

The isolates were processed as per the manufacturer's instructions for ID and AST. The results were interpreted using VITEK 2 software version 08.01, and final results were obtained automatically.

RESULTS

Isolation of *Proteus* Species

Out of 200 clinical specimens of different infection sources, 52 isolates (26%) were identified as *Proteus* spp. They were isolated from 35 (28.45%) urine samples, 9 (36%) wound swabs, 5 (27.77%) burn swabs, 2 (33.33%) vaginal swabs, and 1 (20%) sputum cultures, while results showed complete absence of isolates from eye and ear swabs [Table 1].

The percentages of *Proteus* spp. isolated from various clinical specimens are presented in Figure 1. Of (52) *Proteus* isolates, *Proteus mirabilis* was the most common isolate accounting for 47 (90.4%), followed by *P. vulgaris* 4 (7.7%) and only one isolate (1.9%) of *P. hauseri,* were isolated.

Identification of *Proteus* Isolates

The isolates were first identified as related to the genus *Proteus* by swarming phenomenon on blood agar, the cultures' characteristic smell, and the pale appearance of bacteria (non-lactose fermenting) on the MacConkey agar.

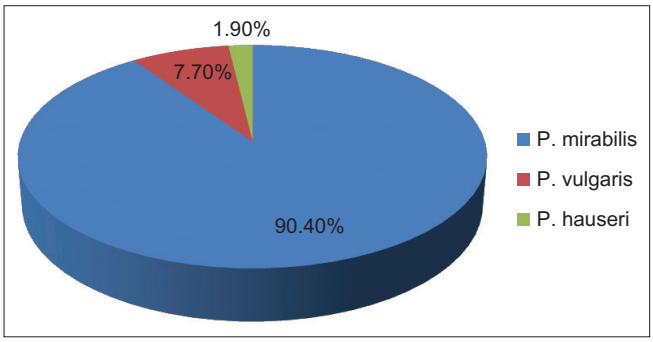

Figure 1: Percentage of *Proteus* species isolated from different specimens

Table 1: Isolation source and percentage of *Proteus* isolates

Isolation source	Number of samples	Number of *Proteus* spp. Isolated per source			Total number of *Proteus* isolates per source	Percentage of *Proteus* isolates per source
		P. mirabilis	*P. vulgaris*	*P. hauseri*		
Urine	123	33	1	1	35	28.45
Wounds	25	6	3	0	9	36.00
Burns	18	5	0	0	5	27.77
Eye	15	0	0	0	0	0.00
Ear	8	0	0	0	0	0.00
Vagina	6	2	0	0	2	33.33
Sputum	5	1	0	0	1	20.00
Total	200	47	4	1	52	26.00

P. mirabilis: *Proteus mirabilis*, *P. vulgaris*: *Proteus vulgaris*, *P. hauseri*: *Proteus hauseri*

Also by microscopic examination of the bacteria, which appeared as straight rods and Gram negative when it stained with Gram stain.

Several conventional biochemical tests were done to characterize the suspected *Proteus* isolates. The results indicated that these isolates were belonged to three *Proteus* species; *P. mirabilis*, *P. vulgaris,* and *P. hauseri*. All the (52) *Proteus* isolates showed positive results to the catalase, urease, and motility, but were negative to citrate and oxidase test. Forty-seven of the (52) *Proteus* isolates gave clearly negative results for indole and salicin fermentation tests and considered as *P. mirabilis*. On the other hand, *P. vulgaris* was represented by (4) isolates when such isolates gave positive results to indole and salicin fermentation tests. However, only one isolate gave a positive result for the indole test and negative for the salicin fermentation test and *P. hauseri* was suspected [Table 2].

Table 2: The biochemical identification results of *Proteus* isolates obtained by conventional tests

Test	*Proteus* isolates		
	*P. mirabilis** (*n*=47)	*P. vulgaris* (*n*=4)	*P. hauseri* (*n*=1)
Oxidase	–	–	–
Catalase	+	+	+
Urease	+	+	+
SIM test			
H2S Production	+	+	+
Indole	–	+	+
Motility	+	+	+
Salicin fermentation	–	+	–
Citrate	–	–	–

*n: Number of isolates, –: A negative result, +: A positive result.
P. mirabilis: *Proteus mirabilis*, *P. vulgaris*: *Proteus vulgaris*, *P. hauseri*: *Proteus hauseri*

For confirmation of the results, VITEK 2 system (ID) GN cards were used and the results are indicated in Table 3.

Antibiotic Susceptibility Determination of *Proteus* Isolates

The susceptibility patterns of the *Proteus* isolates are presented in Table 4. It was found that the more effective antibiotic against isolates was the Meropenem, where all *Proteus* isolates were sensitive to it. Adversely, the less effective antibiotics were tetracycline and tigecycline when they were resisted by all isolates. However, the effect of other antibiotics was variable among the *Proteus* isolates.

Antibiotic resistance profile [Figure 2] revealed that generally a vast of resistance was detected among the *P. mirabilis* isolates against the antibiotics used. It was found that out of (47) *P. mirabilis* isolates, 100% resistance property was found to piperacillin, aztreonam, tetracycline, and tigecycline; more than 90% resistance to piperacillin\tazobactam, ceftazidime, cefepime, imipenem, ciprofloxacin, and trimethoprim/sulfamethoxazole; and about 70% or less resistance pattern was identified to netilmicin, tobramycin, and levofloxacin.

This study also observed resistance of *P. vulgaris* isolates to tetracycline and tigecycline (100%), aztreonam and netilmicin (75%), cefepime and levofloxacin (50%), cefotaxime, amikacin, and ciprofloxacin (25%), while all *P. vulgaris* isolates were sensitive to other tested antibiotics.

On the other hand, *P. hauseri* isolate was found to be resistant to piperacillin, ceftazidime, aztreonam, imipenem, tetracycline, and tigecycline, as illustrated in Figure 2.

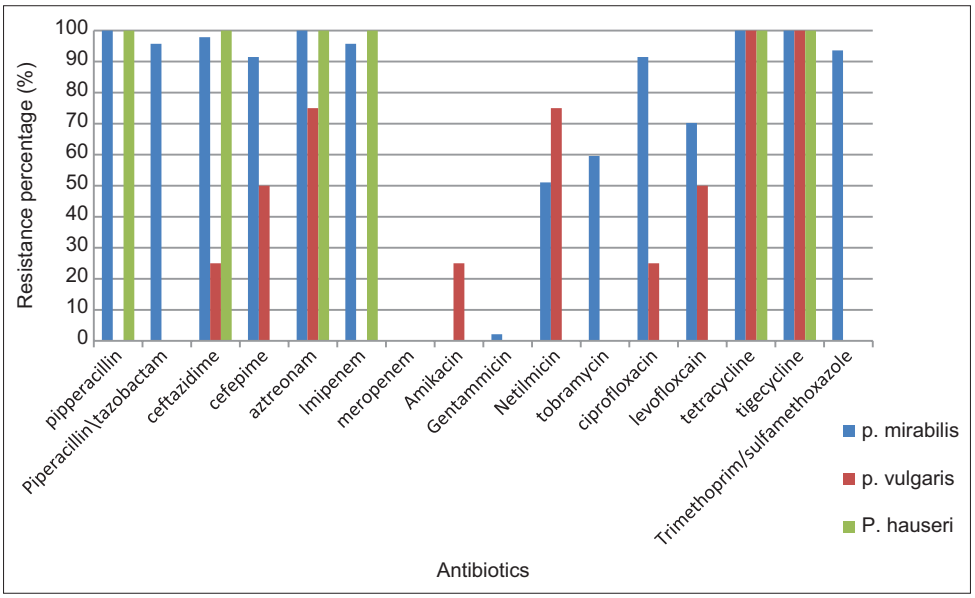

Figure 2: Antibiotic resistance profile of isolated *Proteus* species

Table 3: The biochemical identification results of *Proteus* isolates obtained with (ID) GN cards of VITEK 2 system

Well No.	Symbol/Test	*P. mirabilis*	*P. vulgaris*	*P. hauseri*
2	Ala-Phe-Pro-ARYLAMIDASE	–	–	–
3	ADONITOL	–	–	–
4	L-Pyrrolidonyl-ARILAMIDASE	–	–	–
5	L-Arabitol	–	–	–
7	D-CELLOBIOSE	–	–	–
9	BETA-GALACTOSIDASE	–	–	–
10	H2S PRODUCTION	+	+	+
11	BETA-N-ACETYL-GLUCOSAMINIDASE	–	–	–
12	GlutamylArylamidase pNA	–	–	–
13	D-Glucose	+	+	+
14	GAMMA-GLUTAMYL-TRANSFERASE	+	–	+
15	FERMENTATION-GLUCOSE	–	+	+
17	BETA-GLUCOSIDASE	–	–	–
18	D-MALTOSE	–	+	+
19	D-MANNITOL	–	–	–
20	D-MANNOSE	–	–	–
21	BXYL (BETA-XYLOSIDASE)	–	–	–
22	BETA-ALANINEARYLAMIDASE pNA	–	–	–
23	L-Proline ARYLAMIDASE	–	–	+
26	LIPASE	–	–	–
27	PALATINOSE	–	–	+
29	Tyrosine ARYLAMIDASE	–	–	+
31	UREASE	+	+	+
32	D-SORBITOL	–	–	–
33	SACCHAROSE/SUCRALOSE	–	+	+
34	d-TAGATOSE	–	–	–
35	D-TREHALOSE	+	–	–
36	CITRATE (SODIUM)	–	–	–
37	MALONATE	–	–	–
39	5-KETO-D-GLUCONATE	–	–	–
40	L-LACTATE alkalinization	–	–	+
41	ALPHA-GLUCOSIDASE	–	–	+
42	SUCCINATE alkalinization	–	–	+
43	Beta-N-ACETYL-GALACTOSAMINIDASE	–	–	–
44	ALPHA-galactosidase	–	–	–
45	PHOSPHATASE	+	–	+
46	Glycine Arylamidase	–	–	–
47	ORNITHINE DECARBOXYLASE	+	–	–
48	LYSINE DECARBOXYLASE	–	–	–
49	DECARBOXYLASE bASE	–	–	–
53	L-HISTIDINE assimilation	–	–	–
56	COUMARATE	+	+	+
57	BETA-GLUCORONIDASE	–	–	–
58	O/129 RESISTANCE	+	–	+
59	Glu-Gly-Arg-ARYLAMIDASE	–	–	–
61	L-MALATE assimilation	–	–	+
62	ELLMAN	–	–	+
64	L-LACTATE assimilation	–	–	–

Other well numbers between 1 and 64 not designated in this table are empty. (–) a negative result, (+) a positive result. *P. mirabilis*: *Proteus mirabilis*, *P. vulgaris*: *Proteus vulgaris*, *P. hauseri*: *Proteus hauseri*

DISCUSSION

Two hundred clinical specimens were screened for *Proteus* spp. It was found that 52 isolates (26%) were identified as *Proteus* spp. A near result was recorded by Al-Bassam and Al-Kazaz (2013) who indicated that the total isolation percentage of *Proteus* spp. from different clinical specimens was 28.57%, whereas the results were higher than those obtained by Feglo et al. (2010); Naz and Rasool (2013); Ahmed (2015); and Latif et al. (2017) who mentioned

Table 4: Antibiotic susceptibility patterns of *Proteus* isolates obtained with AST-N326 cards of VITEK 2 system

Antibiotics	Number of *Proteus* isolates with susceptibility								
	P. mirabilis (*n*=47)			*P. vulgaris* (*n*=4)			*P. hauseri* (*n*=1)		
	S	I	R	S	I	R	S	I	R
Piperacillin	0	0	47	4	0	0	0	0	1
Piperacillin\tazobactam	2	0	45	4	0	0	1	0	0
Ceftazidime	0	1	46	3	0	1	0	0	1
Cefepime	3	1	43	2	0	2	1	0	0
Aztreonam	0	0	47	1	0	3	0	0	1
Imipenem	2	0	45	4	0	0	0	0	1
Meropenem	47	0	0	4	0	0	1	0	0
Amikacin	44	0	3	3	0	1	1	0	0
Gentamicin	11	35	1	4	0	0	1	0	0
Netilmicin	23	0	24	1	0	3	1	0	0
Tobramycin	19	0	28	4	0	0	1	0	0
Ciprofloxacin	3	1	43	3	0	1	1	0	0
Levofloxcain	13	1	33	2	0	2	1	0	0
Tetracycline	0	0	47	0	0	4	0	0	1
Tigecycline	0	0	47	0	0	4	0	0	1
Trimethoprim/sulfamethoxazole	3	0	44	4	0	0	1	0	0

**n*: Number of isolates, S: Sensitive, I: Intermediate, R: Resistant. *P. mirabilis*: *Proteus mirabilis*, *P. vulgaris*: *Proteus vulgaris*, *P. hauseri*: *Proteus hauseri*

that *Proteus* spp. from clinical specimens represented (8.4%), (12.6%), (19%), and (12.6%), respectively. The reason for the difference in isolation percentages may be due to the differences in the size of samples, isolation sources, and number of hospitals surveyed.

Of (52) Proteus isolates, 47 isolates (90.4%) of different clinical specimens were identified as *P. mirabilis*. This result was agreed with Gonzalez and Bronze (2018) who reported that *P. mirabilis* causes (90%) of Proteus infections and can be considered a community-acquired infection; Feglo et al. (2010) and Al-duliami et al. (2011), who mentioned that *P. mirabilis* is more widespread than *P. vulgaris* in clinical infections. Furthermore, Auwaerter (2008) declared that *P. mirabilis* is the species most commonly recovered from humans, especially from urinary and wound infections. It accounts for 90% of all infections caused by the *Proteus* species.

As per our knowledge, only two articles have been published regarding the isolation of *P. hauseri*. The first article was by O Hara et al. (2000b), in which only two cases of *P. hauseri* out of 52 isolates were isolated, and they have not mentioned whether swarming was present or not. In the second article, Ostwal et al. (2016) isolated the third case of *P. hauseri* from the stool; there was no swarming on blood agar so that these isolates may be misdiagnosed. Our *P. hauseri* isolate also did not show swarming.

Results showed that the highest percentage of *Proteus* isolates from clinical specimens was isolated from wound swabs specimens representing about 36%. Being wound isolates were the highest percentage in the same trend with many results. This result agreed with similar studies conducted by Yah et al. (2001); Jones et al. (2003); Newman et al. (2006); Feglo et al. (2010); and Pandey and Tyagi (2013). In contrast with studies performed by Orett (1999); Reslinski et al. (2005); and Al-Bassam and Al-Kazaz (2013), which showed *Proteus* spp. to be more commonly in urine than in other clinical specimens.

In addition, high vaginal swabs from women with symptomatic vaginitis showed an isolation percentage of 33.33%. However, the picture is not clear in the case of *Proteus* in women with vaginitis due to small numbers of infected women involved in this study.

Furthermore, results showed a complete absence of isolates from the eye and ear swabs. This may be due to the season of collecting samples and the possible medication taken before sampling.

The effect of different antibiotics on *Proteus* isolates was investigated. Interestingly, these isolates showed different susceptibility toward antibiotics used in this study [Table 4]. It has been found that the majority of the isolates were multidrug-resistant since they were resistant to three antibiotics or more. *Proteus* species can harbor numerous plasmid and integron-mediated determinants of antimicrobial resistance (Hall and Collis, 1998). In line with the findings of this study, resistance of *Proteus* species (*P. mirabilis* and *P. vulgaris*) against antibiotics has been reported by Newman et al., 2006; Mordi and Momoh, 2009; Feglo et al., 2010; Bahashwan and El Shafey, 2013; Kibret and Abera, 2014; and Ahmed, 2015. Although some antibiotics to which *Proteus* species are known to be

sensitive, now they appear to be resistant or less effective. According to De Francesco et al. (2007), etiology and drug resistance change through time as well as may be due to random and improper use of these antibiotics. Our *P. hauseri* isolate was sensitive to meropenem, amikacin, ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole, and some other antibiotics, whereas the *P. hauseri* isolate of Ostwal et al. (2016) was only sensitive to meropenem. O'Hara's *P. hauseri* isolates were sensitive to amikacin, ciprofloxacin, gentamicin, as well as trimethoprim/sulfamethoxazole and resistant to tetracycline, which are close to some extent with our findings.

CONCLUSIONS

In this study, *P. mirabilis*, *P. vulgaris,* and *P. hauseri* are the species implicated in *Proteus* infections in Erbil City. *P. mirabilis* was predominant species among patients with *Proteus* infections and wounds recorded the highest percentage of *Proteus* isolation. Furthermore, a case of rare *Proteus* species – *P. hauseri* – isolated from a patient with urinary tract infection and characterized by no swarming on blood agar.

Results are recommended prescribing of Meropenem in the treatment of *Proteus* species as it is the most effective antibiotic against these bacteria *in vitro*. Moreover, the results indicated that the resistance of *Proteus* species to some antibiotics is increased due to improper use of these antibiotics. Hence, knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred.

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