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A Comparative Bacteriological and Molecular Study on Some Virulence Factor of *Proteus* spp Isolated from Clinical and Environment Specimens

Banaz M. Latif*, Salah. S. Zayn Aleabdyn and Ibraheem S. Ahmed

Iraq

Corresponding author

Abstract

In this study, 300 clinical samples were collected from different hospitals in kirkuk city, 38 isolates of *Proteus spp.* were identified from urine, wounds, Ear swab, burns, stool and vaginal swab by using different bacteriological and biochemical tests. It was found that 26 (68.4%) samples were identifies as *Proteus mirabilis* and 12 (31.5%) samples were *Proteus vulgaris*. Among the 38 isolates of *Proteus spp.*, 18 isolates (47%) were isolated from urine samples; 2 (5.2%) from wounds samples and 4 (10.5%) isolates from burns samples: 10 (26.3%) isolates from Ear swab; 3(7.8%) isolates from stool and 1 (2.6%) isolates from vaginal swab. Out of 38 *Proteus spp.*, 20 (52%) were from female and 18 (47%) from male at different age. Some important virulence factor to *P. mirabilis* isolates from patients was detected by using molecular techniques include PCR and it was found that all isolates gave positive result for *ure R* gene at 225bp 11 (100%) and 16s rRNA gene.

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Keywords

Proteus, PCR, virulence factors gene (ureR, 16srRNA).

Introduction

Proteus spp is Gram negative, facultative anaerobic, rod shaped bacteria. It has swarming motility, urease activity, do not usually ferment lactose**.** Since it belongs to the family of *Enterobacteriaceae*, general behaviors are applied on this genus: It is actively motile, non-spore forming, non-capsulated oxidase-negative, but catalase and nitrase positive. To identify *Proteus spp*, specific tests including positive urease and phenylalanine deaminase tests (Mordi and Momoh, 2009) and (Brooks *et al*., 2004) were used. *Proteus* is widely distributed in the natural environment. It can be found in polluted water and in soil and manure, where it plays an important role in decomposing organic matter of animal origin (Mordi and Momoh, 2009) and (Ro`zalski *et al*., 1997). The genus *Proteus* currently consists of five named species (*P. mirabilis*, *P. penneri*, *P. vulgaris*, *P. myxofaciens* and *P. hauseri*) and three unnamed genomo-species (*Proteus* genomo-species 4, 5, and 6), *P. vulgaris*, *P. mirabilis* and *P. penneri* are opportunistic human pathogens (Guentzel, 1996) and (O'hara *et al*., 2000).

P. mirabilis is often found as free-living organisms that are often found in soil, water, and the intestinal tract of many mammals, including humans. On (Brooks et al., 2004) and (Wassif *et al*., 1995) species level, indole is considered reliable, because it is positive for *P. vulgaris* but negative for *P. mirabilis* (Mordi and Momoh, 2009) and (Betty *et al*., 2007) *P. mirabilis* is the third most common (after *E. coli* and *Klebsiella pneumoniae*) cause of complicated UTI (causing 12% of infections) and the second most common (after *Providencia stuartii*) cause

of catheter-associated bacteriuria in the group of long-term catheterized patients (causing 15% of infections) (Wassif *et al*., 1995).

Isolation and identification

Through the period from September 2013 to March 2014, 300 clinical samples of urine, wounds, burns, ear swabs, stool and vaginal swabs were collected from patients. Samples were transferred to the lab for isolation and identification of *Proteus spp*. by using sterile equipment and media.

All samples were streaked on Blood agar, MacConkey agar. The plates were incubated aerobically at 37ºC for 24 hours. The isolates were identified depending on the microscopical feature by using Gram stain to detect their response to stain, shape and arrangement (Prescott *et al*., 2002) and (Mac *et al*., 2000). In addition, the morphological features on culture media such as Swarming on blood agar, Non lactose fermented growth on MacConkey agar also several of biochemical tests were used to identify the *Proteus* isolates, such as catalase, oxidase tests, indole, methyl red/ Voges-Proskauer (MR-VP) test, citrate utilization tests, urea test, motility test, gelatin liquefaction test and triple sugar iron agar test (Betty *et al*., 2007). In addition to use API 20.

Molecular detection of some virulence factors

We using PCR technique for detection of some virulence gene include (16 SrRNA, ureR).

Extraction of bacterial DNA

This method was applied according to the genomic DNA purification kit that supplemented by the manufacturing company (Geneaid, UK). The suspension containing DNA was stored at -20ºC until used as template for PCR.

PCR amplifications

Detection of virulence genes was performed by amplifying the genes by PCR. Descriptions and sequences of the PCR primers used in this study are show in table 1.

Antibiotic susceptibility test

Kirby-Bauer method was done according to (Betty *et al*., 2007) to carry out antibiotic susceptibility test against 25 different antibiotics. A sterile cotton swab was submerged into bacterial suspension standardized to match the turbidity of the 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml) by preparing serial dilutions of 18 hrs. Brain Heart Infusion culture of tested bacteria, third dilution was used after comparing it with the 0.5 McFarland turbidity standard.

The surface of Mueller Hinton agar plates were spread into four directions by the bacterial suspension, the plates were left for 10 min. to dry. Then, the antibiotic disks were placed by sterile forces on the agar and pressed firmly to ensure the contact with the agar. The plates were incubated at 37ºC for 18-24 hrs.

Inhibition zones developed around the antibiotic disks were measured by using a metric ruler in millimeters according to Clinical Laboratories Standards Institute (CLSI, 2011). The isolate was interpreted as susceptible, intermediate or resistant to a particular antibiotic by comparison with standard inhibition zones.

Results and Discussion

Out of 300 clinical specimens of different infection sources, 38 specimens (12.6 %) were isolated and identified as *Proteus spp.* These results agree with Naz and Rasool (2013) and Feglo *et al*., (2010) who mentioned that *Proteus spp.* from clinical specimens represented (12.6%), and (8.4%) respectively.

Twenty six specimens (68.4%) of different clinical specimens were identified as *Proteus mirabilis*, and12 specimens (31.5%) were identified as *Proteus vulgaris* as in figure 1.

These results were agree with Feglo *et al*., (2010), that mentioned that the percentage isolation for *Proteus mirabilis* was (61%), *Proteus vulgaris* was (30.5%) , but they showed that *Proteus mirabilis* are more widespread than *Proteus vulgaris* in clinical infections, because *Proteus mirabilis* is a part of normal flora of human beings and other mammalians that leads to contamination of water .

According to sex, out of 38 *Proteus spp.*, 20 (52%) were from female and 18 (47%) from male at different age.

These results agreed with (Feglo *et al*., 2010), who mentioned that the percentage of isolation from female was (57%) and from male was (43%) but they not agree

and showed (84.21%) percentage of isolation and (15.78%) isolation from males and females respectively.
Among 38 isolates of *Proteus spp.*, 18 isolates (47.3%) isolated from urinary tract infections (UTI), *Proteus mirabilis* was (61%), *Proteus vulgaris* was (38.8%), These results agreed with Ahmed (2015), who mentioned that *Proteus mirabilis and Proteus vulgaris* was (66%) (30.3 %) isolation from urinary tract infections (UTI). 2 (5.2%) from wounds samples and 4 (10.5%) isolates from burns samples: 10 (26.3%) isolates from Ear swape *Proteus mirabilis and Proteus vulgaris* was (80%) (20 %) These results agreed with (Al-duliami *et al*., 2011), who mentioned that *Proteus mirabilis* are more widespread than *Proteus vulgaris* in clinical infections. 3(7.8%) isolates from stool and 1(2.6%) isolates from high cervical as in figure 2 and table 2.

Virulance factor of *Proteus spp* showin table 3.

Table.1 The primer sequence that used in present study

Genes name	Primer sequence (5-3)	Size bp	Condition	Reference
16 s rRNA	F GGAAACGGTGGCTAATACCGCATAAT R GCAGCGCTAGGTGAGCCTAATGGG	101 bp	95°C 5min 94°C 30sec 60°C30sec 72°C 10sec 72°C 10min	Limanskiĭ *et al. 2005*
ureR	F GGTGAGATTTGTATTAATGG R ATAATCTGGAAGATGACGAG	225 bpb	94°C 4min 94°C 40sec 58°C1min 72°C 20 sec 72°C 10min	Zhang,W *et al.,2013*

Table.2 Percentage of *Proteus mirabilis* isolates from different sources of infections

sample	*Proteus spp*	*P.mirabilis*	Percentage%	*P.vulgaris*	Percentage%
Urine	18	11	61.11	7	38.88
Ear swpe	10	8	80	2	20
Stool	3	2	66.66	1	33.33
Wound	2	1	50	1	50
Burns	4	3	75	1	25
High cervical	1	1	100	0	0
totale	38	26	68.42%	12	31.57%

Table.3 The number of *Proteus* spp isolates particular virulence factor

Virulence Factors	No	Positive%	No	Negative%
Urease	38	100%	0	0%
Swarming	38	100%	0	0%
production Agglutination of RBCs	27	71 %	11	29%
Esterase Production	31	81.5 %	7	18.4 %
Protease production	34	89.4 %	4	10.5 %
Lipase production	27	71 %	11	28.9 %
β-Lactamase production	33	86.8 %	5	13%

Table.4 Antibiotics sensitivity of isolates of *Proteus* spp

Antibiotic	*con*	*Symble*	*No* ***Sensitive***	*% Percentage* ***Sensitive***
Amikacin	**(30)**	**AK**	**30**	78.94%
Ampicillin	**(10)**	**AM**	**6**	15.4%
Amoxicillin+clavulanic acid	**(30)**	**AMC**	**4**	10.52%
Cefotaxim	**(10)**	**CTX**	**5**	13.15%
Ceftazidime	**(10)**	**CAZ**	**3**	7.89%
Ciprofloxacin	**(10)**	**CIP**	**37**	97.36%
Gentamicin	**(10)**	**CN**	**31**	81.57%
Tetracycline	**(10)**	**TE**	**15**	39.47%
Ceftrixon	**(25)**	**CRO**	**25**	65.78%
Nalidixic acid	**(35)**	**N**	**30**	78.94%
Amoxicillin	**(10)**	**AX**	**21**	55.26%
Co-trimoxazol	**(125/33.75)**	**SXT**	**20**	52.36%

Fig.1 Percentage of *Proteus mirabilis* and *Proteus vulgaris* isolation

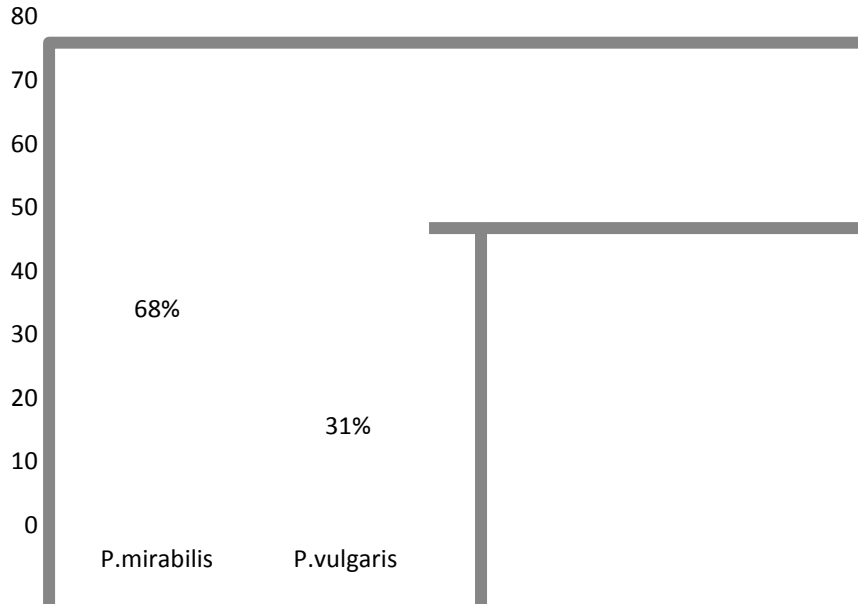


Fig.2 Percentage of *Proteus mirabilis* and isolates from different sources of infections

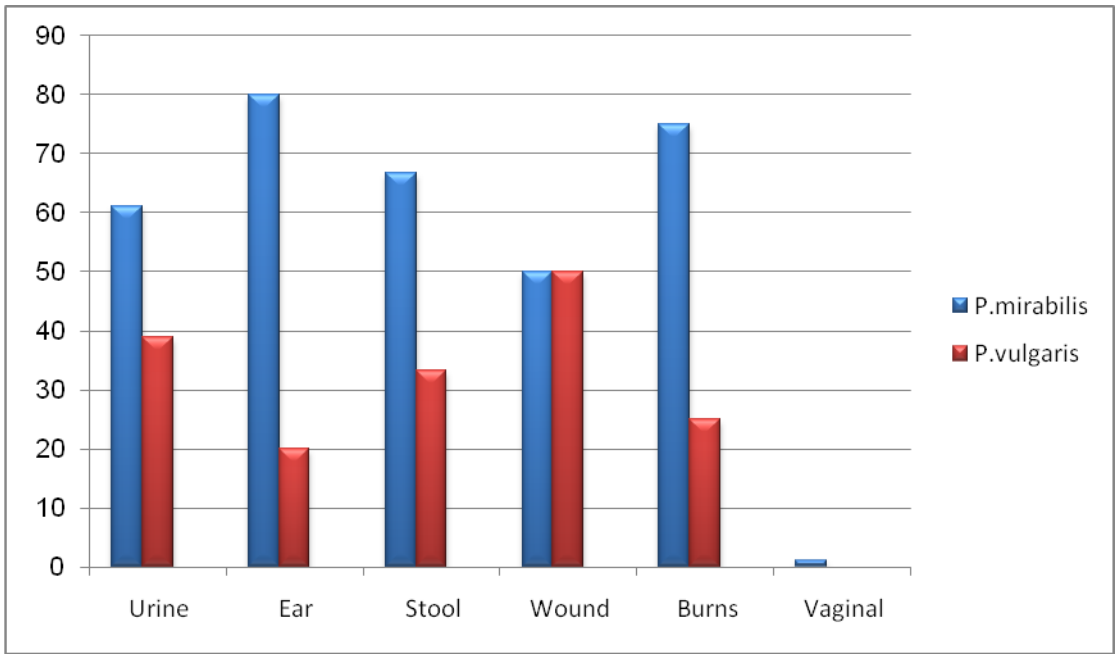
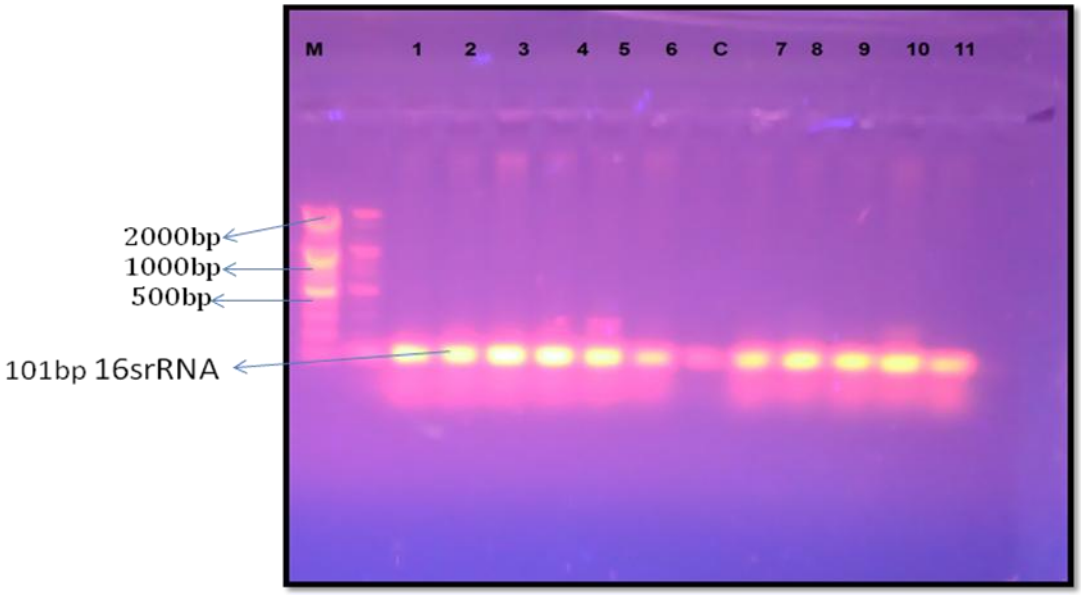
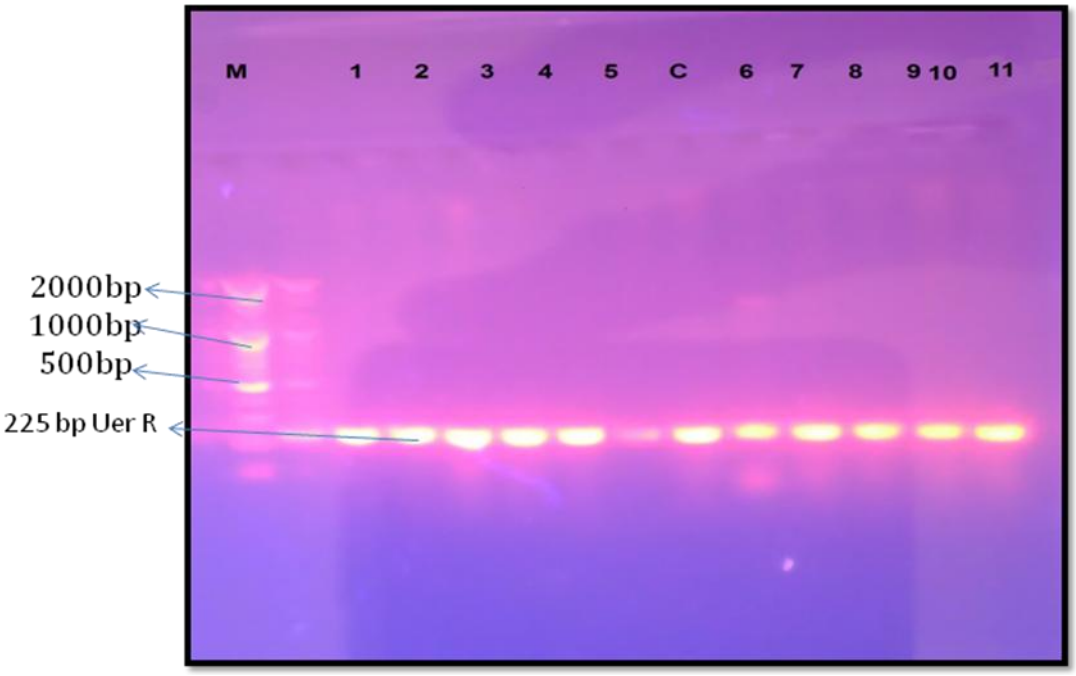


Fig.3a gel electrophoresis of 16 seRNA result



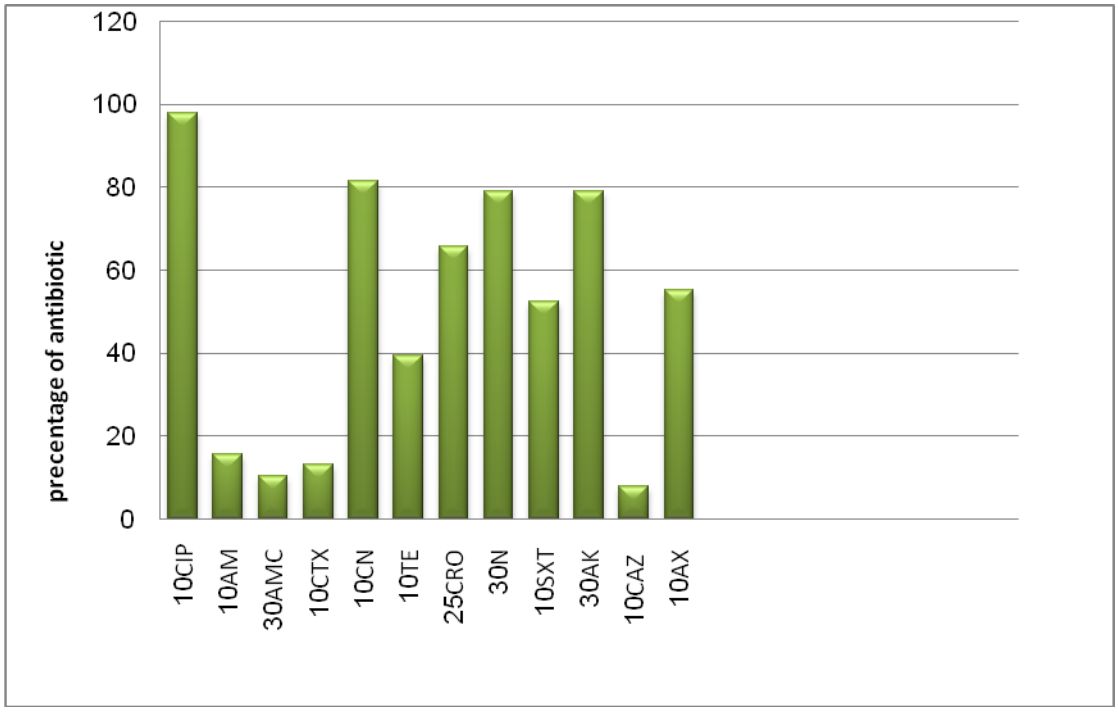
Line (1-11) positive band represents the isolates
M: Ladder with 1500bp
C: Negative control

Fig.3b gel electrophoresis of ureR result



Line (1-11) positive band represents the isolates
M: Ladder with 1500bp.
C: Negative control

Fig.4 Susceptibility of *Proteus* spp isolates to antibiotics



Molecular detection of virulence genes

The results of PCR amplification to specific *16 srRNA* primers indicated that 11(100%) of *Proteus mirabilis* isolates gave positive result at 101bp when compared with allelic ladder,

This result agreed with AI-Dawah (2015). They reported that PCR product was visible of*16 srRNA* gene was (100%) respectively (Figure 3).

The results of PCR amplification to specific *ureR* primers indicated that 11(100%) of *Proteus mirabilis* isolates gave positive result at 225bp when compared with allelic ladder,

This result agreed with AI-Dawah (2015) and Adnan *et al*., (2014). They reported that PCR product was visible of *ureR* gene was (100%) respectively. So the *ureR* gene is responsible for producing urease enzyme in a (100%) rate (Figure 3).

Sensitive antibiotic test

The susceptibility of 38 *P. mirabilis* isolates to 12 antibiotics (Ampicillin, Amoxicillin, Amikacin, Cefotaxime, Ceftazidime, Cefixime, Ciprofloxacin, Ceftrixon, Nalidixic acid, Tetracycline, Gentamicin, Co-trimoxazol) was investigated by using Kirby-Bauer method. Table (4) and Figure-6 indicated that *Poteus spp* isolates had variable degrees of Sensitive towards different Antibiotics.

From the above results, it can be noticed that *Proteus spp* isolates had moderate to low resistant to antibiotics, that inhibit protein synthesis, such as aminoglycosides (Amikacin, gentamycin), Nalidixic acid, and chloramphenicaol, while all isolates showed resistance to Ciprofloxacin, This results agreed with Mordi and Momoh (2009) who found the resistance to Ciprofloxacin was (0%).

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In addition isolates *Proteus spp* showed moderate to high resistance against antibiotics that inhibited the synthesis of cell wall such as ampencillin, ceftazidime and cefotaxim, amoxicillin+clavulanic acid. This was confirmed by the results of Bahashwan and Shafey (2013) hat found a high susceptibility of Ceftazidime and ampencillin in different isolates of *Proteus spp* and moderate to high resistant to some types of first generations of cephlaosporins.

Moreover, the results indicated that the resistance of *Proteus spp* to some antibiotics is increased with prescription of years, because of the wrong and random use of these antibiotics and increasing the rate of *Proteus*

infections. On the other hand, this bacteria had ability to produce β-lactamases, especially extended spectrum β-lactamases (ESBLs), as well as, their ability to transfer genetic elements carrying the genes of these enzymes, and number of mutations occur with these type of enzymes leading to increase resistance to antibiotic especially β-lactam, in addition to other mechanisms such as alteration the target site or alteration the access to the target site by modification of penicillin binding proteins (PBPs) (Rossolini *et al*., 2008; Cantón *et al*., 2012)

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