Detection of Antibiotics Sensitivity at different pH levels for Proteus mirabilis Isolated from patients with Urinary Tract Infections

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Abstract

One hundred and sixty-nine samples of urine have been collected through the period from December/2016 to May/2017. Were for isolation and identification of Proteus mirabilis. Isolated from urine in patients with UTI. Identification was done by growing on different media and biochemical tests as well as the antibiotics susceptibility were studied by using twenty types of antibiotics at acidic pH, neutral pH and alkaline or basic pH by disc diffusion method. These antibiotics were involved Ciprofloxacin, Amikcin, Meromenem, Imipenem, Ampicillin, Rifampin, Gentamicin, Trimethoprim, Tetracycline, Amoxicillin, Sulfamethoxazole, Carbacillin, Rifaximin, Penicillin-G, Oxolinic acid, Bacitracin, Clindamycin, Erthromycin, Novamicin and Aztreomycin. The Ciprofloxacin and Aztreomycin have high activity at both acidic and neutral pH, while the Amikcin, Meromenem, Imipenem and Ciprofloxacin have high activity at basicity pH against these bacteria.

Key words: Urinary Tract Infections, Proteus mirabilis, antibiotics, pH.

الخلاصة

تحديد الحساسية الدوائية عند مستويات مختلفة للأس الهيدروجيني لبكتريا المتقلبة الرائعة المعزولة من مرضى أصابات المسالك البولية

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المقدمة

تم جمع مائة وستون عينة من الادراز خلال الفترة الممتدة من شهر كانون الأول من العام 2016 إلى شهر أيار من العام 2017. وتم استخدام عينة الادراز من مرضى أصابات المسالك البولية ثم تم استخدام هذه العينات على أوساط زرعة مختلفة وتم تلخيصها بواسطة عدة فحوصات كيميائية كذلك درست الحساسية الدوائية لها باستخدام عrlen نوع من المضادات الحيوية. درست الحساسية الدوائية لهذه العينات عند الأس الهيدروجيني الحمضي، والمتعادل والفاعلي باستخدام طريقة انتشار الفرض. كانت أنواع المضادات الحيوية المستخدمة في هذه الدراسة هي السيبروفلكساسين، أميكاسين، ميروبين، إيميبايز، ابيسيلين، ريفامبين، جنتاميسين، تريمثيلوبريم، تتراسيكلين، أموكسيسيلين، سولفاميثوكساسول،
I. Introduction

Urinary tract infections (UTI) medically classified as complicated infections and uncomplicated infections [1]. The uncomplicated urinary tract infections typically affect the individuals who are healthy of otherwise and contain no structural or abnormalities of neurological urinary tract [2]. These infections are differentiated into lower urinary tract infections known as cystitis and upper urinary tract infections known as pyelonephritis [3]. The complicated urinary tract infections are defined as infections associated with factors that compromise the tract of urinary or defense of host, including urinary retention and urinary obstruction caused by neurological disease, renal failure, renal transplantation, immune- suppression, pregnancy and the presence of foreign bodies such catheters or other devices [4].

The urinary tract infections are the most common clinical indication and causes of severe problems for public health and causes economic losses, however; the infections of the urinary tract caused by the range of pathogenic bacteria but most prevalence pathogens included P. mirabilis, E. coli, K. pneumoniae, E. faecalis and Staph saprophyticus, and in addition, problem include increasing the range of antimicrobial resistance among these bacteria [5].

Bacteria Proteus is genera of gram negative bacteria and belonged to the family of Enterobacteriaceae, these bacteria were first time described by Hauser in 1885 and can be distinguished from other genera by their capacity to form swarms shape on agar surfaces media [6]. Proteus mirabilis is pathogens of gram negative bacteria and one of the most common pathogen in clinical specimens and can cause several types of hospital acquired diseases such as infections of the urinary tract, bloodstream and wounds [7]. Proteus mirabilis and many pathogens of gram negative and positive bacteria involved in urinary infections with capacity to form of biofilm [8]. And this increased rate of antibiotic resistance [9]. Some literatures reported that factors associated with antibiotics resistant prevalent in Proteus mirabilis [10].

Treatment and prevent urinary tract infection with successful start by using different drugs types not depends on pathogen susceptibility but also depend on numerous antibiotics and host factors such as pH of the urinary tract [11]. However, there are percent of little works has been done and described the regarding pH and antibiotic activity against pathogens and therefore only the few agents and
uropathogens species have been identified [12] [13]. This is surprising, in addition, that urinary pH varies substantially within and across patients and this relatively easy to clinically adjustment and effect of this on antibiotic activity against gram negative and positive bacteria [14].

**Aim of study**

Determination of the antibiotics sensitivity for bacteria *Proteus mirabilis* isolated from urinary tract infections at acidic, neutral and alkaline levels for the pH.

**II: Materials and Methods**

**Isolation of Proteus mirabilis bacteria**

Total one hundred and sixty-nine samples have been collected from urinary tract infection patients in the period from December/2016 to May/2017. These for isolation and identification of pathogenic *Proteus mirabilis* bacteria. The samples were involved urine samples from urinary tract infection for isolated of these bacteria.

**Identification of Proteus mirabilis bacteria**

The isolates were diagnosed as *Proteus mirabilis* species based on the findings of non-lactose fermenting colonies on macConkey agar with swarming on blood agar plate, characteristic fishy smell, microscopic examination show gram negative pleomorphic bacilli with active motility and the results of biochemical tests were negative for Gram stain, Oxidase and Indol. While, were positive for Catalase, Methyl red, Voscproscouer, Citrate and Urease. In addition, fermentation of glucose (with acid and gas) [15] [16].

**Antibiotic susceptibility test**

Muller Hinton agar plates have been used for identifying the susceptibility of antibiotics, this carried out by technique of disc diffusion method according to Bauer [17]. And the pH of this medium was adjusted at acidic pH with 0.1N of HCL, neutral pH and alkaline pH with 0.1N of NaOH by use of the PH meter.

**III: Results**

The one hundred and sixty-nine samples have been collected from urinary tract infected patients, one hundred and thirty-three samples were positively growing and found the thirty six isolates were identified as *Proteus mirabilis* from all isolates. These bacteria were identified by grown on different media and screened by several biochemical tests.
Figure: 1. Percentage of *Proteus mirabilis* identified isolated from UTI.

Figure (1) the number for total samples was (169) as well as the number and percent of positively growing samples and *Proteus mirabilis* identified samples isolates were (133; 78.69 %) and (36; 21.31 %) respectively from all samples.

Figure: 2. Antibiotics inhibition zones

Figure (2) the antibiotics zones formed on Muller Hinton agar that cultured with *Proteus mirabilis*, as well as explained the Ciprofloxacin antibiotic has largest zone after 24 h from incubation period at 37°C.
Table 1. The number and percent of resistant and sensitive isolates of *Proteus mirabilis* against different types of antibiotic at pH (5.5)

| No. | Antibiotic types  | Number of all Isolates | Number of resistant Isolates | Resistant percent (%) | Number of Sensitive Isolates | Sensitive percent (%) |
|-----|-------------------|------------------------|------------------------------|------------------------|-----------------------------|-----------------------|
| 1   | Amikcin30 μg      | 35                     | 13                           | 37.142%                | 22                          | 62.857%               |
| 2   | Meromenem10 μg    | 35                     | 9                            | 25.714%                | 26                          | 74.285%               |
| 3   | Imipenem10 μg     | 35                     | 18                           | 51.428%                | 17                          | 48.571%               |
| 4   | Ampicillin10 μg   | 35                     | 35                           | 100%                   | 0                           | 0%                    |
| 5   | Rifampin5 μg      | 35                     | 29                           | 82.857%                | 6                           | 17.142%               |
| 6   | Gentamicin10 μg   | 35                     | 20                           | 57.142%                | 15                          | 42.857%               |
| 7   | Ciprofloxacin5 μg | 35                     | 3                            | 8.571%                 | 32                          | 91.482%               |
| 8   | Trimethoprim10 μg | 35                     | 28                           | 80%                    | 7                           | 20%                   |
| 9   | Tetracycline30 μg | 35                     | 35                           | 100%                   | 0                           | 0%                    |
| 10  | Amoxicillin15 μg  | 35                     | 35                           | 100%                   | 0                           | 0%                    |
| 11  | Erthromycin15 μg  | 35                     | 35                           | 100%                   | 0                           | 0%                    |
| 12  | Novamicin30 μg    | 35                     | 27                           | 77.142%                | 8                           | 22.857%               |
| 13  | Aztreomycin30 μg  | 35                     | 4                            | 11.428%                | 31                          | 88.571%               |
| 14  | Sulfamethoxazole25 μg | 35 | 32                           | 91.428%                | 3                           | 8.571%                |
| 15  | Carbnicillin100 μg | 35 | 35                           | 100%                   | 0                           | 0%                    |
| 16  | Rifaximin40 μg    | 35                     | 24                           | 68.571%                | 11                          | 31.428%               |
| 17  | Penicillin-G10 μg | 35                     | 35                           | 100%                   | 0                           | 0%                    |
| 18  | Oxolinic acid2 μg | 35                     | 31                           | 88.571%                | 4                           | 11.428%               |
| 19  | Bacitracin10 μg   | 35                     | 28                           | 80%                    | 7                           | 20%                   |
| 20  | Clindamycin5 μg   | 35                     | 31                           | 88.571%                | 4                           | 11.428%               |

Table (1) the *Proteus mirabilis* isolates at pH = (5.5) were resistant to (Ampicillin), (Tetracycline), (Amoxicillin), (Erthromycin) and (Penicillin) antibiotics, which all have numbered and percent of resistant isolates were (35) and (100%), whereas these bacteria were less resistant to (Ciprofloxacin) and (Aztreomycin) antibiotics at same pH which have numbered and percent of sensitive isolates were (32), (91.482%) and (31), (88.571%) respectively.
Table 2. The number and percent of resistant and sensitive isolates of *Proteus mirabilis* against different types of antibiotic at pH (7)

| No. | Antibiotic types   | pH = 7     | Number of all Isolates | Number of resistant Isolates | Resistant percent (%) | Number of Sensitive Isolates | Sensitive percent (%) |
|-----|-------------------|------------|-------------------------|-----------------------------|-----------------------|-----------------------------|-----------------------|
| 1   | Amikcin 30 μg     |            | 35                      | 8                           | 22.857%              | 27                          | 77.142%              |
| 2   | Meromenem 10 μg   |            | 35                      | 8                           | 22.857%              | 27                          | 77.142%              |
| 3   | Imipenem 10 μg    |            | 35                      | 11                          | 31.428%              | 24                          | 68.571%              |
| 4   | Ampicillin 10 μg  |            | 35                      | 35                          | 100%                 | 0                           | 0%                   |
| 5   | Rifampin 5 μg     |            | 35                      | 29                          | 82.857%              | 6                           | 17.142%              |
| 6   | Gentamicin 10 μg  |            | 35                      | 21                          | 60%                  | 14                          | 40%                  |
| 7   | Ciprofloxacin 5 μg|            | 35                      | 3                           | 8.571%               | 32                          | 91.428%              |
| 8   | Trimethoprim 10 μg|            | 35                      | 28                          | 80%                  | 7                           | 20%                  |
| 9   | Tetracycline 30 μg|            | 35                      | 35                          | 100%                 | 0                           | 0%                   |
| 10  | Amoxicillin 15 μg |            | 35                      | 35                          | 100%                 | 0                           | 0%                   |
| 11  | Erthromycin 15 μg |            | 35                      | 35                          | 100%                 | 0                           | 0%                   |
| 12  | Novamicin 30 μg   |            | 35                      | 27                          | 77.142%              | 8                           | 22.857%              |
| 13  | Aztreomycin 30 μg |            | 35                      | 4                           | 11.428%              | 31                          | 88.571%              |
| 14  | Sulfamethoxazole 25 μg | | 35                      | 30                          | 85.714%              | 5                           | 14.285%              |
| 15  | Carbenicillin 100 μg |       | 35                      | 31                          | 88.571%              | 4                           | 11.428%              |
| 16  | Rifaximin 40 μg   |            | 35                      | 19                          | 54.285%              | 16                          | 45.714%              |
| 17  | Penicillin-G 10 μg |           | 35                      | 33                          | 94.285%              | 2                           | 5.714%               |
| 18  | Oxolinic acid 2 μg|            | 35                      | 28                          | 80%                  | 7                           | 20%                  |
| 19  | Bacitracin 10 μg  |            | 35                      | 16                          | 45.714%              | 19                          | 54.285%              |
| 20  | Clindamycin 5 μg  |            | 35                      | 31                          | 88.571%              | 4                           | 11.428%              |

Table (2) the *Proteus mirabilis* isolates at pH (=7) were resistant to (Ampicillin), (Tetracycline), (Amoxicillin), (Erthromycin) and (Penicillin) antibiotics which all have number and percent of resistant isolates were (35) and (100%), whereas these bacteria were less resistant to (Ciprofloxacin) and (Aztreomycin) antibiotics at same pH which have number and percent of sensitive isolates were (32), (91.482%) and (31), (88.571%) respectively.
Table: 3. The number and percent of resistant and sensitive isolates of *Proteus mirabilis* against different types of antibiotic at pH (8.5)

| No. | Antibiotic types   | pH = 8.5 | Number of all Isolates | Number of resistant Isolates | Resistant percent (%) | Number of Sensitive Isolates | Sensitive percent (%) |
|-----|--------------------|----------|------------------------|-------------------------------|------------------------|----------------------------|------------------------|
| 1   | Amikcin 30 µg      |          | 35                     | 3                             | 8.571%                 | 32                         | 91.428%               |
| 2   | Meropenem 10 µg    |          | 35                     | 4                             | 11.428%                | 31                         | 88.571%               |
| 3   | Imipenem 10 µg     |          | 35                     | 4                             | 11.428%                | 31                         | 88.571%               |
| 4   | Ampicillin 10 µg   |          | 35                     | 35                            | 100%                   | 0                          | 0%                     |
| 5   | Rifampin 5 µg      |          | 35                     | 29                            | 82.857%                | 6                          | 17.142%               |
| 6   | Gentamicin 10 µg   |          | 35                     | 31                            | 88.571%                | 4                          | 11.428%               |
| 7   | Ciprofloxacin 5 µg |          | 35                     | 0                             | 0%                     | 35                         | 100%                  |
| 8   | Trimethoprim 10 µg |          | 35                     | 30                            | 85.714%                | 5                          | 14.285%               |
| 9   | Tetracycline 30 µg |          | 35                     | 35                            | 100%                   | 0                          | 0%                     |
| 10  | Amoxicillin 15 µg  |          | 35                     | 35                            | 100%                   | 0                          | 0%                     |
| 11  | Erthromycin 15 µg  |          | 35                     | 35                            | 100%                   | 0                          | 0%                     |
| 12  | Novamicillin 30 µg |          | 35                     | 12                            | 34.285%                | 23                         | 65.714%               |
| 13  | Aztreomycin 30 µg  |          | 35                     | 15                            | 42.857%                | 20                         | 57.142%               |
| 14  | Sulfamethoxazole 25 µg |    | 35                     | 30                            | 85.714%                | 5                          | 14.285%               |
| 15  | Carbicillin 100 µg |          | 35                     | 31                            | 88.571%                | 4                          | 11.428%               |
| 16  | Rifaximin 40 µg    |          | 35                     | 18                            | 51.428%                | 17                         | 48.571%               |
| 17  | Penicillin-G 10 µg |          | 35                     | 34                            | 97.142%                | 1                          | 2.857%                |
| 18  | Oxolinic acid 2 µg |          | 35                     | 30                            | 85.714%                | 5                          | 14.285%               |
| 19  | Bacitracin 10 µg   |          | 35                     | 29                            | 82.857%                | 6                          | 17.142%               |
| 20  | Clindamycin 5 µg   |          | 35                     | 33                            | 94.285%                | 2                          | 5.714%                |

Table (3) the *Proteus mirabilis* isolates at PH = (8.5) were resistant to (Ampicillin), (Tetracycline), (Amoxicillin), (Erthromycin) and (Penicillin) antibiotics which all have number and percent of resistant isolates were (35) and (100%), whereas these bacteria were less resistant to (Ciprofloxacin) and (Amikcin) antibiotics at same pH which have number and percent of sensitive isolates were (35), (100%) and (32), (91.428%) respectively.
Figure: 3. Comparison between the number of *Proteus mirabilis* resistant isolates at pH (5.5, 7 and 8.5)

Figure (3) the comparison between the number of *Proteus mirabilis* resistant isolates at pH (5.5, 7 and 8.5) were more resistant against (Clindamycin), (Oxolinic acid), (Penicillin-G), (Carbicillin), (Sulfamethoxazole), (Erthromycin), (Amoxicillin), (Tetracycline), (Trimethoprim) and (Ampicillin).

Figure: 4. Comparison between the percent of *Proteus mirabilis* sensitive isolates at pH (5.5, 7 and 8.5)

Figure (4) the comparison between the percent of *Proteus mirabilis* sensitive isolates at pH (5.5, 7 and 8.5) were more sensitive against (Ciprofloxacin), (Imipenem), (Meromenem), (Amikcin) and (Aztreomycin).

Figure (1) showed the percent of *Proteus mirabilis* identified isolates isolated from urinary tract infection was (21.31%), and figure (2) explained the inhibition zones of different antibiotics types with different sizes against these bacteria.
Table (1) found the number and percent of resistant isolates of *Proteus mirabilis* against types of antibiotic at pH (5.5) were higher at, Ampicillin 35(100%), Rifampin 29(82.857%), Trimethoprim 28(80%), Tetracycline 35(100%), Amoxicillin 35 (100%), Erthromycin 35(100%), Novaminic 27(77.142%), Sulfamethoxazole 32(91.428%), Carbnicillin 35(100%), Rifaximin 24(68.571%), Penicill-G 35(100%), Oxolinic acid 31(88.571%), Bacitracin 28(80%) and Clindamycin 31(88.571%). (table: 2) at pH (7) were higher at Ampicillin 35(100%), Rifampin 29(82.857%), Trimethoprim 28(80%), Tetracycline 35(100%), Amoxicillin 35 (100%), Erthromycin 35(100%), Novaminic 27(77.142%), Sulfamethoxazole 30(85.714%), Carbnicillin 31(88.571%), Rifaximin 19(54.285%), Penicill-G 33(94.285%), Oxolinic acid 28(80%), Bacitracin 16(45.714%) and Clindamycin 31(88.571%). And (table: 3) at pH (8.5) were higher at Ampicillin 35(100%), Rifampin 29(82.857%), Trimethoprim 30(85.714%), Tetracycline 35(100%), Amoxicillin 35 (100%), Erthromycin 35(100%), Novaminic 12(34.285%), Sulfamethoxazole 30(85.714%), Carbnicillin 31(88.571%), Rifaximin 18(51.428%), Penicill-G 34(97.142%), Oxolinic acid 30(85.714%), Bacitracin 29(82.857%) and Clindamycin 33 (94.285%).

Table (1) explained the number and percent of sensitive isolates of *Proteus mirabilis* against types of antibiotic at pH (5.5) were higher at Amikcin 22(62.857%), Meromenem 26(74.285%), Imipenem 17(48.571%), Gentamicin 15(42.857%), Ciprofloxacin 32(91.482%) and Aztreomycin 31(88.571%). (table: 2) at pH (7) were higher at Amikcin 27(77.142%), Meromenem 27(77.142%), Imipenem 24(68.571%), Gentamicin 14(40%), Ciprofloxacin 32(91.428%) and Aztreomycin 31(88.571%). And (table: 3) at pH (8.5) were higher at Amikcin 32(91.428%), Meropenem 31(88.571%), Imipenem 31(88.571%), Gentamicin 4(11.428%), Ciprofloxacin 35(100%) and Aztreomycin 20(57.142%).

Figure (3), comparison between the number of *Proteus mirabilis* resistant isolates at pH (5.5, 7 and 8.5). As well as figure (4), comparison between percent of *Proteus mirabilis* sensitive isolates at pH (5.5, 7 and 8.5). These figures showed the presence of differences between responses of these bacteria against different antibiotic types at different pH concentrations.

IV: Discussion

When compared these results (figure:1) with the results for other studies, found the current results were agreed with Salih *et al* who found the percent of the *Proteus mirabilis* isolates from urinary tract infection was (21.7%) [18]. And disagreed with Abuhandan *et al* who found the percent of the *Proteus mirabilis* isolates from urinary tract infection was (8.4%) [19].

And when compared the present results (tables: 1, 2 and 3) with the results for other researchers, found the results for Cernohorska and Chvilova who found the resistant isolates of *P. mirabilis* were against Ampicillin 82(38.5 %), Gentamicin 54(25.4 %), Aztreomycin 8(3.8 %) and no resistance to Imipenem as well as to Meropenem [20]. The results for Pandey *et al* who found highly susceptible to Gentamycin, Amikacin and Ciprofloxacin as well as exhibited resistance to ampicillin [21]. Results for Rashmi who found (100%) sensitive to Imipenem and (50%) to Amikacin [22]. Results for Feglo *et al* who found generally susceptible to Gentamicin and Amikacin while exhibited resistance to Ampicillin
and Tetracycline [23]. And results for Singla et al. who found the number and percent of susceptible Proteus mirabilis isolates were Ampicillin 0(0.0%), Gentamicin 51(71.4%), Amikacin 62(88.5%), Ciprofloxacin 61(87.1%), Imipenem 53(75.7%), Meropenem 67(92.8%), While the resistant isolates were Ampicillin 70(100%), Gentamicin 19(28.5%), Amikacin 8(11.4%), Ciprofloxacin 9(12.8%), Imipenem 17(24.2%), Meropenem 3(4.3%) and Aztreomycin 65(92.8%), [24]. (Singla et al., 2015). In study for Philips who found this bacteria not susceptible to Erythromycin, Ampicillin and Amoxicillin but susceptible to Ciprofloxacin and Gentamicin [25].

The pH for urinary tract can widely change from the acidic environment equal to 4.5 with alkaline environment equal to 8 [26]. And the values outside this range are may be rare and induce damage for tissue and formation of abnormal components like stones and salts [14]. However, Foster and Woodruff are first concluded that pH could influence the activity of Penicillin [27]. Indeed, little work has been done for investigated the effect of pH on antibiotic activity [14].

The fluoroquinolones are widely used in treatment of urinary tract for both pyelonephritis and complicated infections [28]. However, the rates of resistance are still low in Europe and North America, but may trend of increasing resistance and this suggesting changes for its utility [29] [30]. Investigated studies in previous for these agents shown higher activities at highest of pH [11] [13]. This because increased interactions with membrane lipids and porins at high pH and this favorite for increase of antibiotic accumulation inside bacteria [31] [32]. This supports using alkalinizing agents with these antibiotics to increase its bactericidal activity [13]. However, in studying for Irwin et al. who found the elevation pH of media from 5 to 9 caused a decrease in activity of fluoroquinolones against P. mirabilis, but the rates of kill for these antibiotics were most rapid at pH 7 [33].

Gentamicin antibiotic commonly used in urinary tract treatment with differences in its activity between pH (5 and 8) [14]. Studies in previously involving numerous of microorganisms have similar findings [34] [35]. However, because the increase of electrical potential across the membrane of bacteria at higher pH leading to elevate of antibiotic uptake [34]. Studies have shown uptake of this antibiotic is reduced under conditions of alkaline [36].

The trimethoprim and sulphamethoxazole activity against enterococci with higher pH, no depth in analysis concerning these agents and pH could be present [37]. b-Lactams antibiotics are generally not recommended as first line therapy for treatment urinary tract infections unless certain pathogens are implicated [29].

Conclusions

The activity of the antibiotics against Proteus mirabilis bacteria were, Ciprofloxacin equal 91.482% at both acidic and neutral pH, whereas the Amikcin and Ciprofloxacin equal 91.428% and 100% respectively at basicity pH.
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