Bacteriological Study Of Proteus Mirabilis Isolated From Different Clinical Samples

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ABSTRACT

Background: This study focuses on isolating and identifying 17 strains of Proteus mirabilis from a total of 135 clinical samples obtained from patients who visited Hila Teaching Hospital between October 2014 and February 2015. The isolates collected from patients diagnosed with urinary tract infections accounted for 11 cases or 64.7% of the total. In contrast, patients with ear infections contributed 3 cases, making up 17.6% of the total. In addition, the isolates acquired from patients with diarrhea, wound, and vaginal infection each accounted for 1 (5.9%) of the total cases. The collected isolates have been cultivated on specialized media and identified using biochemical reactions. Some virulence factors of all isolates are studied, and the results showed that all bacterial isolates 17(100%) produced hemolysin, urease, Bacteriocin (proticin), and swarming, while 16(94.1%) of isolate produced beta-lactamase. Antibiogram of P. mirabilis isolates has been studied and it has been found that all isolates were entirely resistant to Imipenem 17(100%), while most of the P. mirabilis isolates were sensitive to Ertapenem so the resistant only 3(17.6%). Furthermore, the resistance of isolates to Amoxicillin and Cefotaxime 14(82.3%), also 9(52.9%) of them were resistant to Amoxiclave, 11(64.7%) of them were resistant to Ceftriaxone and 4(23.5%) of them were resistant to Cefepime Clavulanic acid.

Keywords: Proteus Mirabilis, Infections Antibiogram , Virulence factors and Resistant.

INTRODUCTION

Proteus mirabilis is a gram-negative, rod-shaped bacteria, non-spore-forming and facultative anaerobic, belonging to the Enterobacteriaceae family, this bacterium is responsible for a variety of diseases, with a higher prevalence in severe urinary tract infections and bacteremia. It impacts individuals with anatomical defects, immunodeficiency, and ongoing urinary catheterization (¹). In addition to urinary tract infections, P. mirabilis is also linked to opportunistic infections affecting the respiratory system, wounds, burns, skin, eyes, ears, nose, and gastroenteritis.
In addition, it can induce an autoimmune disease in individuals who have a hereditary predisposition to develop rheumatoid arthritis \(^2, 3\). The medical significance of this organism lies in its capacity to generate a diverse range of extracellular enzymes, such as urease. Urease is responsible for the development of bladder and kidney stones, which in turn hinder the effectiveness of antibiotic treatment. Furthermore, hemolysin exhibits cytotoxicity towards urinary tract epithelial cells\(^4\). P. mirabilis exhibits various virulence factors that play a role in infection, such as adhesins, flagella, toxins, quorum-sensing, enzymes, and immunological invasion \(^5\). Urease plays a crucial role in the pathogenesis of \(P.\) mirabilis. This enzyme facilitates the creation of kidney and bladder stones, as well as the process of encrusting or obstructing indwelling urinary structures.

Proteus is found as normal flora in the intestines of humans and animals, it is widely distributed in nature as saprophytes and exists in decomposing animal matter, sewage, manure soil, and feces of humans and animals. It opportunistic pathogen, when it colonizes areas out of its natural habitat, commonly responsible for urinary and septic infections, often nosocomial\(^7\).

In infants, besides urinary tract infections, \(P.\) mirabilis can also cause neonatal meningoencephalitis, \(P.\) mirabilis has a role in empyema and osteomyelitis disease\(^8\). Proteus has been shown to play a role in rheumatoid arthritis (RA). Patients with this disease carry a raised amount of antibodies against \(P.\) mirabilis in the bloodstream, while antigens to other bacteria are not increased in the blood \(^9\). \(P.\) mirabilis can cause an autoimmune effect by mimicry of a human epitope that is only expressed in genetically predisposed humans to develop RA \(^3\). Moreover, \(P.\) mirabilis causes otitis media, this disease is common in children and adults, and in these cases may develop of brain abscess; also it is one of the upper respiratory tract infections like sinusitis and pharyngitis \(^10\). \(P.\) mirabilis have been known to inhabit the skin and mucous of patients and healthy personnel working in these places which may be the primary vectors for pathogenicity\(^11\).

MATERIALS AND METHODS

Isolation and Identification

A total of 135 samples were obtained from patients with various clinical conditions (urine, otitis media, diarrhea, vaginal, and wound infections) who were admitted to Hila Teaching Hospital between October 2014 and February 2015. Every sample was introduced to specific culture media and classified based on biochemical responses, following the diagnostic protocols outlined\(^12\).

Bacteriological Detection of Some Virulence Factors

Hemolysin production: The process involves introducing bacterial isolates into a blood agar medium and then placing it in an incubator at a temperature of 37oC for 24 hours. The presence of a distinct area devoid of red blood cells surrounding the colonies indicates complete hemolysis (\(\beta\)-hemolysis), while a greenish area surrounding the colonies indicates partial hemolysis (\(\alpha\)-hemolysis). No change in the medium indicates no hemolysis (\(\gamma\)-hemolysis) \(^12\).

Urease production:

This test is carried out by inoculating urea medium with bacterial growth. The tubes
were incubated for 18 hrs. at 37°C. The color change from medium to pink indicated a positive result (13).

**Bacteriocin (Proticin) production:**

This test is performed using the cup assay method as described by Al-Qassab and Al-Khafaji (1992)(14).

**Antimicrobial susceptibility test:**

**Disc diffusion test:** After identification by phenotypic methods, antibiotic susceptibility was performed for each isolate by Kirby – Bauer disc diffusion method, the diameter of the zone of inhibition was measured by ml and compared to standard results recommended by (clinical laboratory standards institute documentation) CLSI guidelines.

**Detection of β-lactamase production:**

This test is performed for all isolates that are resistant to β-lactam antibiotics, according to the Rapid Iodometric Method(15).

**RESULTS AND DISCUSSION**

**Isolation of P. mirabilis**

According to this study, the majority of P. mirabilis isolates (64.7%) came from urine, while a smaller proportion (17.6%) came from otitis media. During the stool examination, both wound and vaginal samples showed a prevalence of 1, accounting for 5.9% each. The proximity of the anal hole to the vagina and urethra is the cause of urinary tract and vaginal infections.

**Bacteriological Detection of Virulence Factors:**

**Urease production:**

Urease production by Proteus mirabilis isolates in the present study is (100%), these results are shown in Table (3-1). The results of the present study are in agreement with the results of (16), who reported that (100%) Proteus mirabilis isolates showed strong production of urease from any source.

**Hemolysin production:**

The current study is found that all isolates (100%) of Proteus mirabilis able to produce alpha-hemolysin on blood agar, the results are shown in a table (3-1). The results of this study are in agreement with the results of Liaw et al., (2004) reported cell membrane-associated hemolysin activity as a dominant virulence factor in P. mirabilis (17).

The present study agrees with the result of AL-Jumaa, (2011) that showed all isolates of P. mirabilis (100%) could produce Hemolysine (1), but unlike to reported all isolates produce β-hemolysis. While the result of AL-Salihi shows hemolysin production by P. mirabilis 41(47.7%).

**Swarming behavior:**

All Proteus mirabilis isolates in the current study demonstrated 100% swarming motility when cultured on agar plates. The results are displayed in Table 3-1. The results of this study agree with those of Iwalokun et al. (2004) (18) and EL-Baghdadi et al. (2009)(19), who found that when grown on agar plates, all Proteus isolates (100%) show swarming behavior.
Table (3-1) Virulence factors detected in Proteus mirabilis isolates.

<table>
<thead>
<tr>
<th>Virulence factors</th>
<th>Isolates No.</th>
<th>Isolates percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysin</td>
<td>17</td>
<td>100%</td>
</tr>
<tr>
<td>Urease</td>
<td>17</td>
<td>100%</td>
</tr>
<tr>
<td>Proticin</td>
<td>17</td>
<td>100%</td>
</tr>
<tr>
<td>Swarming</td>
<td>17</td>
<td>100%</td>
</tr>
<tr>
<td>β- Lactamase</td>
<td>16</td>
<td>94.1%</td>
</tr>
</tbody>
</table>

Bacteriocin (Proticin) production:

All isolates in the present study formed proticin (100%) when experienced with sensitive Gram-negative indicator isolates including E. coli, Klebsiella, Salmonella, and Acinetobacter. The results are shown in table (3-1). The present study matches the result of Wilson et al., (1998) who has shown that all isolates of Proteus (100%) which are associated with rheumatoid arthritis formed proticin (20).

Antibiotics susceptibility:

Disc diffusion method:

It has been found that all isolates of P. mirabilis are resistant to Imipenem (100%), while most of the isolates were sensitive to Ertapenem so only 3 isolates (17.6%) are resistant. The isolates showed different percentages of resistance 14(82.3%) to each amoxicillin and Cefotaxime, where some isolates showed resistance to a lesser degree to Amoxiclave 9(52.9%), Ceftriaxone 11(64.7%), and Cefepime Clavulanic acid 4(23.5%). These results are shown in figure (3-2). Most isolates of P. mirabilis have shown resistance to a β-lactam antibiotic group. The effect of Amoxicillin on bacteria is planned as shown in the results which showed that 14(82.3%) of isolates were resistant to this antibiotic agent.

These results of P. mirabilis resistant to this antibiotic are compatible with that of Philips, (2014) who found that (78.6) of the isolates were resistant to Amoxicillin (21). While Karim, (2011) (22) reported that all of P. mirabilis were (100%) confer resistant to Amoxicillin. Quinteros et al., (2003) show that (7%) of isolates are resistant to Cefepime-Clavulanic acid. (23).

AX= Amoxicillin, FEC=Cefepime Clavulan acid, AMC=Amoxiclave, IMP=Imipenem, ETP=Ertapenem, CRO=Ceftriaxone, CTX=Cefotaxime.

Figure (3-2): effect of antibiotics on the growth of Proteus mirabilis isolates.

The present results of resistance to this antibiotic are different from that of Philips, (2014) who found that (75%) of the isolates were resistant to Amoxiclave (21). These results of resistance to this antibiotic differ from that of Wang et al., (2014) who reported that (32.8%) of the isolates were resistant to Imipenem (24). The present study nearly agrees with the result of Bouchillon et al. (2012) that demonstration (73%) of P. mirabilis isolates are resistant to Imipenem. These few differences in results are attributed to the time of the test (25).

The present study approximately agrees with the result of Bouchillon et al. (2012) that demonstration all P. mirabilis isolates are sensitive to Ertapenem (25). This study disagreed with the result of Bouchillon et
al., (2012) (25), that demonstration (45%) of P. mirabilis isolates are resistant to Ceftriaxone. The present study disagrees with the result of Karim (2011) reported that (31.25%) of P. mirabilis were conferring resistant to Ceftriaxone (22).

**Detection of β-Lactamase production:**
This approach relies on the identification of penicillin or cephalosporin acid, which is produced when the amide link in the β-lactam ring of penicillins or cephalosporins is broken down (26). The investigation demonstrates that 16 isolates produce β-Lactamase, as depicted in figures 3–1. In their study, Pagani et al. (2002) discovered that the prevalence of amoxicillin resistance in P. mirabilis was consistently high, with a frequency of 52% (27). According to Luzzaro et al. (2001b), most strains of P. mirabilis are very resistant to beta-lactam antibiotics because they make beta-lactamases (28). Antibiotic resistance genes (ARGs) can be passed down from one generation to the next and can also be disseminated between bacteria through a process called horizontal gene transfer (HGT). Mobile genetic elements (MGEs) facilitate this transfer (19).

**CONCLUSIONS**

1. Proteus mirabilis can be obtained from several clinical sites, including the urinary tract, otitis media, wounds, vagina, and stool.

2. The study found that all of the isolates of Proteus mirabilis had a lot of virulence factors, including hemolysin, urease, proticin, adhesion factors, and swarming activity.

3. All of the strains of Proteus mirabilis exhibited resistance to Imipenem, but the majority of strains demonstrated sensitivity to Ertapenem, Cefepime, and Clavulanic acid.

**REFERENCES**


