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Antimicrobial resistance of clinical *Proteus mirabilis* isolated from different sources

Serry FM, Abdel-Latif HK, Gomaa SE*, Abbas HA
Department of Microbiology and Immunology-Faculty of Pharmacy-Zagazig University- Zagazig- Egypt

* Corresponding author e-mail: drsaly32@yahoo.com

ABSTRACT

Proteus mirabilis is a Gram negative bacteria belonging to the family *Enterobacteriaceae*. It is responsible for a variety of infections such as those of urinary tract, respiratory tract, burns, wounds and diabetic foot ulcers. Bacterial resistance to antibiotics is an increasing problem worldwide. *Proteus mirabilis* shows high resistance to several antibiotics which could lead to multidrug resistance and failure of antimicrobial treatment.

In the current study, *Proteus mirabilis* isolates were identified according to traditional biochemical tests. Antibiotic susceptibility testing was performed by the disk diffusion method. Forty seven *P. mirabilis* were isolated from different sites. Complete resistance was exhibited with tetracycline. High resistance was found with ampicillin, ampicillin-sulbactam, sulphamethoxazole-trimethoprim and chloramphenicol. Intermediate resistance was noted against cefepime, cefotaxime, ceftazidime, cefoperazone, gentamicin, ciprofloxacin and levofloxacin. Low resistance was shown against piperacillin, amikacin, aztreonam, imipenem and meropenem. Multidrug resistance (MDR) was found in 87.2% of the isolates.

The inappropriate use of antibiotics has led to emergence of resistant bacteria which led to ineffective antibiotic therapy. Strict policies must be applied for antibiotic prescription for patients. In addition, susceptibility testing must be performed before antibiotic dispensing.

Key words: *Proteus mirabilis*, Antimicrobial susceptibility, antibiotic resistance, multidrug resistance

INTRODUCTION

Proteus is a genus of Gram-negative bacteria belonging to the family *Enterobacteriaceae*, characterized by their ability to swarm on agar surface **(Jacobsen *et al.*, 2008)**. They can be found in soil, water, and faecally contaminated materials. *Proteus mirabilis* is the most prevalent species being responsible for 90% of all infections caused by the *Proteus* spp. **(Auwaerter, 2008)**. It is involved in many hospital and community acquired infections including those of the urinary tract, respiratory tract, wounds, burns and diabetic foot infections **(O'Hara *et al.*, 2000;Shanmugam *el al.*, 2013).**

Antibiotic resistance is a global health problem that limits the therapeutic options. There are several mechanisms by which bacteria can resist the antibiotics including; antibiotic inactivation by bacterial enzyme, decrease antibiotic entry into bacteria, antibiotic efflux as well as mutation in target site **(Georgios *et al.*, 2014)**. Moreover, bacteria may develop multidrug resistance (MDR) which could lead to ineffective antibiotic therapy and aids in prevalence of persistent infections **(Nikaido, 2009)**.

This study aims to investigate the antimicrobial resistance pattern of *P. mirabilis* isolated from patients with urinary tract infections, diabetic foot ulcers, respiratory tract infections, burn and wound infections

MATERIALS and METHODS

Bacterial isolation and identification

A total of 400 clinical samples were collected from patients admitted to Zagazig University Hospital and Al-Ahrar hospital in Zagazig from different sources. Samples collected were distributed as shown in

(Table 1). The handling of specimens and the isolation were performed following the standard microbiological procedures and the isolated bacteria were identified by Gram staining, colony morphology and using biochemical tests (**Koneman *et al.*, 2006)**.

Antibiotic susceptibility testing

The antibiotic susceptibility test was done using Kirby-Bauer disc diffusion method according to **Bauer *et al.* (1966)**. The antibiotic disks that were used in this study were obtained from Oxoid (Hamphsire, England). These disks were Ampicillin (AM; 10 µg), Piperacillin (PRL; 100 µg), Ampicillin- Sulbactam (SAM; 20 µg), Cefotaxime (CTX; 30 µg), Cefoperazone (CEP; 75 µg), Ceftazidime (CAZ; 30 µg), Cefepime (FEB; 30 µg), Imipenem (IPM; 10 µg), Meropenem (MEM; 10 µg), Aztreonam (ATM; 10 µg), Gentamycin (CN; 10 µg), Amikacin (AK; 30 µg), Tetracyclin (TE; 30µg), Ciprofloxacin (CIP; 5µg), Levofloxacin (LEV; 5µg), Sulphamethoxazole-Trimethoprim (SXT; 5µg) and Chloramphenicol (C; 30µg).

The bacterial suspensions were prepared from overnight cultures on Muller- Hinton agar (Oxoid, Hamphsire, England). Suspensions densities were adjusted to 0.5 McFarland standards approximately (1.5×10^8 CFU/mL). The surface of Muller-Hinton agar plate was evenly inoculated with the Suspensions using a sterile swab. The plates were dried before applying the antibiotic discs. The plates were incubated overnight at 37°C after which the diameters of inhibition zones around the disks were measured. The results were interpreted according to Clinical Laboratory Standards Institute guidelines (CLSI**, 2016)**.

RESULTS

Isolation and identification

Proteus mirabilis was found in 11.75% of clinical samples. The isolates were distributed as shown in (Table 1). *Proteus mirabilis* isolates were Gram-negative rods, lactose non fermenter on Macconkey's agar (pale yellow colonies) and showed swarming motility on nutrient agar plates. Furthermore, they were urease and indole positive and they could produce hydrogen sulphide when grown in triple sugar iron agar**.**

Table 1: Source and frequency of *P. mirabilis* isolates

Source	No. of samples	No. (%) of *P.mirabilis* isolates
Urine samples	125	19 (15.2%)
Surgical wound swabs	112	9 (8%)
Diabetic foot swabs	79	9 (11.4%)
Endotracheal aspirate samples	50	6 (12%)
Burn swabs	34	4 (11.8%)
Total	400	47 (11.75%)

Antibiotic Susceptibility Test (AST)

The antibiotic resistance profile showed varying degrees of resistance to different antibiotics (Table 2). Complete resistance was found with tetracycline (100%). High resistance was exhibited with ampicillin and ampicillin-sulbactam (85.1% each), sulphamethoxazole-trimethoprim (78.8%) and chloramphenicol (72.2%). Intermediate resistance was noted for cefepime, cefotaxime, ceftazidime, cefoperazone (53.2%, 51.1%, 44.7% and 42.6%, respectively), gentamicin (42.6%), ciprofloxacin and levofloxacin (38.3% and 31.9%, respectively).

Low resistance was found with piperacillin and amikacin (25.5% each), aztreonam (14.9%), imipenem (8.5%) and meropenem (6.4%). Frequency of multidrug resistant (MDR) isolates of *Proteus mirabilis* was shown in Table 3. High frequency of MDR was found among the tested isolates (87.2%).

Table 2: Antibiotic resistance profile of *Proteus mirabilis* isolates to different antibiotic disks

Antibiotic disks	No of resistant isolates (%)
Ampicillin	40 (85.1)
Piperacillin	12 (25.5)
Ampicillin-sulbactam	40 (85.1)
Cefotaxime	24(51.1)
cefoperazone	20(42.6)
Ceftazidime	21(44.7)
Cefepime	25(53.2)
Imipenem	4(8.5)
Meropenem	3(6.4)
Aztreonam	7 (14.9)
Gentamicin	20(42.6)
Amikacin	12(25.5)
Tetracycline	47(100)
Ciprofloxacin	18(38.3)
Levofloxacin	15(31.9)
Sulphamethoxazole-trimethoprim	37 (78.8)
Chloramphenicol	34 (72.2)

Table 3. Frequency of multidrug resistant isolates of *Proteus mirabilis*

Number of resistant isolates	Number of Antibiotic classes	Classes of antibiotices
13	6	B-lactams, aminoglycosides, tetracycline, fluoroquinolones, sulphamethoxazole-trimethoprim and chloramphenicol
2	5	B-lactams, aminoglycosides, tetracycline sulphamethoxazole-trimethoprim and chloramphenicol
2		B-lactams, fluoroquinolones, tetracycline, sulphamethoxazole-trimethoprim and chloramphenicol
1		B-lactams, aminoglycosides, fluoroquinolones, tetracycline and chloramphenicol.
14	4	B-lactams, tetracycline, sulphamethoxazole-trimethoprim and chloramphenicol
3		B-lactams, aminoglycosides, tetracycline and sulphamethoxazole-trimethoprim
1		B-lactams, tetracycline, fluoroquinolones and chloramphenicol
3	3	B-lactams, tetracycline and sulphamethoxazole-trimethoprim
1		B-lactams, tetracycline, and chloramphenicol
1		Aminoglycosides, tetracycline and fluoroquinolones

DISCUSSION

Proteus mirabilis is an opportunistic pathogen responsible for variety of infections, mostly prevalent is the urinary tract infections **(Jacobsen and Shirtliff, 2011)**. *Proteus mirabilis* is also common to cause diabetic foot ulcer **(Shanmugam *et al.*, 2013)**. In addition to its capability to cause respiratory tract and wound infections **(Endimiani *et al.*, 2005)**. The present study was performed to investigate the antimicrobial resistance of *P. mirabilis* isolated from different sources.

Forty seven *Proteus miabilis* were isolated in this study with a prevalence rate of 11.75%. This was similar to that observed by **El-Sokkary *et al.* (2015) and Ahmed (2015)**, they reports prevalence rates of 12.4% and 13.2%, respectively. Of note that **Al-Bassam and Al-Kazaz (2013) and Kadhim (2017)** isolated *P.mirabilis* in higher rates (24.8% and 28.49%, respectively). However, **Senthamarai *et al.* (2015), Feglo *et al.* (2010) and Jabur *et al.* (2013)** had lower prevalence rates (2%, 5.2% and 7%, repectively).

The isolates recovered were completely resistant to tetracycline (100%) which agrees with **Ahmed (2015)** who also reported 100% resistance to tetracycline, while resistance rate of 85 % was reported by **Feglo *et al.* (2010)** and 82% was observed by **Newman *et al*. (2006).**

Our study shows that high resistance rates were found with ampicillin, sulphamethoxazole-trimethoprim and chloramphenicol (72%-85%). Those findings were in agreement with that mentioned by **Feglo *et al.* (2010)** where resistance rates of 77-82% were observed with ampicillin, sulphamethoxazole-trimethoprim and chloramphenicol. **Newman *et al*. (2006)** reported resistance rates of 76%, 75% and 73%, respectively to ampicillin, chloramphenicol and cotrimoxazole.

In our study *P.mirabilis* isolates showed intermediate resistance to the tested cephalosporins, fluoroquinolones and gentamicin (31%-53%). Higher resistance rates against ciprofloxacin (51%) were reported by **Kamel *et al.* (2014)**. Only 26.66% observed by **Abbas *et al.* (2013)**, while 40% was concluded by **Kwiecinska-Pirog *et al.* (2013)**. Our results suggest that those antibiotics should not be used in treatment of *P.mirabilis* infections, as it will lead to failure of therapy.

Resistance to ceftazidime and ceftotaxime was higher (44.7% and 51.1%) than **Al-Bassam and Al-Kazaz (2013)** who observed resistance rates of 40% and 30% to ceftazidime and ceftotaxime, respectively. Also, resistance to cefepime was higher than that observed by **Ahmed (2015)** who found that only 20% of isolates were resistant. On the other hand, lower resistance (82.4%) was reported by **Kadhim (2017)**.

Proteus mirabilis isolates show slightly low resistance against piperacillin, amikacin, aztreonam and imipenem (< 30%). Imipenem and meropenem showed the lowest rate of resistance (8.5% and 6.4%) among the tested antibiotics. This result agreed with **Adamus-Bialek *et al.* (2013)**. However, the results conducted by **Serry *et al.* (2014)** stated that lower resistance rates of *P. mirabilis* isolates against amikacin, levofloxacin, ciporofloxacin and gentamicin (2.2 % - 17.8 %), whereas *P.mirabilis* isolates were 100% sensitive to Imipenem.

In this study, 87.2% of *P.mirabilis* isolated were MDR. This result agreed with **Feglo *et al.* (2010)** who found that 84.6 % of *P. mirablis* were MDR, but was not agreed with **Pandey *et al.* (2013)** at which 28.13% *P.mirabilis* isolates were MDR. *Proteus mirabilis* resistance to different antibiotics varied in different studies. This may be due to the antibiotics

abuse in countries from which those isolates were isolated.

CONCLUSION

Several factors could lead to emergence of antibiotic resistace among *Proteus* bacteria. Moreover, failure of antibiotic could result from misuse and abuse of the antibiotics, antibiotic prescription not based on susceptibility testing in addition the use of broad spectrum antibiotics. In order to overcome the problem of development of bacterial resistance to antibiotics, education of people on the antibiotic use and misuse, high restrictions must be applied for antibiotic prescription and susceptibility testing must be done before antibiotic dispensing.

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مقاومة العزلات السريرية لبكتيريا بروتيس ميرابيليس المعزولة من مصادر مختلفه للمضادات العلاجية

فتحي السيد سري، همت كمال عبد اللطيف، سلوى جمعه، هشام عباس
قسم الميكروبيولوجي والمناعة- كلية الصيدلة- جامعة الزقازيق- مصر

بروتيس ميرابيليس هي عبارة عن بكتيريا سالبة الجرام تنتمي إلى عائلة انتيروبكترياسي. وهي مسؤولة عن مجموعة متنوعة من العدوى مثل عدوي المسالك البولية والجهاز التنفسي والحروق والجروح وقرحة القدم السكري. مقاومة المضادات الحيوية مشكلة متزايدة في جميع أنحاء العالم. يظهر بروتيوس ميرابيليس مقاومة عالية للعديد من المضادات الحيوية التي تؤدي إلى مقاومة العقاقير المتعدّدة وفشل المعالجة المضادة للميكروبات. تم التعرف على عزلات بروتيوس ميرابيليس وفقا للاختبارات البيوكيميائية. كما تم إجراء اختبار الحساسية للمضادات الحيوية بطريقة انتشار القرص.

تم تجميع سبعة وأربعون من بكتيريا بروتيس ميرابيليس. تم ملاحظةمقاومة كاملة مع التتراسيكلين. تم العثور على مقاومة عالية مع الأمبيسلين، الأمبسيلين-سلباكتام، السولفاميثكسازول / تريمثوبريم و الكلورامفينيكول ولوحظت مقاومة متوسطة ضد السيفيبيم، السيفوتاكسيم ، السيفتازيديم ، السيفوبيرازون ، الجنتاميسين ، السيبروفلوكساسين والليفوفلوكساسين. تم ايجاد مقاومة منخفضة ضد البيبراسيللين، الأمايكاسين، الأزترونام، الايميبينيم و الميروبينيم تم العثور على المقاومة للأدوية المتعددة في ٨٧,٢ ٪ من العزلات.

الاستخدام غير السليم للمضادات الحيوية يؤدي إلى ظهور بكتيريا مقاومة مما يجعل العلاج بالمضادات الحيوية غير فعال. ولذا يجب تطبيق سياسات صارمة على وصفات المضادات الحيوية ويجب إجراء اختبار الحساسية قبل استخدام المضادات الحيوية.