

## Phenotypic detection of extended –spectrum beta-lactamase production in *Proteus mirabilis* isolation from Patients with Significant Bacteriuria in Najaf provina

Ahmad Alewi Hussein\*

### الخلاصة

جمعت 250 عينة إدرار من أشخاص مصابين بالتهاب المجاري البولية من مراجعين لثلاث مستشفيات (مدينة الصدر الطبية و الحكيم العام والزهراء التعليمي) في النجف الاشرف خلال الفترة من تشرين الاول 2011 إلى كانون الأول 2012, فحصت العينات لتحري عن البكتريا *Proteus mirabilis* تم اعتبار النمو الذي هو  $\geq 10^5$  وحدة مكونة لمستعمرات لكل مل من الإدرار معيار بيلا البكتريا تم تحديد 198 (79.2%) بيلا بكترية, وقد أظهرت نتائج هذه الدراسة ارتفاع مستوى حدوث التهاب المجاري البولية في النساء (65.2%) أكثر من الرجال (34.8%), فأظهرت العزلات *Proteus mirabilis* 52 (26.3%) بينما أظهرت بقية العزلات (73.7%) 146. اختبرت حساسية بكتريا *Proteus mirabilis* اتجاه 11 نوع من المضادات الحيوية بطريقة انتشار الاقراص لـ كيربي- بور وكانت جميع العزلات مقاومة على الأقل لخمس من المضادات الحيوية أي أنها أظهرت نمط المقاومة المتعددة للمضادات الحيوية, وباستخدام طريقة اليود القياسية السريعة فإظهرت النتائج 39 عزلة من بكتريا *Proteus mirabilis* فحص موجب لإنزيم البيتالاكتاميز كما اختبرت بكتريا *Proteus mirabilis* لمعرفة قابليتها على إنتاج البيتالاكتاميز واسعة الطيف وأظهرت النتائج إن 18 عزلة بكترية وبنسبة (34.6%) كانت منتجة لبيتالاكتاميز واسعة الطيف.

### Abstract

250 mid-stream urine samples were collected from patients suspected of urinary tract infection (UTI) who were attended to Three hospitals (Medical Al-Sader City, Al-Hakeem, and Al-Zahra Maternity and Children) in Najaf provina during the period of October 2011 to January 2012 and screened for the presence of *Proteus mirabilis*. The growth of  $\geq 10^5$  colony forming units/ml was considered as significant bacteriuria.

\*Kufa university\ College of science

A total of 198 (79.2%) significant bacteriuria were detected. The study showed higher incidence of UTI in females 129 (65.2%) than males 69 (34.8%) However, 52 (26.3%) isolates of *Proteus mirabilis*, 146 (73.7%) isolates other species pathogens.

The 52 isolates of *Proteus mirabilis* were tested for their antibiotic resistance against 11 antibiotics by the Kirby-Bauer disk diffusion test. All the isolates were found to be resistant to at least 5 antibiotics to which they were subjected. Therefore, all these isolates were considered to be multidrug resistant.

The majority 39 (75.0%) isolates of *Proteus mirabilis* were able to produce  $\beta$ -lactamase enzymes with rapid iodometric method.

All isolates of *Proteus mirabilis* were tested for their ability to produce Extended-spectrum  $\beta$ -lactamase. The result showed that 18 (34.6%) isolates were able to produce ESBLs.

## Introduction

Urinary Tract Infections (UTI) represents one of the most common diseases occurring from the neonate to geriatric age groups encounters in medical practice today (!) It is estimated that about 35% of healthy women suffer symptoms of (UTI) at some stages in their life. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequency (2). The incidence of UTI is greater in women as compared to men which may be either due anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors (3). UTI with *P. mirabilis* usually starts with colonization of the bladder, causing bacteriuria and cystitis but is rarely involved in severe infections (4). In the complicated UTI, bacteria can ascend to the kidney, and cause in acute pyelonephritis, chronic inflammation and renal failure (5 ; 6). The ESBL producing bacteria has been identified in members of Enterobacteriaceae, are increasingly causing urinary tract infection (UTI) both in hospitalized patients. and outpatients (7: 8). The increasing drug resistance among these bacteria has made therapy of UTI difficult and has led to greater use of expensive broad-spectrum drugs. This resistance problem needs a re-newed effort resulting in searching

effective antibacterial agents Urinary Tract Infection represents one of the most antibiotics (9).

The goal of this study was to determine the occurrence of extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Proteus mirabilis* in patients with significant bacteriuria.

## **Materials & methods**

### **2-1-Collection and Handling of Samples**

During the period October 2011 to January 2012., a total of 250 urine samples were taken (by standard mid-stream “clean catch” method) from patients with suspected urinary tract infections. Three hospitals in Najaf (Al-Sadr Teaching, Al-Hakeem, and Al-Zahra Maternity and Children) were included in this study.

Each urine sample was collected from patient into a sterile container, and divided into two portions. One portion was for the direct microscopically examination, for pus cells, red blood cells, casts, and others. The second was streaked on the blood agar and MacConkey agar using standard loop method. The media were incubated at 37°C for 24 hr. culture results were interpreted as being significant and insignificant bacteriuria, according to the standard microbiological procedure. A growth of  $\geq 10^5$  colony forming units/ml was considered as significant bacteriuria (10).

### **2-2- Identification of Bacterial Isolates**

*Proteus mirabilis*. were identified based on colonial morphology, and biochemical reactions according to 11, 12. and 13.

### **2-3- Detection of $\beta$ -Lactamase Production**

Rapid iodometric method was preformed for all bacterial isolates *Proteus mirabilis* as follows:

Several colonies of a young bacterial culture on MacConkey agar, were transferred to Eppendorf tubes containing 100  $\mu$ l of penicillin G solution, and the tubes were incubated at 37°C for 30 minutes. Then, 50  $\mu$ l of starch solution was added and mixed well with the content of the tube. A portion of 20  $\mu$ l of iodine solution was added to the tube which cause the appearance of dark blue

color, rapid change of this color to white (within few second-2minutes) indicated a positive result (12).

#### **2-4- Antibiotic Disk Susceptibility Test**

Antibiotic susceptibility of *Proteus mirabilis*. isolates was studied against the antibiotics by the disk diffusion technique on Muller-Hinton agar, using inhibition zone size criteria recommended by the disk manufacturer and based on the method of Barry (1976). The selection of antibiotic disks and interpretation of zones of inhibition were performed according to the recommendation of the National Committee for Clinical Laboratory Standards (14).

#### **2-5- Detection of ESBL Production**

The methods were performed for detection of ESBLs in significant bacteriuria isolates. All *Proteus mirabilis* isolates that. This tests included:

##### **2-5-1- Disk Approximation Method**

This method was carried out as modified by (15) as follows: Muller-Hinton agar plate was inoculated with an overnight of the test bacterial isolate as recommended for standard disk diffusion susceptibilities test. Disks containing 30 µg cefotaxime, ceftazidime, ceftriaxone, and aztreonam were placed 15 mm (edge to edge) from a disk of augmentin (20 µg amoxicillin plus 10 µg clavulanate). Incubation followed for 16-20 hr at 35°C. Any enhancement of the zone of inhibition between a β-lactam disk and augmentin disk was indicative of presence of an ESBL.

### **Result and Discussion**

#### **4-1: Collection of Sample**

Out of the 250 sample processed 198 (79.2%) showed significant bacteriuria and 52 (20.8%) of which showed Non-significant bacteriuria ( Table 4-1). A total of 129 (65.2%) females and 69(34.8%) males had positive urine culture (significant bacteriuria ). The result showed high incidence of UTI in females then males . The higher incidence of UTI in females might be due to variety of factor , such as the close proximity of the female

urethral meatus to the anus (16), and alternation in vaginal microflora also play critical role in encouraging vaginal with coliforms which may lead to UTI .(2 : 17).

**Table (4-1) Incidence of significant bacteriuria in patient suspected UTI**

Type of culture	No.	Percentage	Female	Male
Significant bacteriuria	198	79.2%	129 (65.2%)	69 (34.8%)
Non-significant bacteriuria	52	20.8%	33 (60%)	19 (40%)
Total	250	100%	156 (74.3%)	54 (25.7%)

The organism grown on the culture of all the 198 urine samples with significant bacteriuria were as follow : 52 (26.3%) isolates of *Proteus mirabilis* , 146 (73.7%) isolates other species pathogens. (Table 4-2).

**Table (4-2): Distribution of etiological agents in patient with significant bacteriuria .**

Bacterial isolate	No. of isolates		Total
	Female	Male	
<i>Proteus mirabilis</i> .	38(73.1%)	14 (26.9%)	52(26.3%)
other species pathogens	91(62.3%)	55(37.7%)	146 (73.7%)
Total	129 (75.9%)	69 (24.1%)	198 (100%)

*Proteus. mirabilis* infects a much higher proportion of patients with complicated urinary tracts, that is, those with functional or anatomical abnormalities or with chronic instrumentation, such as long-term urinary catheterization. In these patients, *Proteus. mirabilis* not only causes cystitis and acute pyelonephritis but also characteristically leads to the production of urinary stones, which complicates further the problems associated with the urinary tract (18).

Consider 19 The *Proteus mirabilis* is the second most common cause of urinary tract infections and is also an important cause of nosocomial infections. Many recurrent causes of bacteriuria and UTI involving *Proteus mirabilis* have been reported (20)

**4-2: Antibiotic susceptibility testing:-**

All the 52 isolated of *Proteus mirabilis* obtained from urine of patient with significant bacteriuria were tested for antibiotics

susceptibility against 11 antibiotics using Kirby –Bauer disk diffusion method (Table 4-3).

The frequency of antibiotics susceptibility of the 52 *Proteus mirabilis* isolated was determined (Table 4-3) , All these isolates showed 100% resistance to ampicillin and amoxicillin In general, over all 63.5% of bacterial isolates were susceptible to ciprofloxacin , 28.8% to gentamycin , 30.8% to trimethoprim , 36.5% to cefotaxime , 40.0% to ceftazidime , 51.9% to ceftriaxone and 69.2 % isolates showed resistance to nalidixic acid , 34.6 % tobramycin and All these isolates showed 100% susceptibility to Imipenem .

The antimicrobial resistance patterns are valuable a guide to empirical therapy and as an indicator of dissemination of antimicrobial resistance determinants (21)  $\beta$ -lactams are the most widely used antibiotics and  $\beta$ -lactamases are the greatest source of resistance to them. An understanding of extended spectrum  $\beta$ -lactamase, detection is therefore valuable. Recent studies conducted by 22 and 23 on ESBL production in members of Enterobacteriaceae isolated from clinical specimens showed 9-50% ESBL producers.

**Table (4-3) Antibiotics susceptible of *Proteus mirabilis* isolates from urine fo patient with significant bacteriuria.**

Type of antibiotic	(%) of resistant isolates	(%) of susceptible isolates
Ampicillin	100%	0
Amoxicillin	100%	0
Cefotaxime	60%	40%
Ceftazidime	48.1%	51.9%
Ceftriaxone	30.2	69.8%
Imipenem	0	100%
Gentamycin	71.2%	28.8%
Tobramycin	34.6 %	65.4%
Nalidixic acid	69.2 %	30.8%
Ciprofloxacin	36.5%	63.5%
Trimethoprim	63.5%	36.5%

#### 4-3: $\beta$ - Lactamase Production

Rapid iodometric method was used for the detection of  $\beta$ -lactamase production in 52 *Proteus mirabilis* isolates which were

obtained from patient with significant bacteriuria . This method depends on the detection of penicilloic acid or cephalosporic acid , result from the breakdown of amid bond in  $\beta$ - lactam ring for each of penicillin's or cephalosporin's (24: 25). In addition , it depend on the fact that hydrolysis products of  $\beta$ - lactams (penicilloic or cephalosporic acid) reduce iodine to iodide, consequently , depolarization starch-iodine (dark blue) complex occurs in an isolates is a  $\beta$ -lactamase producer but not when the enzyme is absent (12).The result form the present study show that 39 (75.0%)  $\beta$ -lactamase- producing *Proteus mirabilis* isolates gave positive reaction after few second to two minutes form the addition of the reagent . The interpretation of these results depends on the concentration of  $\beta$ - lactamase enzyme in the periplasmic space ( 25). Additionally , factors such as temperature and pH also play an important role in enhancement or reduction of enzyme activity (26) These results are Similar results were obtained by ( 27) and (28) shows isolates of *Proteus mirabilis* that able on produce  $\beta$  – lactamases enzyme .Wild-type strains of *Proteus mirabilis* are usually susceptible to ampicillin and other  $\beta$ lactams, which are among the drugs of choice. However, a progressive increase of  $\beta$ -lactam resistance, mediated by the production of acquired  $\beta$ -lactamases, has occurred in this species (29: 30 : 27).

#### **4-4: Frequency of Extended Spectrum $\beta$ -lactamases Production.**

Extended-spectrum  $\beta$ -lactamases (ESBLs) are enzymes which are capable of hydrolyzing penicillins, Extended-spectrum cephalosporins such as (ceftazidime cefotaxime ceftriaxone) and the monobactam antibiotic aztreonam but . Cephamycins (e.g., cefoxitin) or carbapenems (e.g., imipenem, meropenem, and ertapenem) are not affected by these enzymes.( 31: 32 )

ESBLs are inhibited by  $\beta$ -lactamase inhibitors (clavulanic acid, tazobactam, and sulbactam).(33) .

In this the study used method disk approximation test and also known as double –disk test , It was described by (34) .In this test a disk containing amoxicillin clavulanate in placed in proximity to disk containing oxyimino- $\beta$ -lactam and aztreonam antibiotics. The

enhancement of the zone of inhibition of the oxyimino- $\beta$ -lactam is a positive result ( 35). However , this test has served as the reference for detecting ESBL-producing isolates for many year ( 36 : 37).In this investigation ESBL-producing *Proteus mirabilis* isolates ,were identified depending on the enhancement of the inhibition zone of the  $\beta$ -lactam disks (ceftazidime, cefotaxime, ceftriaxone and aztreonam) on the side facing the augmentin disk (amoxicillin –clavulanate 20\10  $\mu$ g/ml). of the total of (52) *Proteus mirabilis* isolates examined in this study, ESBLs were detected in 18 (34.6%)isolates (Table 4-4) .( Figure 4-1)

**Table (4-4): Distribution of ESBL-producing of *Proteus mirabilis* isolates by disk approximation test**

Type of isolate	No.(%) of isolates			
	$\beta$ -lactamase producing	NO $\beta$ -lactamase producing	ESBL-producing	NO ESBL-producing
<i>Proteus mirabilis</i> .	39 (75.0%)	13 (25.0%)	18 (34.6%)	44(65.4%)
Total	52(100%)		52(100%)	

The study suggested that the frequency of ESBL-producing isolates was higher than has been suspected , However . the rate of ESBL-producing *Proteus mirabilis* also was similar to a previous by study (38). Recently, extended-spectrum  $\beta$ -lactamases (ESBLs) active on expanded-spectrum cephalosporins have also started spreading in *Proteus mirabilis*, including most frequently TEM-type derivatives (39 : 40 : 38) .



**Figure (4-1): Disk approximation test to detect ESBLs in isolate of *Proteus mirabilis***

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