

Detection of Escherichia Coli and Proteus Mirabilis Extended -Spectrum
 β -Lactamase Isolated from Urinary Tract Infections

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Abstract

A total of 40 urine specimens were collected from patients admitted to Baaquba Teaching Hospital in Baaquba city for the period from 1/ 12 /2014 to 1/01/2015. From them 16 isolates belonging to members of the family Enterobacteriaceae (40%). isolates were identified as *E.coli* 10 (25%) and three isolates were identified as *proteus mirabilis* 6 (15%). The results showed that only 4 isolates (25%) were detected as ESBL-producers by using disk approximation method *P.mirabilis* and *E. coli* were the species that gave positive results by using this method.

Keywords: Enterobacteriaceae, urinary tract infections, Extended Spectrum β -Lactamases

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الكشف عن انزيمات البييتالاكتاميز واسعة الطيف لبكتريا *Escherichia coli* و *Proteus mirabilis* المعزولة من التهاب المجاري البولية .

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المعهد التقني بعقوبة

الخلاصة

شملت الدراسة 40 عينة جمعت من المرضى المصابين باخماج المجاري البولية من اجناس و اعمار مختلفة وتم جمع العينات من مستشفى بعقوبة التعليمي ، للمدة بين 2014/12/1 ولغاية 2015/1/1. أظهرت نتائج الزرع البكتيري على أوساط أكار الماكونكي وأكار الدم ووسط المثيلين الأزرق والتشخيص المظهري والفحوصات الكيموحيوية أن 16 عزلة تعود لبكتريا العائلة المعوية *Enterobacteriaceae* وبنسبه (40%) وبواقع 10 عزلة (*Escherichia coli* 25%) ، 6 عزلة (*Proteus mirabilis* 15%) . كما أظهرت قابلية العزلات على إنتاج انزيمات البييتالاكتاميز واسعة الطيف باستخدام طريقة الاقراص المتاخمة (Disc Approximation) فقد أعطت عزلتين فقط 4 وبنسبه (25%) نتيجة موجبه لكل من *Escherichia coli* و *Proteus mirabilis* .

الكلمات المفتاحية: بكتريا العائلة المعوية ، أنزيمات البييتالاكتاميز واسعة الطيف ، التهاب المجاري البولية

Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g. cefotaxime, ceftriaxone, and ceftazidime), monobactam(e.g., azteronam), and to a lesser extent cephamycins(e.g. cefoxitin and cefotetan), but do not affect carbapenems(e.g. imipenem and meropenem) (1) . In recent years, several outbreaks caused by ESBLs production in Enterobacteriaceae have been reported , Various types of ESBLs constitute one of the major mechanisms of resistance of clinical gram-negative bacteria to oxyimino-cephalosporins(such as, cefotaxime and ceftazidime) and to monobactam (2). In general, they are most frequently identified in *K.pneumoniae* and *E.coli*; however, many publications have documented their growing

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incidence in other organisms of the family Enterobacteriaceae, including: *Proteus* spp., *Providencia* spp., *Citrobacter* spp., *Enterobacter* spp., *Serratia marcescens*, and others (3).

The emergence of ESBLs has created not only a diagnostic problem but also possess a potential therapeutic challenge for the use of β -lactams in serious infections caused by the Enterobacteriaceae.

Aims of Study:

Isolation and identification of some members of the family Enterobacteriaceae from patients with UTI. Detection of production of Extended-spectrum β -lactamases (ESBLs).

Materials and Methods

Collection of specimens

All specimens were collected from patients admitted to Baaquba Teaching Hospital Wide-mouth containers were used. These were sterilized by hot-air oven at 180C° for 1 hour.

Culture technique

Clinical specimens were inoculated onto 5% blood agar and MacConky agar for the isolation of enteric gram-negative rods (4).

Culture characteristics

For the identification of different enteric gram-negative bacilli, the colonial appearance on simple solid media was studied, after 24 hours incubation at 37C°. The Enterobacteriaceae group yielded large, colorless to gray, shining, convex and opaque colonies. Some genera gave characteristic appearance, e.g. swarming growth of *Proteus* spp. Lactose-fermenting organisms showed pink or reddish colonies when grown on MacConky agar, while the non-lactose fermenters displayed colorless translucent colonies (5).

Biochemical tests

1. Indole test
2. Urease test

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Antimicrobial Susceptibility Test

The most widely used method for susceptibility testing was the disc agar diffusion method. It had the advantage of being simple, economical, and reproducible. The procedure, which was accepted by the National Committee for Clinical Laboratory Standard (NCCLS) was employed, as described by (6).

Preparation of culture medium:

Muller-Hinton medium was employed. The medium was cooled to (45-50)C and poured into Petridishes on a level surface to a depth of 4mm. When the medium was hardened , the Petridishes were placed in the incubator at (15-30) minutes to allow excess moisture evaporate.

Preparation of inoculum:

With a sterile wire loop, the tops of (4-5) isolated colonies of the test organism were picked from the original culture and introduced into a sterile test tube containing 4ml of nutrient broth. The broth was incubated at 37C for about (2-6) hours. The growth was adjusted to a MacFarland (No.0.5) turbidity standard using sterile saline or nutrient broth.

Inoculation of plates:

A sterile cotton wool swab was dipped into suspension, and rotating the swab firmly against the side of the tube above the level of fluid. The swab was streaked in three direction onto the dried surface of a Muller-Hinton plate until the plate was completely and uniformly covered.

Detection of ESBL Production:

Three methods were performed for detection of ESBLs in isolates that cause bacteremia. All bacterial isolates that were positive to β -lactamase production were tested for their ability to produce ESBL enzymes. These tests included:

Disk Approximation Method:

This method was carried out as modified by (7) as follows:

Muller-Hinton agar plate was inoculated with an overnight blood agar culture of the test bacterial isolate as recommended for standard disk diffusion susceptibilities test. Disks containing 30 μ g cefotaxime, ceftazidime, ceftriaxone, and aztreonam wre placed 15 mm

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(edge to edge) from a disk of augmentin (20 μ g amoxicillin plus 10 μ g clavulanate) and then incubated for 16-20 hrs at 35 °C. Any enhancement of the zone of inhibition between a β -lactam disks and augmentin disk gave an indication that the test isolate contains ESBL.

Results and Discussion

Patients and Specimens

From a total of 40 specimens which yielded positive growth, there were 16 isolates belonging to members of the family Enterobacteriaceae (40%). 10 isolates were identified as *E.coli* (25%) and 6 isolates were identified as *Proteus* (15%).

Isolation and Identification of Members of the Family Enterobacteriaceae Causing UTI

Table (1) illustrate the most common members of the the family Enterobacteriaceae isolated from the patients with UTI.

Table (1): Isolation of Enterobacteriaceae isolated from urine specimens of patients admitted at Baaquba teaching hospital

Bacterial species	Number of cases	%
<i>E.coli</i>	10	25
<i>P.mirabilis</i>	6	15
Total No. of isolates	16	40

Escherichia coli was the most common microorganism isolated from UTI, representing 10 isolates (25%). In the present work, it was found that *Proteus spp.* isolated represented 6 isolates (15%). An *E.coli* bacteria were more causes UTI in this study were accounted for (25%) Table (1), came to approach the percentage obtained by (8) which was (30.4%), but do not agree with the results obtained by (9) was (65.59%). The differences in the rates of *E.coli* isolates is probably due to the level of health Hygienic status in different communities. The intestinal bacteria, especially bacteria *E.coli* from more types of intestinal family events for urinary tract infections, and this was confirmed by numerous studies (10). In general, *E.coli* is the causative agent of the vast majority of UTIs, ranging from uncomplicated cystitis to sepsis. Virulence factors present on *E.coli* enable this organism to infect patients without

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genitourinary structural abnormality; whereas, relatively opportunistic organisms that lack virulence factors tend to cause disease in the setting of structural abnormality (11). *Proteus mirabilis* came in second place by (15%), came to approach the percentage obtained by (12) which was (18%), as it was incidence with the findings of the researcher (13) and his group where they found that the incidence of these bacteria was 33.33%, and this is inconsistent with the results referred to by researcher (14) and his group in a study in Southeast Asia about urinary tract infections because the bacteria isolated Variables sex ratio is 8.3%. A lot of studies that the bacterial genus Variables come in second place after the events in bacteria *E. coli* infections of the urinary tract in all ages and sex (15). The prevalence of *Proteus spp.* in the present study indicates that urinary infection was mostly environmental rather than fecal in origin, and agrees with what have been reported by (16), i.e. the UTI contracted in the hospital was usually due to *Proteus*.

Disk Approximation Method:

This method is also known as double disk synergy test; it was first described by (17). In this test, as discussed previously, any enhancement of the zone of inhibition between a β-lactam disks and augmentin disk gave an indication that the test strain contains ESBL whose activity is inhibited by clavulanic acid. It was found that only 4 isolates (25%) were detected as ESBL-producers by using disk approximation method as shown in **Table (3)**. *P. mirabilis* and *E. coli* were the species that gave positive results by using this method.

These results showed that the frequency of ESBL-producing isolates was higher than that reported by other researchers. (18) examined 38 β-lactamase-producing *Klebsiella spp.* isolates obtained from different clinical samples, and found that only 4 *Klebsiella* isolates (10.5%) were identified as ESBL-producers by using disk approximation method. In another local study by (19), her results revealed that out of 60 β-lactam resistant *E. coli* and *K. pneumoniae* subsp. *pneumoniae* isolates, only 11 isolates (18.3%) were detected as ESBL-producers by using disk approximation method. Disk approximation method has served as the reference method for the detection of ESBL-producing strains for a number of years (20). The detection of ESBL-mediated resistance in Gram-negative bacilli is one of the major problems

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in the clinical microbiology laboratories (21). Furthermore, many studies showed that there is a clinical and financial benefit of rapid bacterial identification and early determination of antibacterial susceptibility (22).

Table (3): The Frequency of ESBL-Producing and Non-Producing Isolates by Disk Approximation Method.

Bacterial Isolates	No. of Isolates	No. of ESBL-Producers (%)	No. of Non ESBL-Producers (%)
<i>E. coli</i>	10	2 (20%)	8(80%)
<i>p.mirabilis</i>	6	2(33.33%)	4(66.67%)
Total	16	4(25%)	12(75%)

(23) Reported that from a total of 1699 *Enterobacteriaceae* isolates, only 336 isolates (19.8%) were identified as potential ESBL producers by the double disk synergy test. On the other hand, (24) showed that 35% of examined *Enterobacteriaceae* in Thailand were resistant to third generation cephalosporins. In spite of the fact that the disk approximation method is the most widely used test because it is easy to be used and it can be performed in every laboratory. However, this test may lack sensitivity for some reasons such as: the optimum disk placement is not standardized, the inability of clavulanate to inhibit all ESBLs, the loss of clavulanate disk potency during storage, and the inability of the test to detect ESBLs in strains that also able to produce chromosomal cephalosporinases (25).

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