

Phenotypic detection of some virulence factors of uropathogenic *Escherichia coli* isolated from recurrent urinary tract infection patients

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تعيين بعض عوامل الضراوة مظهريا للبكتريا المعزوله من المرضى المصابين بالتهابات المسالك البولية المتكررة

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الخلاصة

التهابات المسالك البولية احد المشاكل الصحية المتنامية للمرضى المراجعين للمستشفيات في العالم أجريت هذه الدراسة لتعيين بعض عوامل الضراوة لبكتريا (*Escherichia coli*) المسببة للتهاب المسالك البولية المتكررة تضمنت هذه الدراسة 130 مريضا و 50 شخصا غير مريض لغرض المقارنه من كلا الجنسين تراوحت أعمارهم بين 6 إلى 70 سنه من المرضى المراجعين لمستشفى الزهراء التعليمي في محافظة واسط/ الكوت ومستشفى الحريري للجراحات ألتخصصيه/بغداد للفترة بين بداية أيلول 2009 إلى نهاية نيسان 2011 عزلت بكتريا *E. coli* من 53% (68\130) من المرضى المصابين بالتهاب المسلك البولي المتكرر أفضل المضادات الحياتية في حالة التهابات المسالك البولية المتكررة فهي Ciprofloxacin Amikacin, Gentamicin أما بقية المضادات الحياتية فقد كانت الاستجابة لها قليلة. تم التحري عن بعض عوامل ضراوة لبكتريا *E. coli* المسببة لالتهاب المسلك البولي، مظهريا كانت 50 % منتجة للخمّل نمط P, 10.3% منتجة للخمّل نوع S و78% منتجة للخمّل من النوع 1 و36.8% منتجة للخمّل نوع Dr و22.1% منتجة لللايروبكتين وكل العزلات اعطت نتيجة سالبه لإنتاج الهيمولايسين.

Abstract

Urinary tract infections (UTIs) are health growing problem in patients attending some hospitals in worldwide, to shed light on this subject, present study was done to detect some virulence factors of *E. coli* isolated from patients with recurrent urinary tract infection (RUTI) This study included 130 patients with RUTI, and 50 healthy persons as a control of both genders (aged 6-70 years) attending Al-Zahra Teaching Hospital in Al-Kut/Wassite governorate and Al-Harery Teaching Hospital of specialized surgeries/Baghdad during beginning of September 2009 to end of April 2011.

Intracellular bacterial communities (ICBC) (namely *E.coli*) was isolated from (68/130) 53% from patients with RUTI.

These bacteria showed differences in response to antibiotics which was used for treatment, Ciprofloxacin, Amikacin and Gentamicin provide good efficacy against *E.coli* isolated from patients with recurrent UTI.

Results of phenotypical studies on virulence factors of *E.coli* the revealed that 50% of RUTI isolates produced P-fimbria, 10.3% with S-fimbria, 36.8% have Dr-fimbria, 78% have type 1 fimbria and 22.1% of them produced aerobactin and all isolates gave negative results for hemolysin production.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections and are predominantly caused by uropathogenic *Escherichia coli* (UPEC) (1). About 50% of women have at least one symptomatic infection during their lifetime, and many have recurrent episodes. A recurrent urinary tract infection (RUTI) is a symptomatic UTI that follows clinical resolution of an earlier UTI generally, but not necessarily, after treatment (2, 3). Retrospective studies have demonstrated that *E. coli* strains causing UTI may, although appropriately treated and not found in repeated urine cultures can cause a new UTI up to 3 years later. The majority of recurrences are thought to be reinfection, while a minority (5-10%) is relapsed (4, 5, 6). The strong propensity of UTIs to recur, often within a few weeks or months after an initial acute infection, may contribute to additional problems, including renal scarring and an increased risk for developing bladder cancer (7, 8, 9). Adhesive organelles, including type 1, P, and S pili along with Dr adhesions, promote both bacterial attachment to and invasion of host tissues within the UT (10, 11, 1)

Uropathogenic *Escherichia coli* are responsible for up to 85% of these cases (1, 2). Also in Iraq high incidence of bacterial infection was for *E. coli* 80% (3). Uropathogenic *Escherichia coli* express adhesive fibers known as type 1 pili that mediate binding to and invasion of luminal facet cells of the urinary tract (4,6,8). This intracellular niche is conducive to UPEC replication and formation of IBCs with biofilm-like properties (12).

Intracellular bacterial communities exist only transiently before the bacteria dissociate and migrate out of the facet cell (13). Upon infection, the host exfoliates and expels bladder epithelial cells into the urine. Epithelial turnover may cause the quiescent bacteria to revert to an actively replicative form, leading to recurrent bacteriuria (14). This recurrence of UTI with the same serotype might lead to urothelium carcinoma because the IBCs may enhance uroepithelial cells to grow and to divide more than normal rate (15).

Materials and methods

Patients

This study included 130 patients with RUTI of both sexes and 50 healthy persons as a control (aged 6-70 years) attending Al-Zahra Teaching Hospital in Al-Kut/Wassit governorate and Al-Harery Teaching Hospital of specialized surgeries/Baghdad. The All patients did not receive any antimicrobial therapy, at least one week before sampling.

Specimen Collection and Processing

Microbiologically, infection was evaluated by culture only without microscopic examination. The urine specimens were collected according to Vandepitte *et al.* (15). Urine was collected into sterile screw capped test tubes and cultured immediately after collection (in the hospital laboratory), this was done during beginning of September 2009 to end of April 2011.

After that, they were streaked immediately on maCconkey agar (MAC) (Fluka) and eosin methylene blue agar (EMB) (Himedia). The plates were incubated at 37°C for 24-48 hours at ambient air. Only those samples that gave significant growth were considered as infection.

Identification of the Isolates

All isolates of uropathogenic *Escherichia coli* were diagnosed biochemically (16, 17).

Antibiotic Sensitivity Test

It was carried out using agar diffusion method (18).

Mannose resistant hemagglutination (MRHA)

It was carried out to detecting fimbriae other than type-1 fimbriae according to Karr *et al.*, (19).

Mannose sensitive agglutination of Baker's yeast (MS agglutination)

This was carried out to detect the presence of type-1 fimbriae. It was performed according to Schembri *et al.*, (20).

Hemolysin production

The production of hemolysin was tested on human blood agar plates according to Wilson and Gaido (17).

Detection of Aerobactin production

Detecting the production of aerobactin depended on modification of the chemical method of Csaky on (18).

Results and Discussion

Culture Results

The *Escherichia coli* were isolated from 68 patient (52.3%), it was seen that the age groups 30-39 years the most susceptible for infection with recurrent urinary tract infection when comparison with other age group (Table 1), recurrences could be due to a persistent focus of infection especially in young women, but the vast majority is thought to represent re-infection (19,20,21), However, UTIs in men are uncommon until age 50year and are usually indicative of an underlying urologic abnormality (22). Except in the first year of life, acceptance of the diagnosis of UTI in males requires clear documentation since such infection is unusual in the absence of a history of instrumentation or anal intercourse (23).

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Table 1: Culture results of *E. coli* isolated from urine of patients with recurrent UTI according to patient's gender and age.

| Age group (years) | Female | | Male | | Total No. % |
|----------------------|---------------------|---------------------|---------------------|---------------------|----------------|
| | Positive results | Negative results | Positive results | Negative results | |
| < 10 | 3 | 1 | 1 | --- | 5 (4) |

| | | | | | |
|------------------|----|----|----|----|-----------|
| 10-19 | 2 | 2 | 2 | 1 | 6 (5) |
| 20-29 | 14 | 13 | 4 | 1 | 32 (24) |
| 30-39 | 16 | 14 | 2 | 1 | 33 (25) |
| 40-49 | 7 | 5 | 1 | 4 | 17 (13) |
| 50-59 | 6 | 4 | 2 | 5 | 17 (14) |
| ≥ 60 | 3 | 2 | 5 | 9 | 19 (15) |
| Total no. | 51 | 41 | 17 | 21 | 130 (100) |

--- = None, < less than, ≥ equal or more than

Antibiotic sensitivity pattern of the isolates

The results of sensitivity and resistance of all isolates which were tested to antibiotics summarized in (Table 2). This table show antibiotic susceptibility of *E.coli* isolated from 68 patient with recurrent UTI.

In case of recurrent UTI, the isolates were appeared differences in their responses to different antibiotics, they were sensitive to Amikacin (91%), Ciprofloxin (88%), Gentamicin (69%), Cefuroxime(66%), Vancomycin (50.3%), Cefoperazone (47.4%), Rifampin (44%), Cefazidine (35.2%), Nalidixic acid (59%), Cephalothin (20.7%), Oxacillin (9.5%), Amoxicillin (3.2%) and Ampicillin (2%). It was found that recurrent UTI's isolates were susceptible to antibiotics more than that noticed by Bl. C. isolates.

Resistance of bacteria to different antimicrobial agents may develop due to several mechanisms. Antimicrobial resistance promotes the persistence of the pathogens in infected host during antimicrobial treatment and lead to prolong or life threatening infection (24). Also resistance of ICBC (*E.coli*) may be related with its virulence. Resistance of these bacteria to B-lactam group may be controlled by R-plasmid in which has the ability to transfer to other susceptible bacterial cell rapidly through normal gene exchange process such as conjugation (25).

Extended spectrum of β -lactamase are plasmid mediated, since this plasmids can easily transmitted among different members of Enteriobacteriaceae, accumulation of resistance genes results in strains that contain multiresistant plasmid for this reason this bacteria was resistant to a variety classes of antibiotics (26).

Table 2: Antibiotics sensitivity pattern of *E. coli* isolated from recurrent UTI patients.

| Antibiotics | No. (%) of isolates | |
|-------------|---|----------|
| | Recurrent UTI patients' isolates (n) = 68 | |
| | S | R |
| Amikacin | 62 (91%) | 6 (9%) |
| Ampicillin | 1 (2%) | 67 (98%) |

| | | |
|----------------|-------------------|-------------------|
| Amoxicillin | 2 (3.2%) | 66 (96.8%) |
| Cefuroxime | 45 (66%) | 23 (34%) |
| Cefazidine | 24 (35.2%) | 44 (64.8%) |
| Cefoperazone | 32 (47.4%) | 36 (52.6%) |
| Cephalothin | 14 (20.7%) | 54 (79.3%) |
| Ciprofloxacin | 60 (88%) | 8 (12%) |
| Gentamicin | 47 (69%) | 21 (31%) |
| Nalidixic acid | 40 (59%) | 28 (41%) |
| Oxacillin | 6 (9.5%) | 62 (90.5%) |
| Rifampin | 30 (44%) | 38 (56%) |
| Vancomycin | 34 (50.3%) | 34 (49.7%) |

S: sensitive; R: resistant.

Phenotypic *E. coli*' virulence characterization

Uropathogenic *E. coli* virulence factors were detected phenotypically namely, mannose resistant adhesins (use to detect all fimbriae except type 1 fimbriae), mannose sensitive type 1 fimbriae, hemolysin, and aerobactin (Table 3). As describe in following:

The results of detection of type-1 fimbriae by using yeast cells (5%) represent that 53(78%) isolates of recurrent UTI gave positive result, 12(18%) were negative and the rest 3(4%) did not give clear result.

Results of type P-fimbriae detection by using human blood group (O), clarified that 34(50%) of recurrent UTI isolates have this fimbriae.

Detection of type S-fimbriae by agglutination test using cow blood showed that 7(10.29%) of RUTI isolates.

The detection of Dr-fimbriae by using human blood group (O) which give agglutination after mixing with *E.coli* bacterial suspension and then agglutination disappear after adding chloromphenicol in special concentration meaning this isolates have Dr-fimbriae, the result of this study revealed 25(36.76%) isolates of recurrent UTI gave positive reaction respectively.

Uropathogenic *E. coli* have virulence factors, such as different types of fimbria, that promote binding to the epithelium of the vagina and urethra and enhance their ability to cause recurrent UTI (27, 28, 29). Other factors increase resistance to serum bactericidal activity and host defence mechanisms. UPEC can remain dormant in large bacterial reservoirs within the host and can be reactivated to cause recurrent UTI (30). Type1 fimbrea, P-fimbrea, S-fimbrea and Dr-fimbrea may play important role in pathogenesis of UPEC in patients with recurrent UTI because the fimbrea responsible for colonization and attachment of bacteria to the receptor of target cells (29). Erythrocytes and urinary endothelial cells have same receptor for adhesions, for this reason pilited bacteria may play important role in develop of recurrent UTI, because such fimbrea contribute in inflammatory response when phagocytic cells response to inflammatory stimulus (*E.coli*) they become activated and begin to generate large quantities of reactive oxygen

intermediates such superoxide, hydrogen peroxide, hypochlorous acid, singled oxygen and the hydroxyl radical they have detrimental effects causing tissue damage and contributing to the development or progression numerous diseases including cancer (31, 32, 33).

The detection of aerobactin was carried out using spectrophotometer, the results appeared in UPEC isolated from patients with recurrent UTI 15 (22%), because this virulence factor help bacteria to uptake iron which essential in aerobic metabolism and multiplication. Part of the host response to infection is reduce the amount of iron available to the invading UPEC by decreasing the amount of intestinal iron absorption, synthesizing proteins and shifting iron from plasma pool into intracellular storage, the bacteria face a formidable challenge in meeting their iron needing in their infection(34).The hydroxymate siderphore (aerobactin) is the most effective of the several iron chelation employed by the bacteria for the iron acquisition (34).

Table 3: Phenotypes of virulence factors of *E. coli* isolated from recurrent UTI patients.

| Virulence factors | No. (%) of positive <i>E. coli</i> isolates |
|-----------------------------------|--|
| | Recurrent UTI patients' isolates (n) = 68 |
| P-fimbriae | 34(50) |
| S-fimbriae | 7(10.3) |
| Dr-adhesion family | 25(36.8) |
| MS agglutination of Baker's yeast | 53(78) |
| Aerobactin production | 15(22) |

Conclusion

1. Recurrent UTI highly distributed in age group 30-39 years.
2. Amikacin and Ciprofloxacin produce good efficacy in treatment of RUTI while Ampicillin, Amoxicillin and Oxacillin have low effect against it.
3. Type 1 fimbriae more spreaded virulence factor between this bacteria.

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