Isolation Of Escherichia Coli And Klebseilla From Patient With Urinary Tract Infection

عزل بكتريا القولون المعوية والكلبسيلا من المرضى المصابين بالتهاب المجاري البولية

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الخلاصة:
الخلفية:مقاومة بكتريا القولون المعوية والكلبسيلا للمضادات الحيوية تسبب تعقيداً في معالجة التهاب المجاري البولية.
الهدف: ان الهدف من هذه الدراسة هو تقيم فعالية المضاد الحيوي ضد البكتريا المسببة لالتهاب المجاري(بكتريا القولون المعوية) البولية في محافظة النجف الاشرف.
طرق العمل:تم جمع ٨١ عينة ادرار من مرضى مصابين بالتهاب المجاري البولية وتم التحري عن بكتريا اشريشيا القولون والكلبسيلا تم فحص حساسية العزلات للمضادات الحيوية.
النتائج :وجد أعلى معدل للمقاومة (١٠٠٪) للامبيسلين وأموكسيسيلين، بينما تكون المقاومة معتدلة للسيفالوتين.
الاستنتاج:وجد ان بكتريا القولون وبكتريا الكلبسيلا متعددة المقاومة للمضاد الحيوي التي تم اختبارها في هذه الدراسة.

Abstract
Background.Resistance to antibiotics arising in *Escherichia coli* and *Klebseilla* species isolates may complicate therapeutic management of urinary tract infection(UTI) by this organism.
Aim. The aim of this study was to assess antibiotic activity against UTI isolates of *E*.coli in Al- Najaf governorate.
Methodology. Atotal of 81 mid –stream urine samples were collected from patient suspected of UTI and screened for the occurrence of *E*.coli.
Result. Susceptibility of the isolates to antibiotics was test by standard methods. Highest rate of resistance (100%) was found toampicillin and amoxicillin, while moderately resistant to cephalothin .
Conclusion.The present study concluded that *E.coli* resistance to multiple antibiotic were recognized.
Keywords: Urinary tract infection, *Escherichia coli* , *Klebseilla*, Resistant to antibiotic.

INTRODUCTION

A UTI is defined as colonization of a pathogen occurring anywhere along theurinary tract: kidney, ureter, bladder, and urethra. Traditionally, UTIs have beenclassified by the site of infection (ie, pyelonephritis [kidney], cystitis [bladder],urethra [urethritis]) and by severity (ie, complicated versus uncomplicated). Acomplicated UTI describes infections in urinary tracts with structural or functional abnormalities or the presence of foreign objects, such as an indwelling urethral catheter. This model does not necessarily reflect clinical management, however. In children, a simpler and more practical approach is to categorize UTIas a first infection versus recurrent infection. Recurrent infections can be further subdivided into unresolved bacteriuria, bacterial persistence, and reinfection.

The recurrence of a UTI may be caused by several reasons. Unresolved bacteriuriais most commonly caused by inadequate antimicrobial therapy. Subtherapeuticlevels of the antimicrobial agents may be a result of noncompliance, malabsorption, suboptimal drug metabolism, and resistant uropathogens unresponsiveto attempted therapy (1). In these cases, infection typically resolvesafter altering the therapy according to antimicrobial sensitivities determined by aproper urine culture.Bacterial persistence and reinfection occur after sterilization of the urine hasbeen documented. In the case of bacterial persistence, the nidus of infection in the urinary tract is not eradicated. Characteristically, the same pathogen is documentedon urine cultures during subsequent episodes of UTI despite negativecultures after treatment.

The uropathogen frequently resides in a location that isshielded from antimicrobial therapy. These protected sites are often anatomicabnormalities, including infected urinary calculi (2), necrotic papillus or foreign objects, such as an indwelling ureteral stent (3,4)or urethral catheters(5), which once infected may not be sterilized. Identification of the anatomicabnormality is essential because surgical intervention (extirpation) may be necessaryto eradicate the source of infection.

E. coli is the most frequent documented uropathogen. Among neonates, UTI secondary to group B streptococci is more common than in older populations (6). In immune compromised children and children with indwellingcatheters, Candida may be isolated from the urine. Nosocomial infectionsare typically more difficult to treat and are caused by various organisms, including E. coli, Candida, Enterococcus, Enterobacter, and Pseudomonas (7).

Bacterial clonal studies strongly support entry into the urinary tract by the fecal-perineal-urethral route with subsequent retrograde ascent into the bladder. Because of differences in anatomy, girls are at a higher risk of UTI thanboys beyond the first year of life. In girls, the moist periurethral and vaginal area spromote the growth of uropathogens. The shorter urethral length increases thechance for ascending infection into the urinary tract. Once the uropathogenreaches the bladder, it may ascend to the ureters and then to the kidneys by someas-yet undefined mechanism. Additional pathways of infection include nosocomial infection through instrumentation, hematogenous seeding in the setting ofsystemic infection or a compromised immune system, and direct extensioncaused by the presence of fistulae from the bowel or vagina(8).

The urinary tract (ie, kidney, ureter, bladder, and urethra) is a closed, normallysterile space lined with mucosa composed of epithelium known as transitionalcells. The main defense mechanism against UTI is constant antegrade flow ofurine from the kidneys to the bladder with intermittent complete emptying of thebladder via the urethra. This washout effect of the urinary flow usually clears theurinary tract of pathogens. The urine itself also has specific antimicrobialcharacteristics, including low urine pH, polymorphonuclear cells, and Tamm-Horsfall glycoprotein, which inhibits bacterial adherence to the bladder mucosalwall (9).

UTI occurs when the introduction of pathogens into this space is associatedwith adherence to the mucosa of the urinary tract. If uropathogens are clearedinadequately by the washout effect of voiding, then microbial colonizationpotentially develops. Colonization may be followed by microbial multiplicationand an associated inflammatory response.Bacteria that cause UTI in otherwise healthy hosts often exhibit distinctivepropertiesknown as virulence factorsto overcome the normal defenses ofthe urinary system (10, 11). In serotypes of E coli frequently isolated in UTI,bacterial adherence to the uroepithelium is enhanced by adhesins, often fimbriae(pili), which bind to specific receptors of the uroepithelium(12,13).

The interactionof fimbriae with the mucosal receptor triggers internalization of thebacterium into the epithelial cell, which leads to apoptosis, hyperinfection, and invasion into surrounding epithelial cells or establishment of a bacterial focus forrecurrent UTI (14). Uropathogenic strains of E coli have been recognized torelease toxins, including cytolethal distending toxin, alpha hemolysin, cytotoxicnecrotizing factor-1, secreted autotransporter toxin that causes cellular lysis, cause cell cycle arrest, and promote changes in cellular morphology and function(15,16). To promote survival, various uropathogens possess siderophore systemscapable of acquiring iron, an essential bacterial micronutrient, from heme(17). Uropathogenic strains of E coli

have a defensive mechanism that consistsof a glycosylated polysaccharide capsule that interferes with phagocytosis andcomplement-mediated destruction.

The most common form of resistance to β-lactam agents is caused by enzymes that render molecules inactive by opening the β-lactam ring. In Gram-positive,β-lactamase is excreted into the medium and therefore destroys the antibiotics exracellularly (18). In contrast, the β-lactamase of Gram-negative bacteria is located in the periplasmic space where they attack the antibiotic before it can reach its receptor site.

METHODOLOGY:

Present study was carried out in two hospitals in Najaf(Al-Sadder Teaching and Al-Zahra Maternity and children).During the period from July to October 2012, a total of 100 urine samples collected (by mid – strem urine) from patients suspected to have UTI were culture on blood agar (Hi-media Laboratories, India) and CHROM agar (Oxoid, France) using standard method. Gram negative rod isolated in significant counts ($>10^5$cfu/ml) in pure culture were included in the study. Identification of isolates up to the species level was done according to conventional scheme of MacFaddin (19) and was confirmed by an additional biochemical test with API20E miniaturized diagnostic test (Bio Merieux,France).

RESULTS

The present study included a collection of 100 urine samples from two hospitals in Najaf during the period from July to October, 2012 Out of the 100 samples processed; 81 (81%) showed significant bacteriuria. A total of 55(67.9%) females and 26 (32%) males had positive urine culture (significant bacteriuria). The bacterial isolates obtained as a pure and predominant growth from urine samples were only considered for the present study.

All bacteria isolated in this study were identified based on colonial morphology, and biochemical reactions according to MacFaddin (2000). The organisms grown on the culture of all the 81 urine samples with significant bacteriauria were as follows: 43 (53%) isolates of *E. coli*, 26 (32%) isolates of *Klebsiella* spp., 8 (9.8%) isolates of *Proteus* spp., and 4 (4.9%) isolates of *Pseudomonas* spp. The frequency of antibiotic resistance of the 43 *E. coli* isolates. *E. coli* isolates (100%) were found to be resistant to ampicillin and amoxicillin. (94.7%) of*E. coli* isolates were highly resistant to oxacillin, and moderately resistant to cephalothin (22.5%), ciprofloxacin, gentamycin, trimethoprime (46.5%), nitrofurantoin (44.1%), nalidxic acid (37.2%), cefotaxime (39.5%), ceftazidime (34.8%), and chloramphenicol (27.9%). On the other hand, all *E. coli* isolates were susceptible to imipenem.

DISCUSSION

Urinary tract infections are the most common type of the clinical disease produce by the *E.coli*. The results showed a high incidence of UTI in females than males. which might be due to variety of factors, such as the close proximity of the female urethral meatus to the anus (20), and alternations in vaginal microflora that play a critical role in encouraging vaginal colonization with coliforms which may lead to UTI (21, 22). (23) Reported that the bladder infections are 14-times more common in females than males by virtue of the shortened urethra. Moreover, (24) showed that UTI are more common in females, 40% of women have an episode in their lifetime

when they are sexually active.

Numerous in vitro studies have shown the *E. coli* and *Klebsiella* spp. as the most common causes of UTI (25). All *Klebsiella* isolates were identified according to, The development of antibiotics resistance in these isolates is often related to the overuse and misuse of the antibiotics prescribed. Iraq is one of the developing countries where antibiotics sold over the counter, an attitude that encourages self-medication.

In this investigation, the reason of β-lactam resistance of *E. coli* isolates is probably due to the production of TEM β-lactamases, which may be genetically localized on the chromosome or on a plasmid. The TEM-1 is the most commonly encountered β-lactamase in Gram-negative bacteria; up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1 (26). Other studies from many areas reported an increasing resistance of *E. coli* to ampicillin.

In this investigation, although the β-lactamases undoubtedly play a major role in the resistance to β-lactam antibiotics, the high ratio of resistance to ampicillin and amoxicillin was not only attributable to the production of β-lactamase enzymes. The other mechanisms conferring resistance to these compounds is caused by reducing of the activity of β-lactam antibiotics in a resistant cell due to many factors such as; the sensitivity of the antibiotic to β-lactamases, the penetration through the outer membrane, the affinity for the target (PBPs), the amount of β-lactamase, and the affinity of the antibiotic for the β-lactamase . A range of antibiotics have been used for the treatment of UTI caused by *E. coli* and *K.pneumoniae* in Iraq and other countries. However, the widely spread use of this approach has criticized on the ground of drug toxicity and the risk of an increase spread of antibiotic resistance (27).

Bacterial resistance to antibiotics is now widespread and possesses serious clinical threats. The organisms develop resistance to antibiotics by any of the following mechanisms: selection, mutation, phage transduction, and transference. Microbial resistance can be either hereditary in the organism or acquired through the environment. The high resistance in the present study may be due to antibiotic abuse which leads to development of resistant isolates in Iraq. In agreement with the present study, (28) found that 56.8% of clinical *E. coli* isolates in Najaf were resistant to more than five antimicrobial agents.

CONCLUSION:

The study concludedEscherichia coli and K. pneumoniae were the predominant species recovered in patients with significant bacteriuria. Most of the test isolates were resistant to antibiotics, for that reason such organism pose a serious therapeutic problem in Najaf hospitals.

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