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Determination of Antibiotic Resistance Pattern in *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Urinary Tract Infection (Iran-Esfahan 2009-2010)

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Some of gram negative Bacilli such *Klebsiella pneumoniae* and *Escherichia coli* are a relevant opportunistic pathogen that accounts for nosocomial infections. Urinary Tract Infection (UTI) is a on of the most prevalent infection in the worldwide and. Antibiotic resistance in Bacteria is one of the emerging health related problem in the world nowadays. The search were laboratory and performed in Azzahra and Shariaty hospitals in 2009 year in Isfahan, according to statistical formula randomly selected 91 samples from urinary infections. Bacterial identification was performed with microbiological methods. ESBLs determine were in two section, screening and confirming testing were respectively included by Kirby-Bauer's disc diffusion and Combining disc methods and survey antibiogram was performed with by Kirby-Bauer method. From 91 samples frequency of *E.coli and K.pneumoniae* strains was respectively 84/6% and 15/4%. From 77 isolated *E.coli* strains: 76.6% from out hospitalized and 23.4% from hospitalized and from 14 isolated *K.pneumoniae* strains: 57.1% from out hospitalized and 42.9% from hospitalized patients. According to antibiogram result respectively 59.2%, 54.9%, 30.3%, 27.8%, 19.5% and 16.7% of *E.coli* strains into Co-Trimoxazole,Nalidixic acid, Ciprofloxacin, Gentamicin, Ceftazidime, Nitrofurantoin, and respectively 75%, 50%, 40%, 44.5%, 37.5%, 37.5%, 22.3% and 0% of *K.pneumoniae* strains were resistant into Ampicillin,Co-Trimoxazole, Nitrofurantoin, Ceftazidime, Amikacin, Cephotaxime, Imipenem and Ciprofloxacin.The result of this study showed that the rate of nosocomial UTI in hospitalized patients. According to increase of resistance to the common usage drugs in UTI gram-negative bacteria, correct usage of antibiotics is recommended.

Key Words: Urinary Infection, Gram Negative Bacilli, Antibiotic Resistance.

Normally, urine is sterile. It is usually free of bacteria, viruses, and fungi but does contain fluids, salts, and waste products. An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply. The urethra is the tube that carries urine from the bladder to outside the body. Most infections arise from one type of bacteria, *Escherichia coli (E. coli),* which normally lives in the colon. In many cases, bacteria first travel to the urethra. When bacteria multiply, an infection can occur. An infection limited to the urethra is called urethritis. If bacteria move to the bladder and multiply, a bladder infection, called cystitis, results. If the infection is not treated promptly, bacteria may then travel further up the ureters to multiply and infect the kidneys. A kidney infection is called pyelonephritis[1-6].

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The bacterial strains that cause UTIs include: *Escherichia coli* is responsible for most uncomplicated cystitis cases in women, especially in younger women. *E. coli* is generally a harmless microorganism originating in the intestines. If it spreads to the vaginal opening, it may invade and colonize the bladder, causing an infection. The spread of *E. coli* to the vaginal opening most commonly occurs when women or girls wipe themselves from back to front after urinating, or after sexual activity. *Staphylococcus saprophyticus* accounts for 5 - 15% of UTIs, mostly in younger women. *Klebsiella*, *Enterococci* bacteria, and *Proteus mirabilis* account for most of remaining bacterial organisms that cause UTIs. They are generally found in UTIs in older women. Rare bacterial causes of UTIs include *ureaplasma urealyticum* and *Mycoplasma hominis*, which are generally harmless organisms[5-10].

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Symptoms include frequent feeling and/or need to urinate, pain during urination, and cloudy urine. The main causal agent is *Escherichia coli*. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it. When bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI [6-11].

The most common type of UTI is acute cystitis often referred to as a bladder infection. An infection of the upper urinary tract or kidney is known as pyelonephritis, and is potentially more serious. Although they cause discomfort, urinary tract infections can usually be easily treated with a short course of antibiotics with all no significant difference between the classes of antibiotics commonly used.

The most common organism implicated in UTIs (80–85%) is *E. Col*i while Staphylococcus saprophyticus is the cause in 5–10%.

The bladder wall is coated with various mannosylated proteins, such as Tamm-Horsfall proteins (THP), which interfere with the binding of bacteria to the uroepithelium. As binding is an important factor in establishing pathogenicity for these organisms, its disruption results in reduced capacity for invasion of the tissues. Moreover, the unbound bacteria are more easily removed when voiding. The use of urinary catheters (or other physical trauma) may physically disturb this protective lining, thereby allowing bacteria to invade the exposed epithelium [8-13].

During cystitis, uropathogenic *Escherichia coli* (UPEC) subvert innate defenses by invading superficial umbrella cells and rapidly increasing in numbers to form intracellular bacterial communities (IBCs). By working together, bacteria in biofilms build themselves into structures that are more firmly anchored in infected cells and are more resistant to immune-system assaults and antibiotic treatments. This is often the cause of chronic urinary tract infections. Bladder infections are most common in young women with 10% of women getting an infection yearly and 60% having an infection at some point in their life. Pyelonephritis occurs between 18–29 times less frequently. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, urinary tract infection accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations[1-13]. Nearly 1 in 3 women will have had at least 1 episode of urinary tract infections requiring antimicrobial therapy by the age of 24 years. The risk of urinary tract infection increases with increasing duration of catheterization. In non-institutionalized elderly populations, urinary tract infections are the second-most-common form of infection, accounting for nearly 25% of all infections. The condition rarely occurs in men who are younger than 50 years old and who did not undergo any genitourinary procedure. However, the incidence of urinary tract infections in men tends to rise after the age of 50. According to statistics from 1990, the prevalence of urinary tract infections in pre-school and school girls was 1% to 3%, nearly 30-fold higher than that in boys. Also, the statistics from the same year show that approximately 5% of girls will develop at least one urinary tract infection in their school years [1-5].

In what concerns the symptoms of the condition, bacteriuria appears to increase in prevalence with age in women, still being 50 times greater than the one in males. It is estimated that bacteriuria will be experienced by 20 to 50% of older women and 5 to 20% of older men. For prevention measures that studies suggest may reduce the incidence of urinary tract infections. A

prolonged course (six months to a year) of low-dose antibiotics (usually nitrofurantoin or TMP/SMX) is effective in reducing the frequency of UTIs in those with recurrent UTIs [1-5].

Cranberry (juice or capsules) may decrease the incidence of UTI in those with frequent infections. Long-term tolerance, however, is an issue. For post-menopausal women intravaginal application of topical estrogen cream can prevent recurrent cystitis. This however is not as useful as low dose antibiotics[1-5].

A number of measures have not been found to affect UTI frequency including: the use of birth control pills or condoms, voiding after sex, the type of underwear used, personal hygiene methods used after voiding or defecating, and whether one takes a bath or shower[8-13].

Antibiotic resistance is the ability of a micro-organism to withstand the effects of an antibiotic. It is a specific type of drug resistance. Antibiotic resistance evolves naturally via natural selection through random mutation, but it could also be engineered. SOS response of low-fidelity polymerases can also cause mutation via a process known as programmed evolution. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug[14-19].

Antibiotic resistance can also be introduced artificially into a micro-organism through transformation protocols. This can be a useful way of implanting artificial genes into the micro-organism. Antibiotic resistance is a consequence of evolution via natural selection or programmed evolution. The antibiotic action is an environmental pressure; those bacteria which have a mutation allowing them to survive will live on to reproduce. They will then pass this trait to their offspring, which will be a fully resistant generation[14-19].

Several studies have demonstrated that patterns of antibiotic usage greatly affect the number of resistant organisms which develop. Overuse of broad-spectrum antibiotics, such as second- and third-generation cephalosporins, greatly hastens the development of methicillin resistance, even in organisms that have never been exposed to the selective pressure of methicillin *per se* (thus the resistance was already present). Other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion[1-6].

The four main mechanisms by which micro-organisms exhibit resistance to antimicrobials are: Drug inactivation or modification: e.g. enzymatic deactivation of *Penicillin* G in some penicillin-resistant bacteria through the production of â-lactamases.

Alteration of target site : e.g. alteration of PBP—the binding target site of penicillins—in MRSA and other penicillin-resistant bacteria.

Alteration of metabolic pathway: e.g. some sulfonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid[14-19].

The subject of this study was determination of antibiotic resistance pattern in *Klebsiella pneumoniae* and *Escherichia coli* Isolated of urinary tract infection in Iran.

MATERIAL AND METHOD

Clinical isolates

A total of 91 *Enterobacteriaceae* spp. culture isolates from urinary trace infection during the period of 2010, were screened for potential ESBL activity. Based on routine antibiotic disk sensitivity tests, isolates that exhibited intermediate/resistance to any one of the third generation cephalosporins, ceftazidime/ cefotaxime were short listed to detect and confirm ESBL producers [19,20].

Antibiotic susceptibility testing

The susceptibility of the isolates was determined against 8 antibacterial they included, Ceftazidime, Ampicillin, Ceftazidime, Co-Trimoxazole, Amikacin, Nitrofurantoin, Ciprofloxacin and Imipenem (Mast).

Susceptibility and resistance was determined based on the interpretative criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS). *E. coli* ATCC25922, ATCC 35218 and *K. pneumoniae* ATCC 70063 was used as the quality control strain [21].

Screening for ESBLs by double disk synergy test

Enterobacteriaceae cultures that exhibited intermediate/resistance to third generation cephalosporins were screened to detect ESBL producers. A modified double disk synergy test (disk approximation test) first described by Jarlier[22].

Phenotypic confirmatory test by disk diffusion assay

ESBL production was confirmed among potential ESBL producing isolates by phenotypic tests. Sensitivity disks containing third generation cephalosporins with and without clavulanic acid were prepared as follows: ceftazidime 30 mg(Ca), ceftazidime+clavulanic acid 10 mg (Ca +), cefotaxime 30 mg (Ce), cefotaxime+clavulanic acid 10 mg (Ce+);. Disk diffusion assay was carried out as per guidelines of NCCLS and differences in zone diameters between disks with and without clavulanic acid were recorded (Fig. 1) [22].

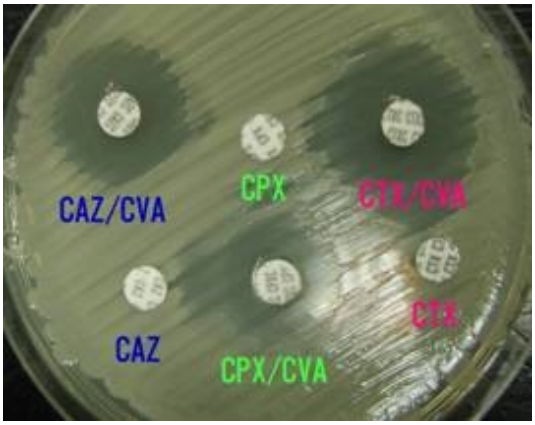

Fig. 1. Confirmatory Test for Detection of ESBLs

RESULTS

From 91 samples frequency of *E.coli and K.pneumoniae* strains was respectively 84/6% and 15/4%. From 77 isolated *E.coli* strains: 76.6% from out hospitalized and 23.4% from hospitalized and from 14 isolated *K.pneumoniae* strains: 57.1% from out hospitalized and 42.9% from hospitalized patients.

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CONCLUSION

Some organisms are intrinsically resistant to many (or even all) antimicrobial agents. Some microbiologists call this "primary resistance". Other organisms acquire resistance, either by mutating or by sharing the resistance genes of resistant organisms. This should not be confused with the term "clinical resistance" which refers to the failure of an antimicrobial to eradicate infection, despite the apparent ability of the agent to kill the 'bug' in vitro. A wide variety of problems can account for clinical resistance, such as impaired host immunity, inadequate drug delivery, and foreign material in the site of a wound[19]. Antimicrobial resistance is an ever-increasing problem in hospitals, especially in intensive care units. The problem is so severe that some authorities believe that we are entering the "post-antibiotic era" where widespread bacterial resistance will render most antibiotics ineffective[19].

According to result there is high prevalence of ESBLs in *E.coli* and *K.pneumoniae* because third generation cephalosporin's are usually first line against to many severely infections disease. Justifiable use of will be an effective means of controlling and decreasing spread of ESBLs strains[22]. Extended spectrum â lactamases (ESBLs) continue to be a major problem in clinical setups world over, conferring resistance to the expanded spectrum cephalosporins. Phenotypic confirmation of ESBLs was carried out by disk diffusion assay as per the recommendations of NCCLS[22]. The zone of inhibition of the antibiotic alone was compared with the zone of inhibition in combination with clavulanic acid. According to NCCLS recommendations a difference of 5 mm increase in zone diameter for either agent tested in combination with clavulanic acid versus its zone diameter when tested alone confirms the presence of ESBLs[22]. The unusually high incidence of ESBLs should be a cause of concern to the regulators of

the hospital antibiotic policy. Over reliance on third generation cephalosporins to treat gram negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics[22].

As ESBLs are frequently encoded by genes located on different transferable genetic elements, a variety of epidemiological situations have been identified, ranging from sporadic cases to large outbreaks. Whereas ESBLs were initially associated with nosocomial outbreaks caused by single enzyme-producing strains, recent studies have revealed more complex situations, with a significant increase in community isolates[23-29].

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