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Full Length Research Paper

Survey frequency of extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from urinary tract infection in Iran

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Extended-spectrum beta-lactamases (ESBLs) are defined as β-lactamase capable of hydrolyzing third generation cephalosporin's and inhibited by β-lactamase inhibitor. Urinary tract infection (UTI) is a one of the most prevalent infection in worldwide and is the second most common infection. Most of UTI are due to *Escherichia coli.* Antibiotic resistance in ESBLs strains is one of the emerging health related problem in the world nowadays. The present study was performed at Tow Tertiary Care Hospitals in Isfahan, Iran during a 14 month period (7th June, 2008 to 6th July, 2010). Standard microbiological methods were performed. In order to validate the ESBLs producing strains, the ceftazidim, ceftazidim clavulanic acid, cefotaxim, cefotaxim clavulanic acid (according to CLSI, 2010) were used by disk diffusion method. Results from 91 samples showed that the frequency of *E. coli* and *Klebsiella pneumoniae* strains was 84/6 and 15/4% respectively, while the Frequency of ESBLs in *E. coli* and *K. pneumoniae* strains was obtained in 27 samples as 35/06% and 5 samples as 35/71%), respectively. According to result, there is high prevalence of ESBLs in *E. coli* and *K. pneumoniae* because the third generation cephalosporins are usually in the first line against too many severe infections. Justifiable use of this method will be an effective means of controlling and decreasing spread of ESBLs strains.

Key words: Extended spectrum beta lactamase, *Escherichia coli, Klebsiella pneumoniae.*

INTRODUCTION

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, 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 (Guideline, 2010; Hanson, 2004; Jepson et al., 2008; Justice et al., 2006; Nicolle, 2001, 2008; Raz and Stamm, 1993; Zalmanovici et al., 2010).

The most common organism implicated in UTIs (80 to 85%) is *E. coli*, while *Staphylococcus saprophyticus* is implicated in UTIs for 5 to 10% (Foster, 2008; Modgil et al., 2006; Roussey et al., 2007; Warren et al., 1999).

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 (Foster, 2008; Modgil et al., 2006; Roussey et al., 2007; Warren et al., 1999). During cystitis, uropathogenic *E. 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 (Foster, 2008; Modgil et al., 2006; Roussey et al., 2007; Warren et al., 1999).

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 to29 times less frequently (Foster, 2008; Hanson, 2004; Modgil et al., 2006; Raz, 1993; Roussey et al., 2007; Warren et al., 1999).

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, 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 (Foster, 2008; Hanson, 2004; Modgil et al., 2006; Raz, 1993; Roussey et al., 2007; Warren et al., 1999).

The condition rarely occurs in men who are younger than 50 years old and who did not undergo any genitor-urinary 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. 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 (Foster, 2008; Modgil, 2006; Roussey, 2007).

Among the wide array of antibiotics, β-lactams are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use (jalalpoor et al., 2009, 2010). The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases. Many of the second and third generation penicillins and cephalosporins were specifically designed to resist the hydrolytic action of major β-lactamases. However, new β-lactamases emerged against each of the new classes of β-lactams that were introduced and caused resistance (Jalalpoor et al., 2009, 2010). The latest in the arsenal of these enzymes has been the evolution of ESBLs. These enzymes are commonly produced by many members of Enterobacteriaceae, especially *E. coli* and *K. pneumoniae* and efficiently hydrolyze oxyimino-cephalosporins conferring resistance to third generation cephalosporins such as cefotaxime, ceftazidime and ceftriaxone and to monobactams such as aztreonam. First isolated in 1983 in Germany, ESBLs spread rapidly to Europe, US and Asia and are now found all over the world. Being plasmid mediated, they are easily transmitted among members of Entero-bacteriaceae thus facilitating the dissemination of resistance not only to β -lactams but to other commonly used antibiotics such as quinolones and aminoglycosides (Bradford, 200; Bush, 1996; Endimiani and Paterson, 2007; Mendelson et al., 2005; Sehulster, 2003; Shukla, 2004).

ESBLs have emerged as a major problem in hospitalized patients worldwide and have been involved in epidemic outbreaks in many institutions in Asia and constitute a serious threat to the current β-lactam therapy as these enzymes cause resistance to most penicillins, cephalosporins and aztreonam. Typically, nosocomial outbreaks were associated with previous antibiotic therapy, especially ceftazidime monotherapy (Bradford, 2001; Bush, 1996; Jain, 2003; Lytsy et al., 2008; Mendelson et al., 2005; Yun, 2002). Hospital colonization by ESBL producing bacteria is usually a complex phenomenon involving many different mechanisms, dissemination of several epidemic strains and dissemination of plasmids and resistant genes. Specific risk factors include prolonged hospital stay, severity of illness, ICU, urinary or arterial catheterization, intubation and mechanical ventilation. ESBLs commonly occur in surgical wards as well as most other areas of the hospital and frequently from patients from extended care facilities (Bradford, 2001; Bush, 1996; Jain, 2003; Lytsy et al., 2008; Mendelson et al., 2005; Yun, 2002).

Since ESBL positive isolates show false susceptibility to expanded spectrum cephalosporins in standard disk diffusion tests it is difficult to reliably detect ESBL production by the routine disk diffusion techniques (Mendelson et al., 2005). Specific detection methods such as double disk potentiation methods recommended by NCCLS have to be adopted. ESBLs are inhibited by β-lactamase inhibitors like clavulanic acid, sulbactam and tazobactam and this property of specific inhibition can be utilized for the detection and confirmation of ESBLs (Bradford, 2001; Bush, 1996; Jain, 2003; Lytsy et al., 2008; Mendelson et al., 2005; Yun, 2002). The aim of the present study was to investigate the prevalence of Enterobacteriaceae producing ESBLs in the urinary trace infection in both hospitalized and non hospitalized patients in Esfahan city in Iran.

MATERIALS AND METHODS

Clinical isolates

A total of 91 Enterobacteriaceae spp. culture isolates from urinary trace infection during the 2008 to 2010 year, 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 (Jalalpoor et al., 2007; Washington et al., 2006).

Screening for ESBLs by double disk synergy test

Enterobacteriaceae cultures that exhibited intermediate/resistance to third generation cephalo-sporins were screened to detect ESBL producers. A modified double disk synergy test (disk approximation test) first described by Jarlier (Agraval and Ghush, 2008; Wikler et al., 2006). Susceptibility and resistance was determined based on the interpretative criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS). *E. coli* ATCC 25922, ATCC 35218 and *K. pneumoniae* ATCC 70063 was used as the quality control strain (Agraval and Ghush, 2008; Wikler et al., 2006).

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 guidelines of NCCLS and differences in zone diameters between disks with and without clavulanic acid were recorded (Figure 1) (Agraval and Ghush, 2008; Wikler et al., 2006).

RESULTS

From 91 samples frequency of *E. coli* and *K. pneumoniae* strains was respectively 84/6 and 15/4%. Frequency of ESBLs in *E. coli* and *K. pneumoniae* strains was respectively 27 samples and 5 samples (Figure 2).

DISCUSSION

According to the result, there is high prevalence of ESBLs in *E. coli* and *K. pneumoniae* because third generation cephalosporin's are usually in the first line against many severe infections. The justifiable use of this method will be an effective means of controlling and decreasing the spread of ESBLs strains. ESBLs continue to be a major problem in clinical setups world over,

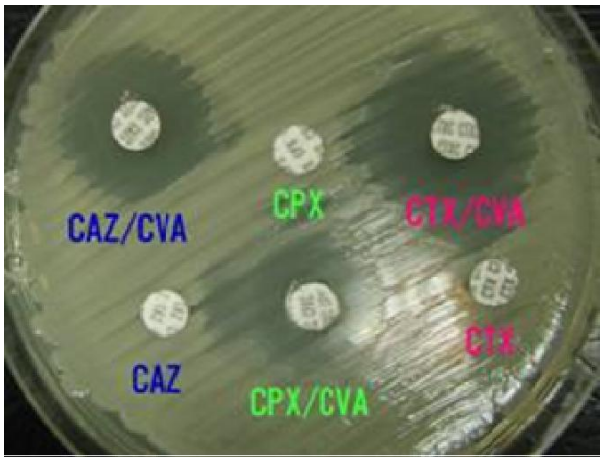

Figure 1. Confirmatory test for detection of ESBLs.

conferring resistance to the expanded spectrum cephalosporins. Phenotypic confirmation of ESBLs was carried out by disk diffusion assay as per the recommendations of NCCLS (Agraval and Ghush, 2008).

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 (Agraval and Ghush, 2008). Our without clavulanic acid were prepared as follows: acid 10 mg (Ce+). Disk diffusion assay was carried out as per study indicates that 35.65% Enterobacteriaceae spp. isolated over a period of one year were ESBLs pro-ducers. 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 (Scheckler, 1998; Shlaes, 1997; Struelens, 1998; Tenover, 1997; Widmer, 2000; WHO, 2001). 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 (Scheckler, 1998; Shlaes, 1997; Struelens, 1998; Tenover, 1997; Widmer, 2000; WHO, 2001).

Antibiotic selective pressure in hospitals may amplify the number of carriers harboring resistant bacteria and enhance the opportunity for these bacteria to cause infections. This fact could also be responsible for the higher prevalence of urinary trace infection carriage of ESBL-producing Enterobacteriaceae in the nosocomial

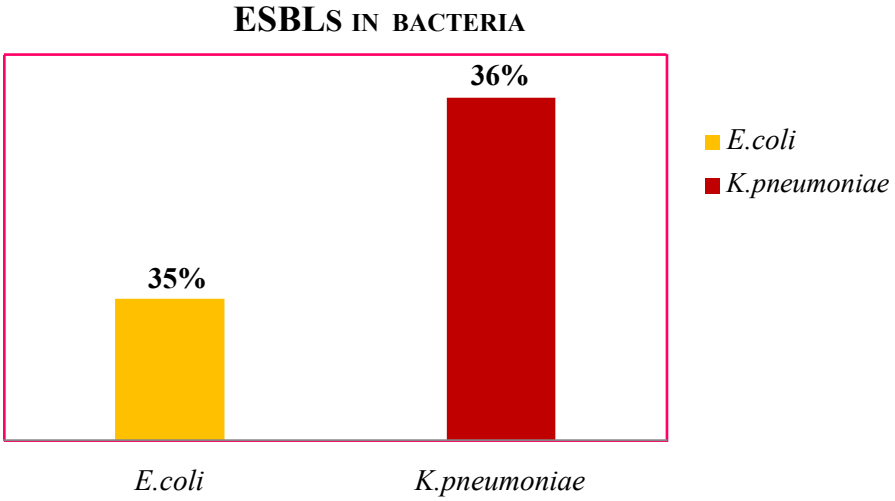

Figure 2. Frequency of ESBLs in *E. coli* and *K. pneumoniae* st.

setting than in the community (this study) or the finding that the rate of ESBL colonization is higher among patients admitted to high-risk units with high levels of antibiotic consumption. A similar situation can be applicable to nursing homes and residents of health care or skilled care facilities, among whom the rates of coloniza-tion with multiresistant pathogens, including ESBL producers, is higher than that among true community patients or healthy volunteers (Scheckler, 1998; Shlaes, 1997; Struelens, 1998; Tenover, 1997; Widmer, 2000; WHO, 2001).

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