BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 10(7)

Antibiotic resistance of *E. coli* isolates from urine samples of Urinary Tract Infection (UTI) patients in Pakistan

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Received June 14, 2014; Revised June 25, 2014; Accepted June 26, 2014; Published July 22, 2014

Abstract:
Drug resistance is becoming alarming with the passage of time worldwide in general and in third world countries in particular. Human urine specimens of patients of urinary tract infection at Sheikh Zayed hospital, Lahore, Pakistan were analyzed for drug resistance in *Escherichia coli*. A total of 69 *Escherichia coli* isolates from human urine specimens were obtained and screened for their antibiograms. A total of seven antibiotic resistance profiles were obtained with over 65% of the isolates showing multi-drug resistance. Very high resistance levels were detected against augmentin and gentamicin (87.5 &77.5 % respectively) while imipenem and tazocin recorded the least resistance levels (32.5% and 12.5% respectively) among the isolates.

Key Words: *E.coli*, Isolate, susceptibility, Antibiotic resistance, Urinart Tract infection.

Background:
Urinary tract infections (UTIs) are among the most common infectious diseases all over the world. Urinary tract infections encompass ranges of morbidity including pyelonephritis and cystitis which are characterized by the existence of microorganism in urinary tract **[1]**. Escherichia coli are a bacterial organism that belongs to the family Enterobacteriaeceae. E. coli is one of the main causes of both nosocomial and community acquired infections in humans. The organism is therefore of clinical importance and can be isolated from various clinical specimens. It is one of the organisms most frequently isolated from urine and blood **[2]**. In addition to Urinary Tract, *E. coli* is the most frequent pathogen associated with Intra-Abdominal Infection (IAI) **[3]**. Understanding the prevailing pattern of antimicrobial resistance is the most important question especially when gram negative isolates continue to demonstrate uncontrolled resistance to various currently in use antimicrobial drugs **[4].**

As an extraintestinal pathogen, *E. coli* is best known for causing urinary tract infection (UTI), bacteremia, and neonatal bacterial meningitis (NBM). The distinctive strains of *E. coli* responsible for most cases of UTI, sepsis, and NBM represent a limited number of virulent clones that are characterized by specific O.K.H serotypes and derive predominantly from *E. coli* phylogenetic group B2, as defined by multilocus enzyme electrophoresis and to a lesser extent from group D **[5]**. It has been observed that antibiotic susceptibility of bacterial isolates is dynamic and varies with time and environment **[6]**. There is a need for a periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities. According to **[7],** *E. coli* is highly resistant to ampicillin, amoxicillin, tetracycline and trimethoprim & sulfamethoxazole. The widespread occurrence of drug resistant *E. coli* and other pathogens in our environment has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide

the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both **[8]**.

Methodology:

Sample collection

69 clinical specimens comprising of urine were screened for *E. coli* in the microbiology laboratory section of Sheikh Zayed Hospital, Lahore. The specimens were processed using standard microbiological methods. All isolates were identified using conventional techniques **[9]**.

Antibiotic susceptibility testing

The specimens were processed for isolation of E.Coli by using standard microbiological techniques and the antimicrobial susceptibility was determined by using Kirby-Bauer disk diffusion technique recommended by Clinical & Laboratory Standards Institute (CLSI), 2010 guidelines **[10].** Susceptibility of isolates to antibiotics were tested against the eight antibiotics, namely ampicillin, nitrofurantoin, gentamicin, amikacin, ciprofloxacin, augmentin, imipenem and tazocin. Inhibition zone sizes were interpreted using standard recommendations.

Ethical issues

In present study, we made an effort to protect the health, privacy and secrecy of personal information and rights to self-determination of all the participants. All ethical issues were considered and the study was conducted after approval from institutional review board and with hospital's permission. Consent was taken from all the volunteer participants of this study to use their urine samples to determine antibiotic resistance patterns of E. coli isolates. The name, personal and medical information of all the participant was kept secret.

Results:

The distribution of *E. coli* from various clinical urine specimens is shown in **Table 1 (see supplementary material).** Total 69 urine samples from UTI patients were screened for *E.Coli* out of which 40 were positive for *E.Coli*. Results of antimicrobial resistance of *E. coli*. A total of eight antibiotics were used against 40 strains (n = 40) of*E. coli(table 1)*. The table indicates the high resistance pattern among strains as most strains are resistant to different antibiotics. The resistance rates for ampicillin (67.5%), nitrofurantoin (52.5%), gentamicin (70%), amikacin (55%), ciprofloxacin (65%), augmentin (77.5%), imipenem (32.5%) and tazocin (12.5%) were observed. High resistance to augmentin and gentamicin was observed whereas more than eighty seven percent of the strains were sensitive to tazocin, 67.5% to imipenem, 47.5% to nitrofurantoin, 45% to amikacin and 35% to ciprofloxacin. In **Table 2 (see supplementary material)** are presented the detailed results of the antibiotic resistance screening tests and summary of the antibiogram profiles.of strains showing antibiotic resistant pattern against different number of antibiotics (1 to 7). But no strain showed resistance against all eight antibiotics. The results show that about 65% of the *E. coli isolates* are multidrug resistant, i.e. are resistant to 3 or more drugs.

Discussion:

The antibacterial drugs most frequently prescribed in UTIs' treatment throughout the world are cephalosporins, semi-synthetic penicillins with or without beta-lactamase inhibitors , trimethoprim- sulfamethoxazole, and quinolones **[11,12]**, however, it was proved that resistance to penicillin, sulfamethoxazole, trimethoprim and cephalotin were 100%, 30.89%, 16.26% and 20.32%, respectively **[13]**. High resistance of *E. coli* to antimicrobial agents tested was observed in this study which is similar to what was observed by who reported very high resistance of *E. coli* isolates to ampicillin and amoxicillin. Resistance to ampicillin observed in this study was similar to what was observed in South Africa, Israel, (62 - 84%) and Hong Kong, Philippines (64 - 82%) **[14]**. They further reported 53% of *E. coli* isolates resistant to nitrofurantoin and 67% to gentamicin. Their findings are in harmony with the reports of this study, showing very high resistance to nitrofurantoin and gentamicin.. The reason for this high resistance to commonly used antibiotics may be due to widespread and indiscriminate use in our environment.

Isolates in this study were (67.5%) sensitive to imipenem. This type of sensitivity of *E. coli* isolates to imipenem has earlier been reported by **[15].** Concern over increasing resistance to β-lactam antibiotics led to the development of β-lactamase inhibitors. These drugs, including clavulanic acid, sulbactam, and tazobactam, have little intrinsic antibacterial activity, but can bind irreversibly to the β-lactamases produced by many bacteria, thereby inactivating the enzymes and leaving the microorganisms sensitive to β-lactamase susceptible antibiotics **[16].** In clinical practice, the β-lactamase inhibitors are often administered in combination with β-lactam antibiotics to extend the spectrum of antibacterial activity of the antibiotics. Tazobactam irreversibly inhibits a broad range of plasmid-mediated and chromosomal bacterial β-lactamases and is considered the most active of currently available β-lactamase inhibitors. The combination of this agent with piperacillin, a β-lactamase-sensitive antibiotic, expands the activity of piperacillin to organisms that produce β-lactamases, including *Enterobacteria*, *Staphylococcus*, and *Bacteroides* species .Isolates in this study were highly sensitive to tazocin (87.5%). From the present study, it appears that tazocin and imipenem are the drugs of choice for serious infections with *E. coli* organisms as has been recommended earlier **[17].**

In recent years, use of fluoroquinolones has increased in many countries and emergence of resistance of bacterial isolates to fluoroquinolones has been observed. Consistent stepwise increase in *E. coli* resistance to ciprofloxacin was observed from 1995 (0.7%) to 2001 (2.5%) by **[18].** Ciprofloxacin resistance in Portugal was 25.8% and Italy 24.3% while in Germany and Netherlands it was 15.2% and 6.8% respectively **[19].** The percentage of ciprofloxacin resistance observed in this study was 65%, which is on the high side. In pervious years, *E. coli* was 100% susceptible to the fluoroquinolones. In 1996, Egri-Okwaji reported 100% susceptibility of *E. coli* isolates to ofloxacin. High resistance of *E. coli* to ciprofloxacin has also been documented by **[14];** they observed that 24% of 189 *E. coli* isolates were resistant to ciprofloxacin. Fluoroquinolone-resistance is typically encoded chromosomally. This resistance against Fluoroquinolones in our study may reflect significant antibiotic pressure in the environment rather than co-carriage of this resistance gene on plasmids.

The reason for the high resistance to ciprofloxacin observed in this study may be due to increasing an irrational consumption rate, transmission of resistant isolates between people and

consumption of food from animals that have received antibiotics. Self-medication and non-compliance with medication and sales of substandard drug may account for the rise in antibiotic resistance observed in this community. The strategy to overcome bacterial resistance is multidirectional. The knowledge that improper exposure of microorganisms to antibiotics results in emergence of resistance strains requires that antibiotics should only be prescribed on receipt of culture sensitivity testing reports to ensure proper and effective therapy. Moreover these must be given in optimal doses, in appropriate combination and for sufficient duration. Such findings are indicative of the overall health-care system within Pakistan with regard to proper post-operative care, hygiene and patient awareness. Since antimicrobial resistant patterns are constantly evolving there is the necessity for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empiric therapies.

Conclusion:

Urinary tract infection (UTI) is most frequent infection after respiratory tract infection. As more than 2/3 of all pathogenic organisms are *E. coli*, antibiotic susceptibility profiles especially of *E. coli* should be considered during the selection of antibiotics for the treatment of UTIs. In the set up of Pakistan and other third world countries, regular urine cultures may be advisable, as treatment failure may likely to occur with frequently prescribed antibiotics.

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Edited by P Kangueane
Citation: **Jafri *et al.*** Bioinformation 10(7): 419-422 (2014)

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Supplementary material:

Table 1: Antibiotic sensitivity/resistance pattern of *E. coli* strains isolated from Urine sample (n=40)

Antibiotic Tested	Sensitive (%)	Resistant(%)
Ampicillin	13 (32.5)	27 (67.5)
Nitrofurantoin	19 (47.5)	21 (52.5)
Gentamicin	12 (30)	28 (70)
Amikacin	18 (45)	22 (55)
Ciprofloxacin	14 (35)	26 (65)
Augmentin	09 (22.5)	31 (77.5)
Imipenem	27 (67.5)	13 (32.5)
Tazocin	35 (87.5)	05 (12.5)

Table 2: Antibiotic resistance profiles of *E. coli* isolated from urine specimens

Number of antibiotics tested	No. & Percent of strains showing resistance
one antibiotic	1 (2.5%)
Two antibiotics	4 (10%)
Three antibiotics	6 (15%)
Four antibiotics	6 (15%)
Five antibiotics	5 (12.5%)
Six antibiotics	7 (17.5%)
Seven antibiotics	8 (20%)

Table 2 shows that number & percentage of bacterial strains showing resistance to antibiotics increases with increase in the number of antibiotics. It may be the confounding effect of more than one antibiotics or a reaction of one antibiotic in combination to the other. In fact, it can also be revealed that individual antibiotic is more specific and effective against a particular species of bacteria.