International Journal of Molecular and Clinical Microbiology 7(2) (2017) 849-854

International Journal of
Molecular and Clinical Microbiology

# Antibiotic resistance pattern and serotyping of *Escherichia coli* producing siderophore in people with urinary tract infection

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ARTICLE INFO

*Article history:*
Received 4 September 2017
Accepted 19 November 2017
Available online 1 December 2017

*Keywords:*
*E. coli,*
*urinary infection,*
*siderophore,*
*antibiotic*

ABSTRACT

Urinary tract infection is one of the most common bacterial infections of human. The most common agent of urinary tract infection is *Escherichia coli*. This study aims to determine the prevalence of uropathogenic *E. coli* urinary infection in human with different antimicrobial resistance, and quantitive and qualitative study of siderophore production and their association with the ability to cause infection with the isolated *E. coli*. All of these studies have been done in Aria hospital at Rasht city (Iran). One hundred thirty samples from patients with urinary tract infection were collected. Serotyping was performed according to agglutination on the slide. Qualitative measurements of siderophore were performed by colorimetric or liquid assay and determination of the type of produced siderophore was also done by Csaky's Assay and Arnow's Assay. Different antibiotics sensitivity tests performed with Kirby Bauer and disk diffusion. From the 130 samples, 33 cases of urinary tract infections were related to *E. coli*. Among them they showed the most susceptibility to nitrofurantoin antibiotic. Most of the serotypes were O1, O29, O126, and O159 .It was revealed that 87.5 % of the samples, were positive for siderophore and according to the OD, the samples of enterobactin siderophore were more than the aerobactin siderophore in all isolates. In general, this study shows that most of the strains produce siderophore and shows that the nitrofurantoin is the best antibiotic to treat urinary tract infections caused by *E. coli*.

## 1. Introduction

*Escherichia coli* is a harmless bacterium in the intestinal flora in a variety of animals, including humans, but sometimes causes fatal diseases in humans, birds and mammals. *E. coli* is often an opportunistic pathogen and common contaminant bacteria in various food sources (Henderson et al.2009). Urinary tract infections are the most commonly acquired hospital infections, so that covering almost 40% of hospital infections. These infections are often not life-threatening, but impose heavy health care costs on the health system. Infectious agents cause a lot of urinary tract infections including fungal and viral agents, but bacterial agents are one of the most important causes of urinary tract infections while it is the reason of 95% of hospital urinary tract infection. The most common bacterial agents that cause urinary tract infections are *Escherichia coli*, *Pseudomonas*, *Proteus mirabilis*, *Klebsiella*, *Enterobacter*, *Staphylococcus*, and *Enterococcus*. The spread of antibiotic resistance among these strains is increasing, while the antibacterial resistance varies from region to region, knowing its pattern

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in the final or experimental treatment of these infections is essential. Awareness of the prevalence of antibacterial resistance among urinary pathogens, especially *E. coli*, for the proper use of antibiotics for proper treatment is one of the goals of this study (Saedi et al., 2013).

The diagnosis of antibiotics that is appropriate for the treatment of urinary tract infections depends on many factors, including the severity of the infection or the primary or secondary infection. Antibiotics that are used for treatment include amoxicillin, cephalosporin, tetracycline and nitrofurantoin, but fluoroquinolones are more common. (Gul et al., 2004). Iron is the most important element needed for bacteria and plays a key role in many biological processes, including the role of iron in photosynthesis, respiration, tricarboxylic acid cycles, oxygen transfer, nitrogen fixation, methanogenesis, regulation Gene expression and DNA making. Iron is the fourth most abundant element in the earth's crust, but due to insolubility in physiological conditions, neutralizing alkaline pH and the presence of oxygen, most microorganisms cannot use it, and under these conditions, the available iron concentration is equal to $10^{-18}$ Molar. Iron is a ferric hydroxide polymer in aerobic conditions and is poorly dissolved in aqueous solutions that are why iron is a growth limiting factor for most microorganisms. The bacteria that colonies in the human body have difficulty in supplying 99.9 percent of intercellular iron of human's body which is not available for bacteria. In addition, extracellular iron found in plasma and lymphatic fluid is strongly associated with Transferrin. Bacteria have methods to overcome this problem. In aerobic conditions, bacteria and fungi produce low molecular weight ligands (siderophore). The siderophore is highly bound to ferric iron and forms the ferric-siderophore complex (Yaghoobi et al., 2010).

## 2. Materials and Methods

During October to December 2016, 130 urine samples from patients with urinary tract infections at Aria Hospital in Rasht (Iran) were collected. The age and sex of these patients were also recorded. It used from Gram strain test, SIM test, MRVP test, Simon citrate test, TSI test and Urease test to detected *E. coli*.

### *2.1. Serotyping of Isolated E. coli*

The serotyping of separated *E.coli* groups was done by means of Mast Assure kit with agglutination method (Table 1). Agglutination in the test and absence of agglutination in the control sample (one drop of normal physiological serum and a loop of microbial suspension) was considered as positive during this period. If test results are positive with polyvalent antiserum, the same test will be done separately for monovalent antiserum of that group. Until was the bacterial serotype to be in detected. Control strain in this study was *E. coli* O:157-H: 7 ATCC 43895.

**Table 1.** Polyvalent and monovalent antiserum found in the Mast Diagnostic Kit

| Polyvalent Antiserum | Monovalent Antiserum |
|---|---|
| Polyvalent 1 | O1,O26,O86a,O111,O127a ,O128 |
| Polyvalent 2 | O44,O55,O126,O146,O166 |
| Polyvalent 3 | O18,O114,O142,O151,O157,O158 |
| Polyvalent 4 | O6,O27,O78,O148,O159,O168 |
| Polyvalent 5 | O20,O25,O64,O153,O167 |
| Polyvalent 6 | O8,O15,O115,O169 |
| Polyvalent 7 | O28ac,O112ac,O124,O136,O144 |
| Polyvalent 8 | O29,O143,O1152,O164 |

### *2.2. Qualitative measurement of siderophore by Plate Assay method:*

First, a CAS Agar (Chrome Azurol Sulfonate) (Sigma Aldrich Company) culture medium was made. The isolates were inoculated into a spot in a CAS agar blue medium and incubated at 37°C for 24 hours. The size of the colored (yellow-orange) halo around the colony indicated the overall activity of sidrophore.

### *2.3. Quantitative evaluation of siderophore by Liquid Assay*

Cultures were grown in Malt Extract Broth (Merck Company) at 37°C/24 hours under static conditions as well as under shaker conditions (100 rpm) at 37°C/24 hours. The cells were removed by centrifugation at 3000 rpm for 15 mins. 0.5 ml of the culture supernatant was then mixed with 0.5 ml CAS solution and 10µl shuttling solution (sulfosalicylic acid). The color obtained was determined using the spectrophotometer at 630 nm after 20 mins of incubation. Necessary blank (minimal medium)

& reference solution (minimal medium + CAS dye + shuttle solution) were used during the determination.

*2.4. Determine the type of siderophore produced by Uropathogenic E. coli:*

**Csaky's assay**: For detection of hydroxamates like aerobactin of *E. coli* 1 ml supernatant of culture was hydrolyzed with 1 ml of 6 H2SO4 in a boiling water bath/6hrs or 130°C/30mins. To this was added 3 ml Na-acetate for buffering, 1 ml sulfanilic acid & then 0.5 ml iodine soln. After 3-5mins, excess iodine is destroyed with 1 ml of Na-arsenite soln. 1 ml of alpha naphthyl amine was then added and water was used to make up vol to 10 ml. Color was allowed to develop for 20-30mins. Absorbance was measured with the help of uv-vis spectrophotometer at 526nm.

**Arnow's assay:** For detection of catheclates like enterobactin of *E. coli* 1 ml culture supernatant was mixed after each orderly addition.1 ml HCl followed by 1 ml nitrite-molybdate (catechol's prod yellow color) & then 1 ml NaOH (color changes to red). Color was stable for 1hour & absorbance was measured at 510 nm using a uv-vis spectrophotometer.

The severity of the orange areas was different among different isolates and this indicates the different amounts of siderophore produced by different isolates. The change in the blue color of the chromium azersulfonate solution tested in orange indicates the presence of a siderophore.

Siderophore content in the liquor were calculated by using following formula:

$$\% \text{ Siderophore units} = \frac{Ar - As}{Ar} \times 100$$

Where Ar = Absorbance of reference at 630 nm (CAS reagent)

As = Absorbance of sample at 630 nm.

*2.5. Antibiotic susceptibility test*

For all isolates of *E. coli* by Kirby Bauer, Disk Diffusion method was performed according to CLSI (2016) instructions in plates containing Mueller Hinton Agar.

## 3. Results

According to the result of tests, 33 samples were *E. coli*. Considering the age and sex of people with urinary tract infection caused by *E. coli*, the results of 33 patients, women were 19 and men were 14, the number of women was 57.5% higher. The distribution between age groups and the number of patients was concluded that in the age group of 70-79 years, with 24.3 percent, the most cases were detected and in women aged 80-89 years with the highest rate of 26.5 percent and in Men in the age range of 79-70 years (35.7%) made up the largest number of patients (Table 2).

**Table 2.** Study of the number of people with uropathogenic urinary tract infection according to age and sex

| Age (year) | Number | Percent |
|---|---|---|
| 1-9 | 2 | 6.05% |
| 10-19 | 0 | 0% |
| 20-29 | 0 | 0% |
| 30-39 | 4 | 12.1% |
| 40-49 | 3 | 9.1% |
| 50-59 | 4 | 12.1% |
| 60-69 | 4 | 12.1% |
| 70-79 | 8 | 24.3% |
| 80-89 | 6 | 18.2% |
| ≥90 | 2 | 6.05% |
| Total | 33 | 100% |

In the test, the siderophore production was determined using the Plate Assay, which generated 29 samples of the 33 specimens, that is, 87.9% of the *E. coli* produced the siderophore (Fig. 1).

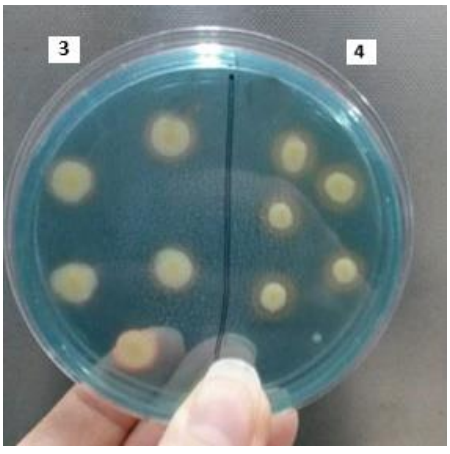


**Figure 1.** Positive production of siderophore in CAS Agar medium and formation of orange halo zone

In the quantitative study of siderophore, the highest amount of siderophore produced by *E. coli* isolates (96.97%) was found, and the ODs of the samples of the enterobarcin siderophore were higher in all isolates than the aerobactin siderophore (Table 3).

**Table 3.** The type of siderophore produced by Uropathogenic *E. coli* according to the amount of OD of clinical samples

| UPEC | Percent of siderophore | Enterobactin (510 nm) | Aerobactin (526 nm) |
|---|---|---|---|
| Maximum | 96.7% | 0.234 | 0.123 |
| Moderate | 81.10% | 0.123 | 0.039 |
| Minimum | 62.74% | 0.055 | 0.000 |
| Standard | 90.17% | 0.231 | 0.123 |

The prevalence of UPEC serotypes isolated from the urine of patients in the studied population in 34 UPEC isolates isolated from the culture of patients with urinary tract infections with O26, O55, O1 O15, O8, O29, O20, O25, O143 O159, O6, O18, O166, O126, O44, and O128. Serotypes of O1, O126, O159 and O29 were the most frequent strains with 3 cases (9.09) and 1 strain was not typed. O6 was the standard serotype (Table 4).

**Table 4.** Frequency of UPEC serotypes isolated from patients' urine

| *Frequency Serotype* | *Number* | *Percent* |
|---|---|---|
| O1 | 3 | 9.09 |
| O55 | 2 | 6.06 |
| O26 | 1 | 3.03 |
| O128 | 2 | 6.06 |
| O44 | 2 | 6.06 |
| O126 | 3 | 9.09 |
| O166 | 2 | 6.06 |
| O18 | 2 | 6.06 |
| O6 | 2 | 6.06 |
| O159 | 3 | 9.09 |
| O29 | 3 | 9.09 |
| O20 | 2 | 6.06 |
| O25 | 1 | 3.03 |
| O143 | 2 | 6.06 |
| O15 | 1 | 3.03 |
| O8 | 1 | 3.03 |
| UT | 1 | 3.03 |
| Total | 33 | 100 |

In the study of antibiotic susceptibility, the highest resistance was 88.8% for nalidixic acid and 78.8% for ciprofloxacin and tetracycline respectively, and the lowest resistance was 3% for nitrofurantoin (Table 5).

**Table 5**. Antibiotic resistance test

| ***Disk Name*** | ***Sensitive*** | ***percent*** | ***Resistant*** | ***Percent*** | ***Moderate*** | ***percent*** |
|---|---|---|---|---|---|---|
| Cefixime | 4 | 12.1% | 23 | 69.7% | 6 | 18.25% |
| Nitrofurantoin | 31 | 94% | 1 | 3% | 1 | 3% |
| Ceftriaxone | 8 | 24.2% | 22 | 66.8% | 3 | 9% |
| Gentamycin | 19 | 57.6% | 8 | 24.2% | 6 | 18.2% |
| Sulfamethoxazole | 12 | 36.4% | 21 | 63.6% | 0 | 0% |
| Nalidixic acid | 2 | 6.1% | 29 | 88.8% | 2 | 6.1% |
| Ciprofloxacin | 6 | 18.2% | 26 | 78.8% | 1 | 3% |
| Tetracycline | 2 | 6.1% | 26 | 78.8% | 5 | 15.1% |
| Ceftazidime | 11 | 33.3% | 17 | 15.6% | 5 | 15.1% |
| Cefotaxime | 7 | 21.7% | 21 | 63.2% | 5 | 15.1% |

## Statistical comparison

Data analyzed by SPSS-23 software and Chi-square statistical test (Fischer exact test). The sample size was less than 50, so the Fisher's exact test was used.

Variables: The bacterial serotypes considered as independent and main variables, antibiotic resistance considered the dependent variable. Sex, age, siderophore, considered as interfering variables. No statistically significant relationship was found between the serotypes (Sig greater than 0.05).

In the statistical comparison with different serotypes and sexuality of patients for all tasted antibiotics, a significant relation found in ceftriaxone (Sig less than 0.05) and the severity of this significant (0.81) is significant) and cefotaxime (Sig less than 0.05) and the severity of this significant (0.84) is significant) antibiotics. In the statistical comparison with different serotypes and the age groups of patients of all antibiotics tested, an significant relation found in sefotaxime ((Sig less than 0.05) and the severity of this significant (0.84)).

In the statistical comparison with different serotypes and siderephore for all tasted antibiotics, significant relation found in ceftriaxone (Sig less than 0.05) and the severity of this significant (0.81) is significant), ciprofloxacin (Sig less than 0.05) and the severity of this significant (0.73) is significant) and cefotaxime (Sig less than 0.05) and the severity of this significant (0.84) is significant) antibiotics. For all the antibiotics tested for different serotypes and siderophore. However, there was no significant relation with other antibiotics.

## 4. Discussion

Urinary tract infections are the most commonly reported nosocomial infection among humans. According to studies, 90% of urinary tract infections are caused by UPEC, due to increase in mortality, it is vital to study on hospital infections and determining antibiotic resistance patterns of bacteria that cause infections (Haeidari et al., 2013). Presence of virulence factors in UPEC, strengthens the concept of the relationship between UPEC and pathogenesis of the urinary tract. In Escherichia coli, the enterobactin and aerobactin siderophores have the greatest effect on iron extraction systems for iron extraction (Vagrali, 2009). Nowroozi et al investigated 100 samples of E. coli urinary tract infection at Jahrom city and concluded that the incidence of urinary tract infection in women is higher (64%), which probably is due to Shortness of the urethra and the proximity of its outer mouth to the vagina and anus in women (Nowroozi et al., 2006). In the 2005 Mehnert-KAY study, the highest urinary tract infection in women was reported (Mehnert, 2005). In this research, after examining the samples, the number of people with urinary tract infection caused by Escherichia coli was more common in women and (57.5%) and the highest rate of infection was in the age group of 70-79 years. In a study published by Mohajeri et al. In Kermanshah in 2011, of 200 strains of Escherichia coli, all of which were sensitive to ampicillin and imipenem, and 27 percent of the samples were sensitive to cefotaxime, 22.5 percent to ceftazidime and 26 percent of the sample were resistant to ciprofloxacin (Mohajeri et al., 2011). In the study of Dormanesh et al., in 2013, isolated strains of E. coli isolated from children showed that the isolates had the highest resistance to gentamicin antibiotics (95.1%), ampicillin (91.1%), amikacin (85.4%) and ciprofloxacin (83.8%) (Dormanesh et al., 2013). In this study, antibiotic nitrofurantoin with 94% sensitivity was the most appropriate antibiotic. According to the resistance of the esophagus, the samples of the tested nalidixic acid antibodies (88.8%) and ciprofloxacin (77.8%) and tetracycline (8.77%), we conclude these antibiotics are not suitable for treatment. Due to the comparison of different percentages of antibiogram results in different studies, it should be noted that the resistance to different antibiotics is different based on the therapeutic patterns that occur in different regions, so regional differences in different parts of the world or even a country, It provides different therapeutic responses to antimicrobial drugs. The origin of these differences can be seen in the genetic differences between individuals and strains and differences in other fields. In a study conducted by Rashki et al. In patients with urinary tract infection caused by Escherichia coli in southern Iran at 2014, after serotyping, it was concluded that serotype O2 (16.43) and O6 (16.43) and after of that, O18 (13.69) had the highest number of strains (Rashki et al., 2014).In a study by Kauffmann and colleagues at 1943, the highest O2 and O6 serotype was reported with 12% (Kauffmann et al.,1943).In a study conducted by Zhao et al. at 2009, the largest O1 serotype was reported (Zhao et al., 2009).In this study, O1 and O29 and O126 and O159 serotypes with 9.09% were observed as the most abundant strains. In a study conducted by Gokan et al. at 2010 on the production of microorganisms, they produced siderophore from all clinical specimens from *Escherichia coli*, and the production of enterobactin

siderophore was higher than that of aerobactin (Gokan et al., 2010). Henderson et al. (2009) conducted quantitative methods for evaluating siderophore on *E. coli*. In studies on the production of enterobactin and aerobactin siderophores, it was observed that, as in this study, the highest amount of siderophore produced was of the type of enterobactin (Henderson et al., 2009). In this study, 29 samples of 33 cases produced siderophore, and in general, enterobactin siderophore was more commonly observed. In summary, the outcome of various studies indicates different antibiotic resistance patterns in different geographic regions, as well as high resistance to antibiotics that are commonly used and the appearance of degree of resistance to newer antibiotics. The proper use of antibiotic therapy by avoiding the administration of unnecessary antibiotics and preventing the appearance of antibiotic resistant strains (acquired resistance) is very necessary.

## Acknowledgement

Financial support from the Islamic Azad University (Lahijan–Iran) and hospital laboratory (Rasht–Iran) to carry this work in greatly acknowledged by all the authors.

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