Detection of Quinolones Resistance in *Ureaplasma urealyticum* Clinical Isolates

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Introduction

Ureaplasma spp. are one of the sexually transmitted pathogens that considered etiology agent of urethritis, prostatitis, bacterial vaginosis, cervicitis, and pelvic might be led to infertility in men and women [1-4]. For treatment of infections caused by this bacteria fluoroquinolones are most used antibiotics. This antibiotics killing Ureaplasma through the inhibition of DNA replication. Nowadays there are increasing report on acquired resistance to quinolones [5]. The mechanism of fluoroquinolone resistance in Ureaplasma are depend on point mutations in DNA gyrase and topoisomerase IV [4-7].

In order to figure out the resistance rate of *U. urealyticum*, it is necessary to analyze the mutation rates. Therefore, we have determined the mutation rate of *U. urealyticum* to quinolone antibiotics by using PCR and sequencing of GyrA and GyrB subunits of DNA gyrase and ParC, ParE subunits of topoisomerase IV in clinical isolates.

Materials and Methods

Bacterial isolates

This study was done on sample has been described previously [2]. Samples were gathered from pregnant women, referred to obstetrics and gynecology section or prenatal clinic in Beasat Hospital, Sanandaj, Iran. For all women endocervical samples were collected using a cotton swap into sterile 15-mL falcon tubes containing 5 mL of phosphate-buffered saline and placed at 70°C until DNA extraction.
DNA extraction

Samples were transferred to laboratory in standard condition. DNA extraction were done by Kit instruction (High pure PCR Template Preparation; Roche, Germany). Extracted DNA were stoked -70°C until PCR test.

PCR test

PCR was performed in a final volume of 20 ml for ParC and parE and gyrA genes with primers as shown in Table 1.

The PCR were done in a Thermocycler (Eppendorf, Hamburg, Germany) with program including; initial denaturation 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 64°C for 30 seconds, and extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes. In order to observe the amplified PCR products, gel electrophoresis were accomplished in 1.5% gel agarose stained by ethidium bromide, and then photographed after visualized by UV light.

Sequencing

PCR products were sent to Sina Clone co for sequencing. Results of sequencing were analyzed Using Chromas pro V 2.1.1 software. Using online software including FASTA and European Bioinformatics Institute (EMBL-EBI) data were Aligned and point mutation detected.

Result

The study carried out, to investigate the prevalence of quinolone resistance mutations in 30 samples for parC, parE and gyrA genes as shown in Table 2. The results of gene sequencing showed that the substitution of amino acids in codon 83 parC happened. Ser83LUC amino acid substitution caused by movement S to L in 5 samples. Aspartic acid 82 Asparagine change caused by amino acid substitution D to N happened in 4 cases. The results of the gyrA gene sequencing showed that the amino acid substitution in codon 104 occurred in 2 sample. GUL104LYS amino acid substitution change occurred in 5 samples.

Table 1 Primers were for PCR amplifications in clinical samples.

<table>
<thead>
<tr>
<th>5’ to 3’ sequence</th>
<th>primers</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTGATGGTGATAGTGCAG</td>
<td>gyrA-F</td>
<td>gyrA</td>
</tr>
<tr>
<td>TAGAATCTAGTGGGT</td>
<td>gyrA-R</td>
<td></td>
</tr>
<tr>
<td>CCGTCTACCGAGTATTTT</td>
<td>ParC-F</td>
<td>parC</td>
</tr>
<tr>
<td>TCTGATAAACGCATGGAAG</td>
<td>ParC-R</td>
<td></td>
</tr>
<tr>
<td>TCGAAACGTCCCTGGAATGT</td>
<td>ParE-F</td>
<td>parE</td>
</tr>
<tr>
<td>AGCCTTTTCATGCAACCAC</td>
<td>ParE-R</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Point mutations results in studied samples by sequencing.

<table>
<thead>
<tr>
<th>Genes</th>
<th>No</th>
<th>Yes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>GyrA</td>
<td>28 (93%)</td>
<td>2 (7%)</td>
<td>30</td>
</tr>
<tr>
<td>ParC</td>
<td>24 (40%)</td>
<td>6 (60%)</td>
<td>30</td>
</tr>
<tr>
<td>Par E</td>
<td>24 (40%)</td>
<td>6 (60%)</td>
<td>30</td>
</tr>
</tbody>
</table>

Discussion

Many pathogenic bacteria that infect the genital tract of women, including genital mycoplasmas, Neisseria gonorrhoeae, Chlamydia trachomatis, Enterobacteriaceae, Gram-positive cocci, and Gardnerella vaginalis. Mycoplasma families can cause chronic and subclinical genital infections that may have a negative impact on female fertility [1, 8-10]. Ureaplasma is one of the main causes of Non gonococcal urethritis (NGU) in men, also in pregnant and non-pregnant women causing premature delivery, spontaneous abortion, premature birth, vaginitis and cervicitis. Today, drug resistance is a major problem in most countries. This resistance is steadily rising due to the limited number of effective drugs, also is a threat in the control program [5,11-15].

DNA topoisomerase IV has two subunit that encoded by parC, and parE genes. Amino acids substitution such as D82N, S83L, and E87L in parC gene causes resistance to quinolones. There are also other mutations in the gene parC amino acid asparagine to aspartic acid is substituted in Area 82 and Area 87 is glutamic acid to leucine [16-19]. In our study the mutation frequency in resistance gene parC was 20%.

Kawai in 2015 a study U. urealyticum, out of 158 isolates, 23.4% quinolone resistance in parC gene mutation that leads to changes in gene found a specific area called s83l [7]. parE gene is necessary for bacterial chromosome partitioning. The amino acid sequence encoded by the gene parE and parC have many similarities. Mutations in the C-terminal amino acids encoded by this gene causes resistance to quinolones. A quinolone resistance in U. urealyticum, can occurred due to amino acids replacement in ASP435 to Asn.

DNA gyrase encoded by gyrA and gyrB genes. Replacement of amino acids Q104K in gyrA gene causes resistance to quinolones. In gyrA gene in the 104 position lysine is replaced by glutamine [7,13,16,19-21]. In our study the mutation rate in resistance gene gyrA was 6.6%.

Conclusion

Quinolones are most common antibiotics effective in treatment infections caused by a U. urealyticum. Therefore early detection of resistance genes is essential to correct treatment regime to prevent the spread of resistant strains. Study also showed us parC gene mutation at position 82 and in position 104 of gyrA gene as a marker for resistance to quinolones in isolated U. urealyticum.

List of Abbreviations

Ureaplasma urealyticum(U. urealyticum), DNA gyrase genes (gyrA, gyrB, parC and parE).

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

The authors declare that there are no conflicts of interest regarding the publication of this article.
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Authors' Contributions

Amir Safari carried out the study and collected data. Rashid Ramazanzadeh supervised the study, participated in designing and conducting it, and prepared the original version of the manuscript. All authors studied and approved the content of the present manuscript and participated in revising the paper.

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References


