Prevalence of dfr, int and sul Genes in Cotrimoxazole Resistance Klebsiella pneumoniae Isolated from Two Hospitals of Iran

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Extensive use of antimicrobial agents such as cotrimoxazole has been associated with raising of antimicrobial resistance. Current study is focused on assessing the prevalence of cotrimoxazole resistance in klebsiella pneumoniae and the frequency of related genes. 155 isolates of klebsiella pneumoniae were collected during Mar. 2007 to Apr. 2012 from Ilam hospitals and Milad hospital of Tehran. Antibiotic susceptibility test done to screening resistance isolates according to Kirby-Bauer method. sul1, sul2, sul3, dfrA1, dfrA5, and Int1 genes were detected by PCR. Among 155 species, forty isolates (26%) were resistance to cotrimoxazole. Frequency of sul1 gene was 32 isolates (80%) and 24 isolates of dfrA1 (60%), none isolates of dfrA5 (0%), 28 isolates of int (70%), 25 isolates of sul2 (62.5%), and no isolates of sul3 (0%) has been detected. 17 (42.5%) isolates have sul1 and sul2 simultaneously, and 18 (45%) isolates have int1 and dfrA1. 11 isolates have sul1, sul2, int1 and dfrA1 genes concurrence by 27.5% frequency. Our study shown resistance to cotrimoxazole in klebsiella isolated from Ilam hospitals and Milad hospital of Tehran is moderate and Sul genes have the highest frequency in resistance isolates.

Key words: Cotrimoxazole, sul1, Resistance gene, Klebsiella, Tehran, Ilam.

Klebsiella is a facultative anaerobic gram negative rod shaped bacteria belongs to the Enterobacteriaceae family. Most of the members of this genus are nonmotile. K. pneumoniae can cause various disease such as lungs inflammation and hemorrhage with necrosis that sometimes produces a thick, bloody, mucoid sputum. Sulfonamides primary recruited at 1930s in clinical and veterinary medicine to treat bacterial infections and to decrease level of emergence resistance, Sulfonamides have generally been combined with Diaminopyrimidines1. Chromosomal mutation is one of the mechanisms of antibiotic resistance that have been occurring in the absence of antibiotic, but the secondary resistance mechanism, is the genetic interchanges that is mainly conducted by plasmids2. This kind of resistance in bacteria has been reported also against Sulfonamides. This agent act as a structural analogue of Para-aminobenzoic acid could bind dihydropteroate synthase (DHPS), a catalytic enzyme in the folic acid biosynthesis pathway, resulting in the inhibition of dihydrofolate acid formation3. Sulfonamides
interfere with the formation of folic acid in bacteria, by competitively inhibit the bacterial enzyme dihydropteroate synthase. Sulfonamide is a selectively act on prokaryotic bacterial cells, Sulfonamide cannot interact with mammalian cells because these cells do not synthesize folic acid, and thus have no dihydropteroate synthase target enzyme. Resistance to Trimethoprim is caused by modifications in the target enzyme dihydrofolate reductase (dfr) encoded by dfr-genes. So far 28 dfr-genes are described and they are usually associated with integrons. Two plasmid-borne genes, sul1 and sul2, have been found to be associated with the very common Sulfonamide resistance in Gram-negative bacteria.

MATERIALS AND METHODS

155 K. pneumoniae have been collected from hospitals of Ilam and Milad of Tehran. Antibiotic susceptibility for Ampicillin, Tetracycline, Ciprofloxacin, Cotrimoxazole has been done by Disk Diffusion method based on recommendations of the National Committee for Clinical Laboratory Standards (NCCLS). DNA extraction and PCR Cotrimoxazole resistance genes sul1, sul2, sul3, dfrA1, dfrA5 and Int1 detected by PCR. The presence of class 1 integrons (Int1) in each strain was assessed by using class 1 specific primers. A fresh bacterial colony was suspended in 100 mL of sterile distilled water and boiled at 100°C for 10 min. After centrifugation, 3 μL of supernatant was used for PCR assays with the primers described in Table 1. Amplification of DNA was performed in thermal cycler (Eppendorf, Germany). PCR elongation times and temperature conditions were described in Table 1. PCR products were electrophoresed in 1.5% agarose gels and visualized under UV light.

RESULTS

Total of 155 isolates were collected from patients admit to different wards of Milad hospital (Tehran) and Ilam hospitals (Imam khomeyni, and Shahid Mostafa), Iran. Out of 155 strains isolated from urinary tract infections 40 isolates (57/14%), 58 (82/1%), 47 (67/1%), 8 (11/4%), 34 (47/51%) were resistant to Cotrimoxazole, Ampicillin, Tetracycline, Nitrofurantoin, and Ceftriaxone, respectively. Out of 32 (80%) strains having sul1 and 28 strains (70% int1,25 strains (62.5%) have sul2, 24 have dfrA1(60%), and none of them having dfrA5 and sul3 (0%) genes (Fig. 1). Seventeen (42.5%) strains contain simultaneously sul2 and sul1 genes. Eighteen(45%) of the isolates contain int1+ dfrA1 genes, and eleven (27.5%) of isolates have sul1,sul2, int1 and dfrA1 with together.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer F</th>
<th>Primer R</th>
<th>Size</th>
<th>Annealing temperature</th>
<th>Ref:</th>
</tr>
</thead>
<tbody>
<tr>
<td>sul 1</td>
<td>F: 5' - CCGGCTGGGCTACCTGAACG -3'</td>
<td>R: 5' - GCCGATCGCGTGAAGTTCCG-3'</td>
<td>432bp</td>
<td>55°C</td>
<td>In this study</td>
</tr>
<tr>
<td>sul 2</td>
<td>F: 5' - GCCGCTCAAGGCAGATGGCATT -3'</td>
<td>R: 5' - GCCTTGGATACCGGCACCGT -3'</td>
<td>293bp</td>
<td>53°C</td>
<td>In this study</td>
</tr>
<tr>
<td>sul 3</td>
<td>F: 5- CAGATAAGGCAATGGAGCATGCTCTGC - 3</td>
<td>R: 5- GATTTCGTGAACCTGAAATCTATT -3'</td>
<td>569bp</td>
<td>55°C</td>
<td>In this study</td>
</tr>
<tr>
<td>dfrA5</td>
<td>F: 5- ACGGAGTGATGTTGTCGCG -3</td>
<td>R: 5- TCTGTAAGATCTCCGGCCGCC -3</td>
<td>279bp</td>
<td>53°C</td>
<td>[8]</td>
</tr>
<tr>
<td>dfrA1</td>
<td>F: 5- TGGAGTGATGTTGTCGCG -3</td>
<td>R: 5- AACATACCACTTCCGGGCTC -3</td>
<td>343bp</td>
<td>In this study</td>
<td></td>
</tr>
<tr>
<td>Int1</td>
<td>F: 5- GCCTGTCGGGCTGACGTGATC</td>
<td>R: 5- CCGATGTTGCGATTACCTCAT -3</td>
<td>585bp</td>
<td>56°C</td>
<td>[8]</td>
</tr>
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</table>

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DISCUSSION

Overall resistance percentage of isolates against Trimethoprim/Sulphamethoxazole, Ampicillin, Tetracycline, and Ciprofloxacin was very high, and the highest resistance was estimated against Ampicillin (81.25%), after that 26% of isolates were resistant to Cotrimoxazole. Based on our results sul 1 gene has the highest prevalence in klebsiella strains resistant to Cotrimoxazole isolated from Iran. Frequency of sul 1 (80%) was higher than sul 2 (62.5%) and sul 3 (0%) that are in accordance with other studies conducted worldwide. Our observed trends in Sulfamethoxazole-resistant allele distributions (sul1 >sul2 >sul3 ) different from previous studies. There have been few studies of the genetic distributions underlying Trimethoprim resistance in the world. Our result shown the similarity to result in other studies about the frequency of dfrA1 in isolated sample. Previous study found that qnr is located in complex Int4 family class 1 integrons, which are also known as complex sul1-type integrons because of the presence of duplicate qacE and sul1genes. Class 1 integrons playing an important role on antibiotic resistance dissemination in many multidrug resistance gram negative bacteria, including many of zoonotic serovars of Salmonella enteric and other Enterobactericeae. Prevalence of sul1 in present study is 32(80%) isolates. Some studies shown most of isolates have dfrA1/A15/A16 genes but in present study 24 (60%) of isolates have these genes. Other genetic mobile elements also have acting as sources of sul genes. For example, in SMX resistant Vibrio cholerae serogroup 0139, it has been reported that the sul2 gene was part of a cluster located on a newly discovered genetic element of the integrative conjugative element group named SXT. Resistance genes of SXT exist in a composite transposon-like structure and were probably acquired recently. In current study the prevalence of dfrA1 is more than dfrA5, while in other study similar result has been shown. Existence of sul genes in various types of clinical and environmental isolates indicates that these genes have an universal function of carrying and spreading Sulfonamide resistance in bacteria. High frequency of sul genes, plasmid related resistance and rising of prevalence of SXT resistance in K. pneumoniae isolates indicates that continuous surveillance programs should be implemented in hospital and clinical settings to better control and treatment of related diseases, and monitoring the trends of SXT resistance in gram negative bacteria.

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