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 cotromoxazole in klebsiella isolated from Ilam hospitals and Milad hospital of Tehran is moderate and S*ul* 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 *Diaminopyrimidines*[1]. 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 plasmids[2]. This kind of resistance in bacteria has been reported also against *Sulfonamides*, This agent act as a structural analogue of Para-amino-benzoic acid could bind *dihydropteroate synthase* (DHPS), a catalytic enzyme in the folic acid biosynthesis pathway, resulting in the inhibition of *dihydrofolic acid* formation[3]. *Sulfonamides*

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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[4]. Two plasmid-borne genes, *sul1* and *sul2*, have been found to be associated with the very common *Sulfonamide* resistance in Gram-negative bacteria[5].

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)[6].

DNA extraction and PCR

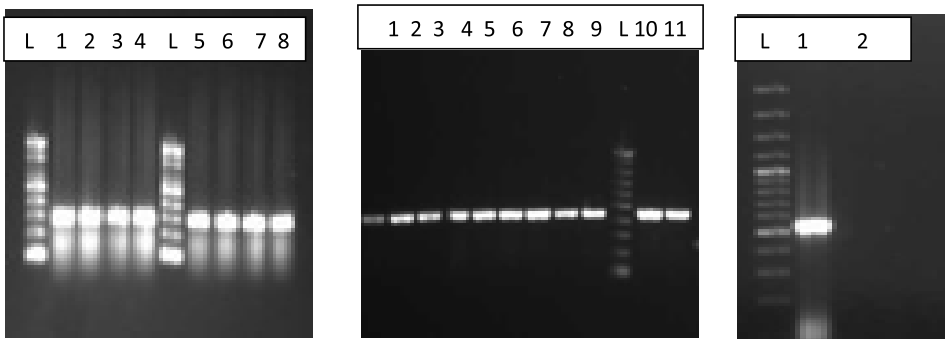
Cotrimoxazole resistance genes *sul*1, *sul*2, *sul*3, *dfrA1*, *dfrA5* and *Int1* detected by PCR. The presence of class 1 integrons (*Int1*) in each strain was assessed by using class 1 specific primers. A fresh bacterial colony was suspended in 100 mL of sterile distilled water and boiled at 100°C 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[7].

RESULTS

Total of 155 isolates were collected from patients admit to different wards of Milad hospital (Tehran) and Ilam hospitals (Imam khomayni, 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 1. Primers used for PCR detection of *sul1*, *sul2*, *sul3* ,*dfrA1*,*dfrA5* and *Int1* genes

Gene	Primer	Size	Annealing temperature	Ref:
sul 1	F: 5' - CGGCGTGGGCTACCTGAACG -3' R: 5' - GCCGATCGCGTGAAGTTCCG-3'	432bp	55°C	In this study
sul 2	F: 5' - GCGCTCAAGGCAGATGGCATT -3' R: 5' - GCGTTTGATACCGGCACCCGT -3'	293bp	53°C	In this study
sul 3	F: 5- CAGATAAGGCAATTGAGCATGCTCTGC - 3 R: 5 - GATTTCCGTGACACTGCAATCATT -3'	569bp	55°C	In this study
dfrA5	F: 5- ACGGAGTGATTGGTTGCGG -3 R:5- CTCTGTAAATCTCCCCGCC -3	279bp	53°C	[8]
dfrA1	F: 5- TGGAGTTATCGGGAATGGC -3 R:5- AACATCACCTTCCGGCTCG -3	343bp		In this study
Int1	F: 5- GCCTGTTCGGTTCGTAAGCT -3 R: 5- CGGATGTTGCGATTACTTCG -3 R: 5-TTGAGGCTGGGTGAAGT-3	585bp	56°C	[8]



A **B** **C**

Fig. 1. Electrophoresis of PCR product of sul gene on 1% agarose gel, A: L (Ladder 100 bp), sul 1= 432 bp (lane 1-7),lane 8, positive control B: L (Ladder 100 bp), sul2= 293 bp (lane 1-9),C: L (Ladder 100 bp), lanes 10 and 11,posirtive control, sul3= 569 bp (lane 1),lane 2, negative control

DISCUSSION

Overall resistance percentage of isolates against *Trimethoprim/Sulphametoxazole, 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[9, 10]. 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[11]. Previous study found that *qnr* is located in complex *Int*4 family class 1 integrons, which are also known as complex *sul*1-type integrons because of the presence of duplicate *qac*E and *sul*1genes[12,13]. 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*[14]. 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[15]. 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 *sul*2 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[16]. In current study the prevalence of *dfr*A1 is more than *dfr*A5, while in other study similar result has been shown[13]. 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[17-21].

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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