Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 04, pp.139-143, April, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ANALYSIS OF GENETIC DETERMINANTS INVOLVED IN ANTIBIOTIC RESISTANCE IN CLINICAL STRAINS ISOLATED FROM URINE SAMPLES OF RENAL TRANSPLANTATION RECIPIENTS

Dr.Kian Ghanati[1] , Fatemeh. Fallah [1] Ph.D, Mohammad.Hosseini moghaddam [2] M.D, Latif.Gachkar [2] M.D, Abdollah.Karimi [1] M.D, Saadat. Adabian [1] Ph.D, Raheleh Sadat.Sajadinia[*] [3] M.D

[1] The Paediatric Infection Research Centre, Shahid Beheshti university of Medical Sciences, Tehran, Iran
[2]Department of General and Transplantation Surgery of Labafinejad hospital, Tehran, Iran
[3] Shahid Beheshti university of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article History:
Received 28th January, 2012
Received in revised form
18th February, 2012
Accepted 25th March, 2012
Published online 30th April, 2012

Key words:

Urinary Tract Infection (UTI),
Renal transplant recipients,
Antibiotic resistance,
Integron.

ABSTRACT

Background: *Urinary Tract* Infection (UTI) is one of the most important infections among renal transplant recipients; however, the threat caused by the acquisition of antibiotic resistance by pathogenic bacteria has been growing.
Objectives: The aim of this study was to investigate the antibiotic sensitivity of 25 strains isolated from urine samples of renal transplant recipients and evaluate the presence and prevalence of resistance determinants such as Integron in them.
Methods: The patients enrolled into the study had undergone renal transplantation in the Department of General and Transplantation Surgery of Labafinejad hospital, 70 to 90 days before urine samples were collected. Susceptibility of 25 bacterial strains [10 Enterococcus faecalis, 4 Coagulase-negative Staphylococcus 2 Streptococcus sp., 2 Klebsiella pneumoniae, 2 E.coli, 2 Acinetobacter baumannii, 1 Stenotrophomonas maltophilia, 1 Proteus mirabilis and 1 Citrobacter freundii] to 13 antibiotics: Gentamycin, Tetracycline, Ceftazidime, Co-trimoxazole, Imipenem, Ciprofloxacin, Norfloxacin, Cefalotin, Amikacin, Chloramphenicol, Nalidixic Acid, Amoxicillin and Nitrofurantoin were determined by the Kirby-Bauer disk diffusion method. In addition, the existance of Integrons in resistant isolates was assessed by PCR.
Results: It was demonstrated that TEM gene in gram-negative bacterial strains (100%) and Entrococcus (55.6%) and ctx gene in other gram-positive bacterial strains (72.3%) are responsible for antimicrobial resistance more than any other gene. Furthermore, it was revealed that class 1 and 2 Integrons exist in all resistant isolates while class 3 Integrons only exist in Entrococcus.
Conclusion: The results indicate that Integrons may contribute significantly to the spread of antibiotic resistant bacteria among renal transplantation patients.

INTRODUCTION

Following the researches for understanding the principles of antibiotic resistance among bacteria, it was investigated that the acquisition and dissemination of resistance determinants is carried out through a mobile genetic element such as a plasmid or transposon that as a result of studying their sequences, Integrons are identified (1-4). Integrons are observed in the Genome of many gram-positive and gram-negative bacteria and can be transferred horizontally from strain to strain by conjugation (1-5). Four classes of Integron are identified so far, whereas 3 classes are associated with multi-drug resistance among different strains (6, 7). Dissemination of antibiotic resistance genes has led to the rapid emergence of antibiotics resistance among clinical isolates of bacteria, therefore we decided to study the Antibiotic sensitivity of strains isolated from urine samples of renal transplant recipients and evaluate the presence and frequency of Integrons, as one of the most important resistance genes, by PCR.

Corresponding author: rahelehsajadinia@yahoo.com

MATERIALS AND METHODS

200 patients (aged between 10-70 years) who were enrolled into the study had been transplanted in the Department of General and Transplantation Surgery of Labafinejad hospital, within a period of 15 months (June 2009- September 2010). The mean time scince the day of transplantation, till the day when the urine samples were collected was 80 ± 10 days. First morning, midstream urine samples were collected from patients (Patients were provided with information forms where they could find instruction on how a urine specimen should be correctly collected) and quantitive urine culture was performed where all urine samples were plated on to the

Table 1. Primers tested in *PCR*

Target gene	Primers	Oligonucleotid sequences (5´ to 3´)	References	Expected size(bp)
TEM	TEM-F TEM-R	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	10	867
SHV	SHV-F SHV-R	GGTTATGCGTTATATTCGCC TTAGCGTTGCCAGTGCTC	10	867
OXA	OXA-F OXA-R	ACACAATACATATCAACTTCGC AGTGTGTTTAGAATGGTGATC	10	885
CTX	CTX-MU1 CTX-MU2	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	11	593
DHA	DHA-1U DHA-1L	CACACGGAAGGTTAATTCTGA CGGTTATACGGCTGAACCTG	12	970
VEB	VEB-1A VEB-1B	CGACTTCCATTTCCCGATGC GGACTCTGCAACAAATACGC	13	1014
Intl-1	Intl1-F Intl1-R	GGTCAAGGATCTGGATTTGG ACATGCGTGTAAATCATCGTC	14	500
Intl-2	Intl2-F Intl2-R	CACGGATATGCGACAAAAAGGT GTAGCAAACGAGTGACGAAATG	14	740
Intl-3	Intl3-F Intl3-R	CTTCATTCACGCACTATTAC TAACTTGACCGACAGAGG	14	827
VIM	VIM-F VIM-R	AGTGGTGAGTATCCGACAG ATGAAAGTGCGTGGAGAC	15	225
Foxup	FOXUP1F FOXUP1R	CACCACGAGAATAACC GCCTTGAACTCGACCG	16	1148
NHAmp	NHAmpF NHAmpR	ATTCGTATGCTGGATCTCGCCACC CATGACCCAGTTCGCCATATCCTG	16	396
PER	PER-A PER-B	GGGACARTCSKATGAATGTCA GGGYSGCTTAGATAGTGCTGAT	16	692

Table 2: The average resistance of isolated Entrococcus to antibiotics

Antibiotic	% resistance
Gentamycin	75.3
Tetracycline	0
Ceftazidim	33.4
Co-trimoxazole	35.6
Imipenem	37.2
Ciprofloxacin	50.7
Norfloxacin	56.8
Cefalotin	37.7
Amikacin	44.12
Chloramphenicol	75.1
Nalidixic Acid	38.5
Amoxicillin	61.2
Nitrofurantoin	12.5

Table 3: The average resistance of isolated Gram-negative bacterial strains to antibiotics

Antibiotic	% resistance
Gentamycin	11.11
Tetracycline	23.4
Ceftazidim	66.7
Co-trimoxazole	87.3
Imipenem	88.62
Ciprofloxacin	22.23
Norfloxacin	25.2
Cefalotin	88.9
Amikacin	16.8
Chloramphenicol	0
Nalidixic Acid	28.9
Amoxicillin	33.6
Nitrofurantoin	0

culture mediums.In cases where the number of growing colonies of bacteria exceeded 10^5 CFU/ml (significant bacteriuria) or the number of growing fungal colonies exceeded 10^4 CFU/ml (significant funguria) the samples were further worked up.

The isolates were screened for antimicrobial susceptibility using Kirby-Bauer disk diffusion methodology (NCCLS,Wayne,Pa), the group of antimicrobials tested were Gentamycin (10 µg), Tetracycline (30 µg), Ceftazidime (10 µg), Co-trimoxazole (25 µg), Imipenem (10µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Cefalotin (30µg), Amikacin (30µg), Chloramphenicol (30µg), Nalidixic Acid (30 µg), Amoxicillin (10µg) and Nitrofurantoin (32 µg). Antibacterial susceptibility was confirmed by the standard disk diffusion method (8). Transposon and plasmid DNA were extracted as described by Enne et al (9). Antibiotic resistance genes were detected with primers listed in Table 1. PCR amplification was carried out in a total volume of 25 µl for 30 cycles as follows: initial denaturation at 94°C for 5 min and

cycles consisting of denaturing at 94°C for 30 s, annealing at 53-65 °C for 30 s and a final extension at 72 °C for 10 min. The expected size of each product was ascertained by electrophoresis in a 2% agarose gel with appropriate molecular size markers (100 bp DNA ladder; MBI Fermentas). The amplified fragments were then digested with appropriate restriction enzymes. The size of generated fragments is shown in Table 1.

RESULTS

Urinary Tract Infection (UTI) was detected in 33 (16.5%) patients (12 of them had symptomatic infection and 21 asymptomatic). Gram-positive bacterial strains were isolated in most cases of urinary tract infection: Entrococcus faecalis in 10, Coagulase-negative Staphylococcus in 4 and Streptococcus spp. in 2. Gram- negative strains were isolated in 9 cases: Klebsiella pneumoniae in 2, E.coli in 2, Acinetobacter baumannii in 2, Stenotrophomonas maltophilia, Proteus mirabilis and Citrobacter freundii each in 1 case.Yeasts were isolated in 8 cases as well. Entrococcus isolated in this study were highly resistant to Gentamycin and Chloramphenicol (Table 2). However, gram-negative isolates were extremely sensitive to Chloramphenicol and Nitrofurantoin and highly resistant to Cefalotin, Imipenem and Co-trimoxazole (Table 3).

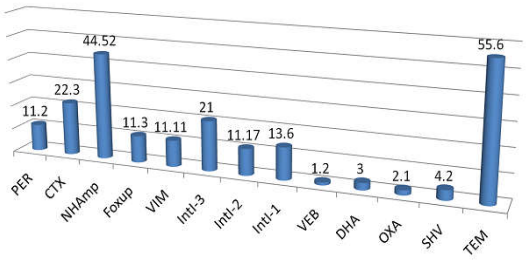

Figure 1: Frequency of genes responsible for antibiotic resistance in Entrococcus

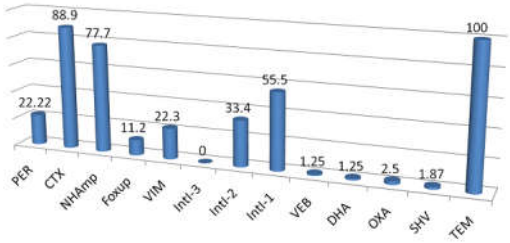

Figure 2: Frequency of genes responsible for antibiotic resistance in Gram-negative bacterial strains

It was revealed that TEM gene in Entrococcus (55.6%) and gram-negative bacterial strains (100%) and ctx gene in other gram positive bacterial strains (72.3%) are responsible for antimicrobial resistance more than any other gene. Furthermore, it was shown that the prevalence of class 1, 2 and 3 Integrons is 55.5%, 33.4% and 0% in gram negatives, 13.6%, 11.17% and 21% in Entrococcus and 8.12%, 6.75% and 0% in other gram positives, respectively (Figure 1, 2 and 3).

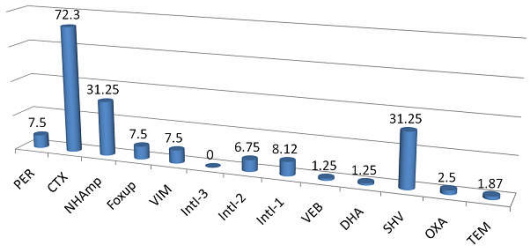

Figure 3: Frequency of genes responsible for antibiotic resistance in Gram-positive bacterial strains

DISCUSSION

Improvements in graft survival have led to widespread acceptance of renal transplantation as the preferred treatment for the majority of patients with End Stage Renal Disease (ESRD) (17 and 18). However, urinary tract infection (UTI) is still one of the main causes of death or transplant rejection in these patients. The fact that UTI is the most common infection among renal transplantaion recipients (6-68%), make the situation even worse (19 and 20). In 2005, Shirazi et al. reported that the occurrence of UTI following renal transplantation in Iran is 37% (21), however, in this study, only about 16.5% of renal recipients, suffered from UTI following renal transplantation, which seems to be due to appropriate nursing services in the Department of General and Transplantation Surgery of Labafinejad hospital such as administrating proper UTI prophylaxis. Nearly 80% of renal transplant recipients have at least one episode of infection during the first year after transplantation, and the infection is the highest during the first three months (22), as if in our study, the highest rate of UTI occurrence was reported during the first to fourth months (40%) and then during the fourth to sixth months after transplantation, which is consistent with other reported studies.

Gram-negative strains are considered to be responsible for the majority of UTIs in renal transplant recipients (more than 70%) and E.coli is among the most common gram-negative strains causing UTI (19 and 20). Chaung et al demonstrated that E-coli, entrococcus, Staphilococcus and klebsiella are respectively 29%, 24%, 16% and 10% responsible for UTI. Thus; E-coli and then entrococcus are the main strains responsible for UTI during 6 to 12 weeks after renal transplantation (23).In agreement with this finding, Khosroshahi et al. and Senger et al. demonstrated that E-coli is responsible for 53.3% and 61.3% of UTIs occurred in renal transplant recipients in Iran and Turkey, respectively (20 and 24), whereas, in this study, entrococcus was the most common strain isolated from the urine samples of renal transplant recipients suffering from UTI (30.3%) while E-coli was responsible for only 6.06% of UTIs as well as Klebsiella. It should be noted that in this study, Staphylococcus was responsible for 12.12% of UTIs among renal transplant recipients which was similar to the research by Chaung et al (23). In agreement with the finding of Chaung et al., in our study a high antibiotic resistance was observed in most of the isolated strains (23). Investigating the genes responsible for antimicrobial resistance in entrococcus (as the most common strain responsible for UTI) revealed that TEM gene plays an important role in antimicrobial resistance (55.6%) as this gene

initiates high resistanc to a wide range of β-lactamases. Furthermore, it was indicated that the prevalence of class 1, 2 and 3 Integrons, is 13.6%, 11.17% and 21%, in entrococcus, respectively. As previously noted, Integrons of class 1 were more prevalent than those of class 2 (25 and 26). In a research by Fluit et al., it was shown that the prevalence of class 1 Integron and also the increase in its prevalence in the genome of resistant E-coli, kelebsiella oxytoca, Enterobacter aerogenes and Enterobacter cloacae, were higher compared with the other classes of Integrons, during 1993, 1996 and 1999 (25). In our study, it was demonstrated that class 1 Integron is significantly associated with antimicrobial resistance which is consistent with previous studies. Lei Shi et al. demonstrated that class 2 and 3 Integrons do not exist in the genome of bacteria isolated from urine samples of renal transplant recipients suffering from UTI (22), however, in this study class 2 Integron was detected in the genome of gram negatives (33.4%), entrococcus (11.17%) and the other gram positives (6.75%) while, class 3 Integron was detected only in entrococcus (21%). It should be considered that Integrons are not restricted to pathogens, as they can be detected in a wide range of commensal bacteria as well. Skurnik et al. demonstrated that Integrons can be detected in the genome of E-coli, isolated from healthy subjects free of recent antibiotic exposure. The prevalence of Integrons in isolates received from farmers who were in touch with pigs, were reported to be 18.5% for class 1 Integron and 7.4% for class 2 Integron, and for the employees working in Insurance Company, were reported to be 12.2% for class 1 Integron and 4.1% for class 2 Integron (26). The aim of this study was to define the role of Integrons as one of the most important genetic elements responsible for antibiotic resistance among the strains isolated from urine samples of renal transplant recipients. Our observations could support recommendation for certain changes in the UTI treatment strategy in renal transplant recipients.

Acknowledgement

We thank the head and honored members of the Department of General and Transplantation Surgery of Labafinejad hospital and the Paediatric Infection Research Center of Shahid Beheshti University of Medical Sciences and our colleagues, Mr.Saadat Darabian, Ms.Zari Gholinejad and Ms.Sara Toosi, for their assistance and helpful suggestions during this study.

REFERENCES

1. White PA, McIver CJ, Rawlinson WD. Integrons and gene cassettes in the entrobacteriaceae. Antimicrob Agents Chemother 2001; 45: 2658-2661
2. Nogrady N, Gado I, Toth A, Paszti J.Antibiotic resistance and class 1 integron patterns of non-typhoidal human Salmonella serotypes isolated in Hungary in 2002 and 2003.Int J Antimicrob Agents 2005;26: 126-132
3. Partridge SR, Recchia GD, Scaramuzzi C, Collis CM, Stokes HV, Hall RM. Definition of the attI1 site of class 1 integrons. Microbiology 2000; 146: 2855-2864
4. White PA, McIver CJ, Deug YM, Rawlinson WD. Characterisation of two new gene cassettes, aadA5 and dfrA17, FEMS Microbiol lett 2000; 182: 265-269
5. Loon H, Box A, Verhoef J, Fluit AC. Evaluation of genetic determinants involved in β-lactamase and multi-resistance in a surgical ICU, Int J Antimicrob Agents 2004; 24: 130-134
6. Arakawa Y, Murakami M, Suzuki K, Ito H, Wacharotayankun R, Ohsuka S, et al. A novel integron-like element carrying the metallo-beta-lactamase gene bla_{IMP}. Antimicrob Agents Chemother 1995; 39: 1612-1615.
7. Leverstein-Van Hall M, Blok H, Donders A, Paauw A, Fluit AC, Verhoef J. Multidrug resistance among Entrobacteriaceae is strongly associated with the presence of integrons and is dependent of species or isolate origin, J Infect Dis 2003;187: 251-259
8. National Committee for Clinical Laboratory Standards, Methods for disk antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa, 2003
9. Enne V, Livermore D, Stephens Hall L. Persistence of sulphonamide resistance in Escherichia coli in the UK despite national prescribing restriction. Lancet 2001; 357: 1325-1328
10. Oliver A, Weigel L.M, Rasheed J.K, McGowan J.E, Raney.P, Tenover.F.C, Mechanisms of decreased susceptibility to cefpodoxime in *Escherichia coli*, Antimicrob Agents Chemother, 2002; 46(12): 3829–3836
11. Pagani L, Dell'Amico E, Migliavacca R, D`Andrea M.M, Giacobone E, Amikosante G. Multiple CTX-M-type extended-spectrum β-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in Northern Italy, J Clin Microbiol, 2003; 41(9): 4264–4269
12. Pai H, Kang C, Byeon J, Lee KD, Park WB, Kim H.B,et al. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae*, Antimicrob Agents Chemother 200;48(10): 3720–3728
13. Jiang X, Zhang Z, Li M, Zhou. D, Ruan.F, Lu.Y. Detection of extended-spectrum β-lactamases in clinical isolates of *Pseudomonas aeruginosa*, Antimicrob Agents Chemother 2006; 50(9): 2990–2995
14. Machado E, Cantón R, Baquero F, Galan JC, Rollan A, Peixe L, et al . Integron content of extended-spectrum-β-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain, Antimicrob Agents Chemother 2005; 49(5): 1823–1829
15. Giakkoupi P, Xanthaki A, Kanelopoulo M, Velahaki A, Miriagou V, Kontou S, et al. VIM-1 metallo-β-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals, J Clin Microbiol 2003; 41(8): 3893–3896
16. Coudron P, Hanson N, Climo M, Occurrence of extended-spectrum and ampC beta-lactamases in bloodstream isolates of *Klebsiella pneumoniae* isolates harbor plasmid-mediated FOX-5 and ACT-1 AmpC beta-lactamases, J Clin Microbiol 2003; 41(2): 772–777
17. Akbar S, Jafri S, Amendola M, Madrazo BL, Salem R, Bis KG.Complications of renal transplantation. Radiolographics 2005; 25: 1335-1356
18. Chiang, Y., Chen C, Wu C, Chu SH, Chen Y, Liu KL, et al .De novo cancer occurrence after renal transplantation: a medical center experience in Taiwan, Transplant 2004; 36: 2150-2151
19. Säemann M, Hörl W.H. Urinary tract infection in renal transplant recipients, Eur J Clin Invest 2008;38:58-65

20. Senger SS, Arslan H, Azap OK, Timurkaynak F, Cagir U, Haberal M. Urinary tract infections in renal transplant recipients. Transplant Proc 2007; 39:1016–1017
21. Shirazi MH, Ranjbar R , Hemati F , Sadeghifard N. Bacterial Infections in Renal Transplant Recipients, Iran J Public Health 2005; 34:62-65
22. Shi L, kou Y, Li L, Miyoshi S. Analysis of Genetic determinates involved in multiresistance in clinical strains isolated from renal transplantation recipients in Guangzhou, china. J Health Sci 2007; 53: 185-189
23. Chuang P, Parikh CR, Langone A. Urinary tract infections after renal transplantation: A retrospective review at two US transplant centers, Clin Transplant 2005; 19: 230-235.
24. Khosroshahi HT, Mogaddam AN, Shoja MM. Efficacy of high-dose trimethoprim-sulfamethoxazol prophylaxis on early urinary tract infection after renal transplantation.Transplant Proc 2006; 38:2062–2064
25. Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility and epidemiology. Eur J Clin Microbiol Infect Dis 1999; 18: 761-770
26. Skurnik D, Menach A, Zurakowski D, Mazel D, Courvalin P, Denamur E. Integron-associated antibiotic resistance and phylogenic grouping of E.coli isolates from healthy subjects free of recent antibiotic exposure. Antimicrob Agents Chemother 2005; 49(7): 3062-3065

****