ARTICLE

EVALUATION OF AMINOGLYCOSIDES RESISTANCE GENES AMONG BETA LACTAMASEPRODUCING ESCHERICHIA COLI

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ABSTRACT

The aim of this study was to determine the occurrence of Aminoglycoside resistance in relation to extended-spectrum B-lactamase(ESBL)-producing Escherichia coli in Tabriz. Escherichia coli (E. coli) is a member of human and animals gut flora. It is also one of the major causes of nosocomial and other infections. Aminoglycosides are one of the antibiotics groups which are used for treatment of E. coli infections and yet resistance to aminoglycosides has increased in the recent years. ESBL-producing E.coli are specifically concerning because they confer resistance to a large number of antibiotics including third generation cephalosporin. The present study investigated the prevalence of genes encoding aminoglycoside-modifying enzymes in ESBL producing E. coli strains isolated from intestinal or extra-intestinal infections.Combined disk procedure was carried out for detection of beta lactamase production using ceftazidime and ceftazidime/clavunic acid. The frequency of aminoglycoside modifying enzymes encoding genes aac (3)-IIa, ant(2')-Ia, aph(3')-IIa, aph(3')-Ia, and aac(3)-IVwas analyzed by the PCR method.The results of this study indicated that the genes encoding aminoglycoside-modifying enzymes are prevalent in E.coli isolates in the study region, which highlighted the necessity of considering preventive measures to control dissemination of these resistance genes.

KEY WORDS
Escherichia coli; Antimicrobial Agents; Aminoglycoside, Beta lactamase

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INTRODUCTION

Three major groups of E. coli are including commensal, intestinal pathogenic (diarrheagenic) and extra intestinal pathogenic (ExPEC) [1, 2]. Commensal E. coli can also be a causative agent of extraintestinal infection [3,4]. E. coli is the most common causative agent of disease in humans worldwide including the urinary tract, biliary tract, intravenous catheters, skin and soft tissue and wounds [5,6]. Each of these infections can lead to life-threatening septicemia, and E. coli are one of the main pathogens of nosocomial infections [7]. Appearing resistance in enterobacteriaceae is a crucial problem that requires immediate attention. The aminoglycosides and B- lactams are two immense families of antibiotics that are regularly used in clinical settings.

β-lactam antibiotics prevent bacteria through interruption of cell wall biosynthesis [8,9].

Resistance associated with production of extended-spectrum B- lactamases (ESBLs) is a specific problem in the handling of E. coli infections [10]. Infections with ESBL producing organisms are related to higher rates of mortality, morbidity and health care costs. ESBLs are most often plasmid mediated [11,12]. ESBL-producing E.coli, are not only specifically concerning because they confer resistance to a large number of antibiotics including third generation cephalosporins, but also their prevalence has been rising in community and hospital settings during recent years [13,14]. The aminoglycosides were first characterized in 1944, they act by binding to the 30S subunit of the prokaryotic ribosome resulting in interruption to protein synthesis. Resistance to aminoglycosides can be through decreased aminoglycoside uptake or enzymatic modification of the aminoglycoside through acetylation (AAC), adenylation (ANT) or phosphorylation (APH) 8. Since bacteria producing ESBL usually offers some resistance to other antibiotics and the genes of resistance are located on plasmids, it is believed that producing ESBLs are also related to resistance to aminoglycoside. The aim of this study was to determine the existence of aminoglycoside resistance genes in relation to ESBL producing strains.

MATERIALS AND METHODS

A total of one hundred seven (n=107) *E.coli* isolates (35 urines, 36 Diarrheal stools and 36 wounds) were collected from Emam Reza hospital (Tabriz, Iran)and were re-cultured and identified by biochemical tests in laboratory of microbiology department [15].

Antibacterial susceptibility tests of all isolated *E.coli* were performed by Kirby-Bauer disk diffusion method using following antibiotic disks (MAST Ltd UK) acording to Clinical and Laboratory Standards Institute

(CLSI) instructions: amikacin(Ak 10µg), cefotaxime(CTX 30µg), ciprofloxacin(CIP5µg), ampicillin(AM 10µg), (SXT1.25/23.75µg), imipenem (IPM10µg), choleramphenicol (C 30µg), kanamycin (K 10µg), ceftazidime (CAZ30µg), gentamicin (GM10µg), trimethoprimsulphamethoxazol[16].

Detection of ESBL-producing E.coli

All resistant isolates to beta lactame antibiotics were cultured on Muller-Hinton agar (MH) and combined disk tests were performed by placing disks of ceftazidime (CAZ), and CAZ/Clavulanic acid (MAST Ltd UK) on MH plates. *ESBL* production was interpreted if the zones produced by the disks with clavulanate were ≥ 5mm larger than those without inhibitor [17].

DNA extraction and PCR screening for anti-microbial resistance genes

CTAB protocol was used as DNA extraction method. DNA of all isolates confirmed for *ESBL* production and aminoglycoside resistance was extracted [18] and then aminoglycosides resistant genes were detected by PCR method utilizing the specific primers shown in [Table 1][19].

The PCR mixture (total volume, 20µl) included 1.5µl template DNA, 2µl of 10× PCR buffer plus $MgCl_2$, 0.4µl of Taq DNA polymerase, 0.4µl dNTP ,14.9µl H_2o and 0.4µl of each primer.

DNA amplification was carried out in a DNA thermal cycler (Gradient Eppendrof) by using the following conditions: Initial denaturation step at (94◦c for 6 min), followed by 30 to 40 cycle repetitions of denaturation (94◦c for 50 s), annealing (50-60 ◦c for 50s) and extention (72◦c for 80s) with a final extension at 72◦c for 8 min[20].

After electrophoresis of PCR products in %1.2 (w/v) of agarose for 70-80 min at 100v, gels stained with ethidium bromide (0.5 mg / ml) and images were taken by UV transIlumination

RESULTS

Out of 107 isolates27 were resistance to aminoglycoside (25.23%) and 72 were Beta-lactamase producing (67.29%). 18 of 27 aminoglycoside resistant *E.coli* produced Beta-lactamase (66.67%) by combined test[Fig. 1]. Bacterial isolates displayed the highest level of resistance to ampicillin (46.72 %) and trimethoprim-sulphamethoxazol (46.72%) and the lowest resistance to imipenem (0%) and amikacin (1.86%). The resistance features of 107 isolates of *E.coli* to the 10 antimicrobial disks examined is shown in [Table 2].Prevalence of aminoglycoside resistance genes among 18 *ESBL* producing *E.coli* are as follows: ant(2")-Ia 6 (%33.33), aph(3')-Ia 6 (%33.33), aac(3)-IIa 3 (%16.67), aph(3')-IIa 3 (%16.67), aac(3)-IV 0 (%0)[Fig.2].

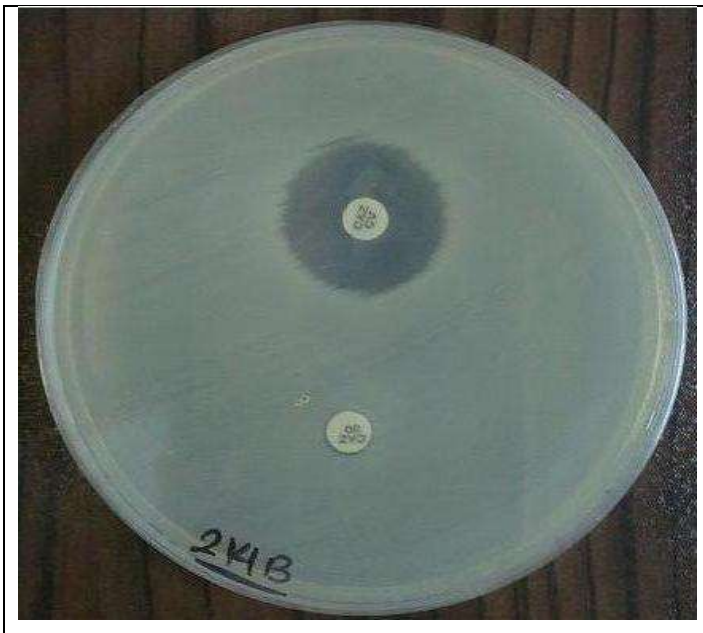

Fig. 1: Representative of E. coli isolate showing ESBL production confirmed by an increase in zone size of more than 5 mm for ceftazidime (CAZ) with and without clavulanic acid

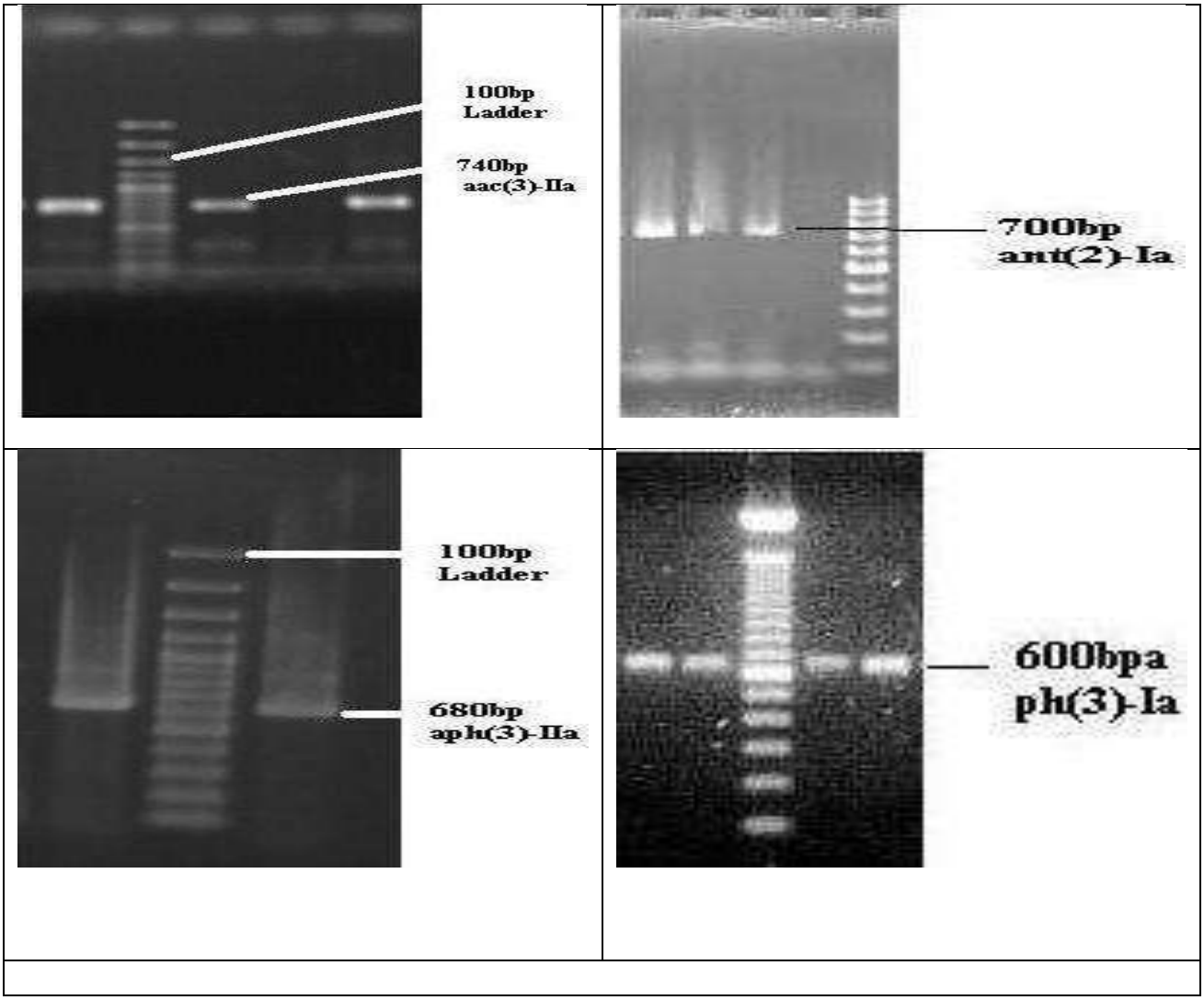

Fig 2: Agarose gel electrophoresis of PCR products from *E. coli* isolates tested for four aminoglycoside resistance genes: aac(3)-IIa (740 bp), ant(2")-Ia (700bp), aph(3')-IIa (680 bp), aph(3')-I (600 bp). 100 bp Ladder was used in all PCR

Table 1: Primers used for the amplification of Aminoglycoside resistance genes among *E.coli* isolates

Target Gene	Primer Sequence	Annealing temperature	Cycle	Product size Bp	References
aac (3)-IIa	F: CGGAAGGCAATAACGGAG R: TCGAACAGGTAGCACTGAG	50	35	740	19
ant(2')-Ia	F: TCCAGAACCTTGACCGAAC R: GCAAGACCTCAACCTTTTCC	52	35	700	19
aph(3')-IIa	F: GAACAAGATGGATTGCACGC R: GCTCTTCAGCAATATCACGG	56	35	680	19
aph(3')-I	F: ATGGGCTCGCGATAATGTC R: CTCACCGAGGCAGTTCCAT	55	35	600	19
aac(3)-IV	F: GTGTGCTGCTGGTCCACAGC R:AGTTGACCCAGGGCTGTCGC	50	35	627	19

Table 2: Susceptibility of 107 *E.coli* isolated from different clinical specimens

Antibiotic	Resistance (No)	%
SXT1	50	46.72
AM	50	46.72
CIP	27	25.23

CTX	21	19.62
C	18	16.82
K	13	12.16
GM	12	11.21
CAZ	10	9.34
AK	2	1.86
IMP	0	0
GM; Gentamicin (10µg), K; Kanamycin(10µg), AK; Amikacin (10µg), IPM; Imipenem(10µg), C; Choleramphenicol (30µg), CAZ; Ceftazidime (30µg), SXT; Trimethoprim sulphamethoxazol (1.25/23.75µg), CIP; Ciprofloxacin(5µg), AM ;Ampicillin (10µg), CTX; Cefotaxime (30µg)		

DISCUSSION

In the last two decades, there has been increased resistance to *β*-lactam antibiotics due to plasmid mediated acquisition of *ESBL*s [21,22,23]. Aminoglycosides despite having side effects and problems associated with the increasing resistance of microorganisms to these drugs, as well as in the treatment of bacterial infections are most valuable [24]. Prevalence data collected from German in 2007, reported that the percentage of ciprofloxacin-resistant *E.coli* was (21.9%) compared to (25.23%) in our investigation [25,26]. In current study, the majority of isolates (46.72%) were resistant to ampicillin and trimethoprim-sulphamethoxazol that was higher than the results obtained from USA (39.1%) and Europe (29.8%) [27,28,29]. In this study low numbers of isolates were resistant to amikacin, ceftazidime and gentamicin (1.86%, 9.34% and 11.21% respectively) while the rate of resistance against these antibiotics in India (51%, 56% and 64% respectively) was much higher in compare with our results [Table 2][29].

Resistance to imipenem was not detected in either isolates,therforeimipenem is the choice of treatment of infections caused by *E.coli* isolates. We found a high rate of *ESBL* producing isolates (67.29%) compare to (13.5%) in nursing homes in Northern Ireland [30]. According to liturature resistance against gentamicin, kanamycin and tobramycin is coded by ant(2")-Ia gene [31] , resistance to gentamicin is coded by aac(3)-IIa gene, and also concurrent aph(3')-Ia and aph(3')-IIa inactivated kanamycin and neomycin[2]. The results of our study showed that ant(2")-Ia (33.33%), aac(3)-IIa(16.67%), aph(3')-Ia(33.33%), aph(3')-IIa(16.67%) and aac(3)-IV 0 (%0) were the most commonly detected aminoglycoside resistant genes in clinical isolates of *E.coli* by PCR. The resultsobtained in different regions are shown in [Table3] indicates that there are a lot of variety inthe percentage of genes from a region to another region in the world.

E. coli resistance to aminoglycosides is increasing over time, depending on situation of antibiotics consumption in the society, which requires further study. To prove the increasing prevalence of resistance to aminoglycosides, annually repeated surveilance is necessary [24,32,34]. Major differences in the findings related to antibiotic resistance, as well as the genes diversity of resistance to aminoglycosides indicate that imporant differences are due to diverse isolates and various geographic regions and most of aminoglycoside resistant *E. coli* are*β*-lactamase producer.

In conclusion, our results showed that Prevalence of the aac(3)-IV gene was lower than other studies and resistance by aac(3)-IV gene was not observed in this research. The high antibiotic resistance against all antibacterial disks examined were found except for imipenem and amikacin which prescription of this antibiotic can be effective for the treatment of *E.coli* infections of human in our area. Also high resistance against ampicillin and trimethoprim-sulphamethoxazol indicated that these antibiotics in the past were commonly used in our area.

Table 3: Showing the results obtained for aminoglycoside resistance genes in various geographic regions

Scientists	%	Genes	Year	References
Haldorsen and Simonsen	2.85	Ant(2")-I	2014	35
Soleimani and Aganj	78.87	Ant(2")-I	2014	31
Soleimani et al	47.88	Ant(2")-I	2012	36
Soleimani and Aganj	47.88	Aac(3)-IIa	2014	31
Maynard and Bekal	33%in human,17%in animal	Aac(3)-IIa	2004	2
Soleimani et al	54.83	Aac(3)-IIa	2012	36
Jakobsen et al	63.15	Aac(3)-IIa	2008	37
Jakobsen et al	0	Ant(2")-I	2008	37
Maynard et al	75% in pigs	Aac(3)-IV	2003	19
Maynard and Bekal	50	Aph(3')-I	2004	2
Maynard and Bekal	0	Aph(3')-IIa	2004	2
Maynard and Bekal	0	Ant(2")-I	2004	2
Saenz et al	17	Aph(3')-I	2004	38
Saenz et al	35.29	Aph(3')-IIa	2004	38
Vinue et al	66.67%(2 of 3)	Aph(3')-I	2008	39
Maynard et al	87	Aph(3')-Ia	2003	19

Maynard et al	15	Aph(3')-IIa	2003	19
Maynard et al	34	Aph(3')-I	2003	19
Maynard et al	8	Aph(3')-IIa	2003	19

CONFLICT OF INTEREST
There is no conflict of interest

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FINANCIAL DISCLOSURE
None

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