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Research Article

Different Features of *Escherichia coli* and *Klebsiella pneumoniae* in Children and Adults

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Abstract

Introduction: This study aimed to describe the association between age groups and antimicrobial susceptibility patterns, as well as integron presence in *Escherichia coli* and *Klebsiella pneumoniae* isolates from Tabriz, Iran.

Materials and methods: Equal numbers of isolates from adults and children, 140 for *E. coli* and 150 for *K. pneumoniae*, were examined for susceptibility to 13 routine antibiotics. Integron existence in multidrug resistant (MDR) isolates was also determined using PCR-RFLP.

Results: Significant age-related differences were observed in the resistance rates of *K. pneumoniae* toward cotrimoxazol, nalidixic acid, ciprofloxacin, and norfloxacin. For *E. coli,* age-related differences in the resistance rates to tetracycline, chloramphenicol, ciprofloxacin, and norfloxacin were significant. PCR-RFLP results revealed the presence of class 1 integron (*intI*1) in 24.5% and 19.2% of MDR *E. coli* in children and adults, respectively. In *K. pneumoniae*, 72.9% of isolates from children and 84% from adults were positive for *intI*1. The prevalence of class 2 integrons was significantly associated with age, in both *E. coli* and *K. pneumoniae.* No class 3 integrons were detected in this study.

Conclusions: The resistance rates differ across age groups. Moreover, this study is the first to demonstrate age-related differences in integron presence, especially for class 2 integrons, in *E. coli* and *K. pneumoniae*.

Keywords: Integron, *E. coli*, *K. pneumoniae*, Children, Adult

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1. Introduction

With the widespread use of antibacterial agents, problems in the treatment of infections with drug resistant organisms, particularly those showing resistance to three or more different classes of antibiotics (multidrug resistant- MDR), have increased [1, 2].

In addition to intrinsic resistance, the acquisition of resistance genes by horizontal transfer through plasmids, transposons, and integrons plays an important role in the dissemination of resistance genes, as well as in the development of multidrug resistance in bacterial populations [3]. Integrons have been identified as a mechanism used by some of bacteria to collect antibiotic resistance genes and express multiple resistance phenotypes in synergy with transposons [4, 5]. These elements encode a site-specific recombinase (IntI) that belongs to a distinct family of the tyrosine recombinase superfamily, responsible for the insertion of gene cassettes at *attI*, and also provides the promoter responsible for the expression of the gene cassettes that usually encode the antibiotic resistance. The movement of cassettes is catalyzed by site-specific recombination and results in the dissemination of resistance genes [4, 6, 7]. Although the integron platforms are defective in self-transposition, they are often associated with transposons and/or conjugative plasmids, which can serve as vehicles for their transmission [4]. There are two main groups of integrons: chromosomal and mobile integrons. Mobile integrons are divided into five classes, based on their *intI* sequences. Of these groups, classes 3, 4, and 5 are rare [8]. Many reports on this are available from different countries [9–15], revealing antibacterial resistance arising due to the presence of integrons in gram-negative bacteria, especially in *E. coli* and *K. pneumoniae*. However, there is a paucity of literature concerning comparisons of integron carriage in *E. coli* and *K. pneumoniae* obtained from different age groups. Thus, this study aimed to investigate antibacterial resistance patterns and their relation to the presence of integrons in *E. coli* and *K. pneumoniae* isolated from various clinical specimens from child and adult patients.

2. Materials and Methods

2.1. Clinical isolates

During a period of one year, from 2015 to 2016, 70 *E. coli* and 75 *K. pneumoniae* were isolated from various clinical specimens including urine, blood, wound exudates, bronchial secretions, sputum, cerebrospinal fluid (CSF), and catheter and pleural fluid of children admitted to the Children's Hospital. The same number and types of isolates were obtained from adult patients (age ≥14 years) admitted to the Sina Hospital. Both hospitals used in this study are referral educational centers for infectious disease in the northwest of Iran.

2.2. Antimicrobial susceptibility testing

All isolates were tested for their susceptibility to routine antimicrobial agents using the standard disk agar diffusion method [16]. The antimicrobial agents employed in the susceptibility tests were: gentamicin (10 µg), amikacin (30 µg), amoxicillin (10 µg), ceftazidime (30 µg), cephalothin (30 µg), imipenem (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), co-trimoxazole (25 µg), tetracycline (30 µg), chloramphenicol (10 µg), and nitrofurantoin (300 µg) (Mast Co., Merseyside, UK). *E. coli* ATCC 25922 was used as the control strain.

2.3. Detection of integrons

The template DNA for PCR was prepared by the boiling method. Briefly, bacteria were harvested from 1.5 ml of an overnight Luria-Bertani broth (Sigma Aldrich, Germany), suspended in sterile distilled water, and incubated at 95 ˚C for 10 min. Following centrifugation for 5 min at 11,500 × *g*, the supernatant was stored at -20 ˚C and used as template DNA stock.

To determine whether the MDR isolates of *E. coli* and *K. pneumoniae* carried integrons, the conserved regions of integron-encoded integrase genes *intI1*, *intI2*, and *intI3* were amplified with the degenerate primer pair hep35: 5′-TGCGGGTYAARGATBTKGA TTT-3′ and hep36: 5′-CARCACATGCGTRTARAT-3′, where B = C, G, or T, K = G or T, R = A or G, and Y = C or T [17]. Primers were provided by Alpha DNA (Montreal, Canada). The PCR was performed in a 25 µl reaction mixture containing 2 µl of DNA template, 50 pmol of each oligonucleotide primers, 0.2 mM of deoxynucleoside triphosphates (Takara, Japan), 1.5 mM of $MgCl_2$ (Takara), 2.5 µl of 10X PCR buffer (100 mM Tris-HCl, pH 8.3 and 500 mM KCl), and 2.5 U of *Taq* polymerase (Takara). The PCR was performed in an Eppendorf thermal cycler as follows: 10 min at 94 ˚C (initial denaturation), followed by 30 cycles of denaturation at 94 ˚C for 30 s, annealing at 55 ˚C for 30 s, and extension at 72 ˚C for 45 s, with a final extension step at 72 ˚C for 10 min. A tube containing the PCR reaction with no DNA template was used as a negative control for all PCRs. The expected amplicons (491bp) were ascertained by electrophoresis on agarose gels (1.5% w/v gels in TAE buffer) at 100-120 V for 30-55 min.

To determine integron classes, the PCR products were further analyzed by restriction fragment length polymorphism (RFLP) analysis, following digestion with either *Rsa*I or *Hinf* I, according to the manufacture's instructions (MBI, Fermentas, Lithuania). The size and number of the generated fragments were taken into consideration as suggested by White *et al*. [17].

2.3.1. Statistics

Statistical analysis was performed using SPSS software for Windows, version 19. A chi-square test was used to assess any association between the results from the two age groups. *P* values less than 0.05 were considered significant.

3. Results

Out of total 145 bacterial isolates (70 *E. coli* and 75 *K. pneumoniae*) obtained from children, 100 isolates [68.9% (n = 48; 68.5% *E. coli* and n = 52; 69.3% *K. pneumoniae*)] were isolated from hospitalized patients, while the remaining isolates (n = 45) were obtained from outpatients.

The sources of tested isolates were as follows: urine (n = 43; 61.4% *E. coli* and n = 36; 48% *K. pneumoniae*), stool (n = 14; 20% pathogenic *E. coli*), blood (n = 5; 7.1% *E. coli* and n = 7; 9.3% *K. pneumoniae*), wound (n = 3; 4.2% *E. coli* and n = 19; 25.3% *K. pneumoniae*), catheter (n = 3; 4.2% *E. coli* and n = 5; 6.6% *K. pneumoniae*), and CSF (n = 2; 2.8% *E. coli*). The mean age of the children was 35.8 ± 14.9 months.

In the adult population, 55.4% (n = 39) of *E. coli* and 76% (n = 57) of *K. pneumoniae* were obtained from hospitalized patients and urine constituted the major source of these isolates (n = 63; 90% for *E. coli* and n = 49; 65.3% for *K. pneumoniae*), followed by wound (n = 2; 2.8% of *E. coli* and n = 16; 21.3% of *K. pneumoniae*) and blood (n = 2; 2.8% of *E. coli* and n = 5; 6.6% of *K. pneumoniae*). The mean age of the adults was 30.1 ± 4.3 years.

When the antibacterial susceptibility patterns of the *E. coli* and *K. pneumoniae* isolates obtained from the two different age groups were compared, considerable differences were observed in the resistance rates of *K. pneumoniae* isolates to cotrimoxazol, nalidixic acid, ciprofloxacin, and norfloxacin, between children and adults. In the case of *E. coli,* differences between the resistance rates of child and adult isolates against tetracycline, chloramphenicol, ciprofloxacin, and norfloxacin were statistically significant (Table 1).

In terms of the prevalence of the multidrug resistance phenotype, no difference was observed when comparing isolates of *E. coli* obtained from children and adults [n = 60 (85.7%) vs. n = 58 (82.8%), respectively]. The multidrug resistance phenomenon was also common in *K. pneumoniae*, as observed in isolates obtained from children (n = 74; 98.4%) and adults (n = 75; 100%), though no difference was found between isolates obtained from the two age groups. All MDR isolates were examined for the presence of integrons and their types by PCR-RFLP. The observation revealed that in both *E. coli* and *K. pneumoniae*, the class 1 integron had a dominant presence; none of the

Table 1: Antimicrobial susceptibility patterns of *E. coli and K. pneumoniae* according to age groups.

	E. coli			*K. pneumoniae*		
	Adult isolates (n = 70)	Child isolates (n = 70)		Adult isolates (n = 75)	Child isolates (n = 75)	
Antibiotic	No. (%) of resistant isolates	No. (%) of resistant isolates	*P value*	No. (%) of resistant isolates	No. (%) of resistant isolates	*P value*
Gentamicin	24 (34.2)	23 (32.8)	0.98	55 (77.3)	56 (74.7)	0.39
Amikacin	24 (34.2)	23 (32.8)	0.98	55 (77.3)	56 (74.7)	0.39
Amoxicillin	69 (98.5)	70 (100)	0.31	75 (100)	74 (97.7)	0.31
Ceftazidime	31 (44.2)	34 (48.5)	0.87	59 (78,7)	68 (90.7)	0.1
Cephalothin	53 (75.7)	56 (82.8)	0.25	59 (78,7)	66 (88)	0.22
Imipenem	1 (1.4)	1 (1.4)	1	12 (16)	8 (10.7)	0.36
Nalidixic acid	37 (52.8)	48 (68.5)	0.15	32 (42.7)	59 (78,7	0.01*
Ciprofloxacin	26 (37.1)	41 (58.5)	0.01*	23 (30.7)	40 (53.3)	0.04*
Norfloxacin	28 (40)	43 (61.4)	0.02*	20 (26.7)	39 (52)	0.01*
Co-trimoxazole	55 (78.5)	47 (67.1)	0.12	68 (90.7)	75 (100)	0.02*
Tetracycline	51 (72.8)	52 (74.2)	0.28	52 (69.3)	45 (60)	0.32
Chloramphenicol	10 (14.2)	19 (27.1)	0.02*	35 (46.7)	52 (69.3)	0.07
Nitrofurantoin	6 (8.5)	12 (17.1)	0.23	72 (96)	73 (97.3)	0.36
* Significant values						

Table 2: Prevalence of integrons in MDR isolates according to age groups.

	E. coli			*K. pneumoniae*		
	Adult isolates (n = 57)	Child isolates (n = 61)		Adult isolates (n = 75)	Child isolates (n = 74)	
Integron	No. (%) of positive isolates	No. (%) of positive isolates	*P value*	No. (%) of positive isolates	No. (%) of positive isolates	*P value*
IntI1	15 (24.5)	11 (19.2)	0.48	54 (72.9)	63 (84)	0.07
IntI2	6 (9.8)	0 (0)	0.01*	6 (8.1)	14 (18.7)	0.05*
* Significant values						

isolates contained the *intI*3 gene (Figure 1). The prevalence of integrons among *E. coli* and *K. pneumoniae* are shown and compared across adults and children in Table 2. As demonstrated, chi-square and Fisher's exact tests showed a significant difference between the prevalence of class 2 integrons, but not class 1 integrons, in both *E. coli* and *K. pneumoniae* isolates obtained from adults and children.

4. Discussion

Antimicrobial resistance rates in bacteria can vary between groups of patients according to their age. However, detailed comparisons of rates or trends in children and adults

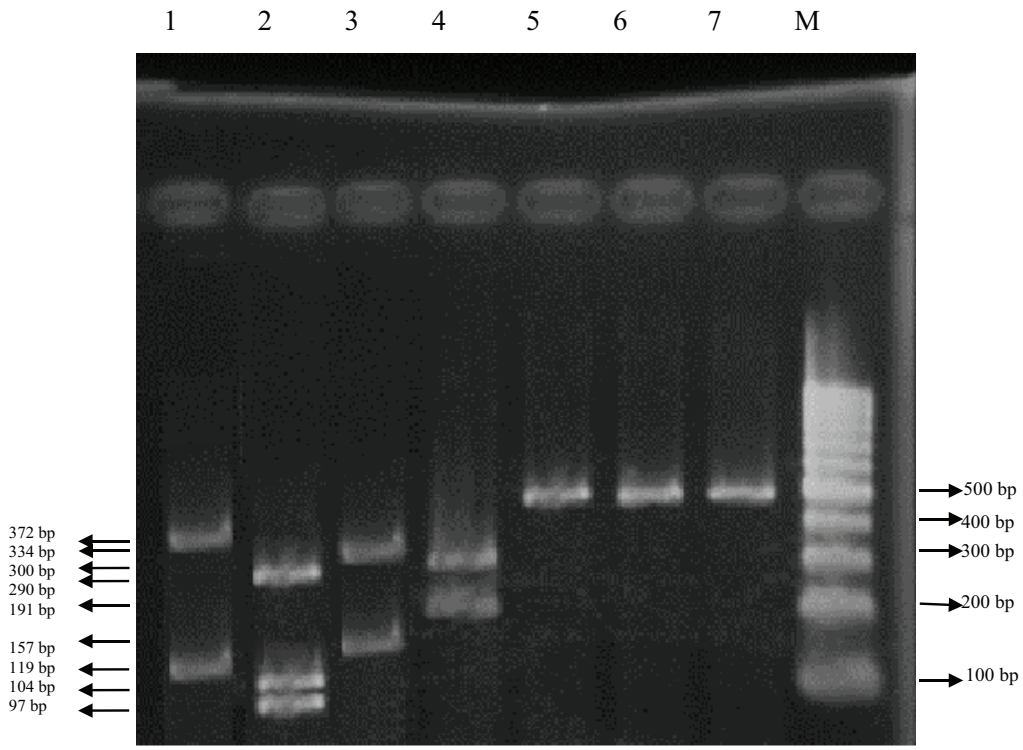

Figure 1: PCR-RFLP of *intI* products. Lane 1, *Rsa*I-treated product in the control strain represents a class 3 integron; Lane 2, *Hinf*I-treated product in the control strain represents a class 3 integron; Lane 3, *Hinf*I-treated product represents a class 2 integron; Lane 4, *Rsa*I-treated product represents a class 2 Integron; Lane 5, *Rsa*I-treated product represents a class 1 integron; Lane 6, *Hinf*I-treated product represents a class 1 integron; Lane 7, PCR product of *intI* amplification, Lane M, 100 bp DNA ladder.

are limited. The present study therefore investigated clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* with respect to their antimicrobial resistance and integron carriage.

With the exception of two isolates (one *E. coli* isolated from a child and one *K. pneumoniae* isolated from an adult), all isolates tested for their resistance to various antibiotics were resistant to amoxicillin, which is in line with reports from Iran and other countries [9, 18]. This marked high rate of resistance can be linked to the production of beta-lactamases by some of the bacteria, including *E. coli* and *K. pneumoniae*.

Another striking feature was observed when antibiotic susceptibility patterns were compared between isolates (for both *E. coli* and *K. pneumoniae*) obtained from children and adults. This assessment revealed that the resistance rate to the two main fluoroquinolones, ciprofloxacin and norfloxacin, was higher in isolates obtained from adults than in isolates obtained from children ($P < 0.05$). Similar results have been reported in an investigation from Spain [19].

Fluoroquinolones are an important group of antimicrobial agents widely used in the treatment of various infectious diseases, due to their excellent clinical activity against most bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*. Their use in

children, however, has been limited as a result of possible fluoroquinolone-induced toxicity[20]. This restraint further explains the lower resistance rates observed in isolates obtained from this group.

We also found that chloramphenicol resistance (in the case of *E. coli*) and nalidixic acid resistance (in the case of *K. pneumoniae*) was significantly greater among adults isolates than in those from children. These observed differences could be also due to the limited usage of chloramphenicol and nalidixic acid in pediatric infections.

In this study, *K. pneumoniae* isolates from adults were significantly more resistant to cotrimoxazol in comparison to isolates obtained from children. This result is consistent with the study of Tonkic *et al*. on *K. pneumoniae* in Croatia [21].

Imipenem (IMP) demonstrated the greatest *in vitro* activity against the bacteria tested in this study, with 98.5% of *E. coli* isolates, both from children and adults, being susceptible to imipenem. This rate of susceptibility is similar to the rate reported by Yildirim *et al*. from Turkey [22]. Additionally, the same study reported an IMP susceptibility of 98.4% for *K. pneumoniae*, which is higher than our calculated rate (84% in children isolates and 89.3% in adult isolates). Imipenem is the drug of choice for the treatment of serious infections caused by ESBL-producing microorganisms, so emergence of resistance to this antibiotic is alarming and requires special consideration.

The emergence of multiple drug resistant *Enterobacteriacea* is a great problem in clinical settings due to limited therapeutic options. Our results show a high proportion of multidrug resistance among *E. coli* strains, especially in isolates from children. We showed that 81.4% of *E. coli* isolates obtained from adults in our study were MDR, while in children this was 87.4%. A high incidence of multidrug resistance in *E. coli* isolates from children has been previously documented in another part of Iran [9, 23]. However, the calculated incidence of the MDR phenotype in our study was even higher. In the case of *K. pneumonia*, all of the isolates we tested were MDR (except for one isolate from a child). The rate of MDR *K. pneumoniae* in our study is extremely high, as compared to the 53% and 59.8% MDR rates reported from Malaysia and Tanzania, respectively [10, 24].

The widespread use of antibiotics, coupled with the transmissibility of resistance determinants mediated by plasmids, transposons, and gene cassettes in integrons, are major contributing factors in the emergence of multidrug resistant pathogens [25].

According to the PCR-RFLP results of the current study, the existence of class 1 integrons was observed in 24.5% of child and 19.3% of adult MDR isolates of *E. coli*, which was lower than those rates reported in other studies [9, 13, 15, 26].

In a study from the south of Iran, the prevalence of class 1 integrons in uropathogenic *E. coli* isolates of children was reported as 6.25%, which is lower in comparison to our results [23].

We also found a significantly higher frequency of the *intI2* gene in the multidrug resistant *E. coli* isolates obtained from children (9.8%), compared to adult isolates (0%). This finding is surprising, since one can assume that higher rates of resistant and integron-containing bacteria exist in adult patients, due to the selective pressure of antibiotics consumed during a lifespan. Although some previous studies have shown variations in integron prevalence in *E. coli* isolates from clinical specimens, no study of different age groups in the same region was available for comparison with our current study. Only one published study has reported a significantly higher prevalence of class 1 integrons in commensal *E. coli* isolates of healthy antibiotic-naive children, compared to healthy elderly persons [27].

In the present study, a positive test result for class 1 integrons was seen in 72% of child and 84% of adult isolates of *K. pneumoniae*. These findings strongly indicate a wide distribution of class 1integrons in clinical isolates of *K. pneumoniae*, which will limit and threaten the efficacy of antimicrobial therapy.

We also found that *intI2* was significantly more prevalent among adult isolates of *K. pneumoniae*, suggesting a role for class 2 integrons in the development of multidrug resistance in *K. pneumoniae* isolates from adults.

Overall, our study revealed that the resistance of *E. coli* and *K. pneumoniae* isolates toward some antibiotics, including ciprofloxacin, norfloxacin, chloramphenicol, nalidixic acid, and cotrimoxazol, significantly differs between child and adult patients. Moreover, our results show significant differences between the prevalence of class 2 integrons in child and adult isolates of *E. coli* and *K. pneumoniae*, which we report for first time in our region.

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Conflicts of Interest

There is no conflict of interest.

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