# Antibiotics Resistance and Integron Class 1 among Commonsal *Escherichia coli*

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**الخلاصة**

الأحياء المجهرية المعوية المتعايشة هي مخازن للجينات المقاومة للمضادات الحياتية خصوصا البكتريا القولونية (أي كولأي). وكان الهدف من هذه الدراسة هو التعرف على مدى انتشار الانتكرون الصنف 1 و المقاومة للمضادات الحياتية بين عزلات الأي كولاي المتعايشة. وكذلك، هذه الدراسة تهدف للكشف عن علاقة الانتكرون الصنف 1 مع المقاومة للمضادات الحياتية (مقاومة لمضاد حياتي واحد أو اكثر) و التي قد تنقل أفقيا الى بكتريا أخرى . ثلاثة مائة و واحد عزلة من الاي كولاي تم الحصول عليها من أشخاص أصحاء (أعمارهم من 1 -80 سنة). تم اختبار حساسية جميع ألعزلات ضد 16مضاد حياتي باستخدام طريقة الانتشار القرصي وتم الكشف عن الانتكرون بواسطة PCR. اكتشف الانتكرون الصنف 1 في 112 عزلة (37٪) من أصل 301 عزلة. ولاحظ مقاومة عالية لمعظم المضادات الحياتية خصوصا الامبسلين و الاموكسلين و لينكومايسين و سيفالكسين في حين كانت المقاومة لأميكاسين والجنتاميسين أقل شيوعا. المقاومة لسيفوتاكسيم و سيبروفلوكساسين و أوكسي تتراسكلين والتتراسيكلين وميثوبريم ترتبط بشكل واضح (P<0.05) مع العزلات الحاوية على الانتكرون الصنف 1. كل العزلات الحاوية على الانتكرون الصنف 1 كانت مقاومة لأكثر من خمسة مضادات حياتية ومرتبط بشكل كبير مع العديد من أنماط المقاومة. في الختام، ازدياد مقاومة المضادات الحياتية بين عزلات الأي كولاي المتعايشة في هذا المجتمع وكذلك ازدياد انتشار الانتكرون الصنف 1والذي مرتبط بوضوح مع المقاومة لمضاد حياتي واحد أو أكثر .

**Abstract**

Normal intestinal microbiota are a reservoir for antibiotic resistance (genes) especially *Escherichia coli* (*E. coli*). The goal of this study was to investigate the prevalence of integrons class 1 and antibiotic resistance among commensal *E. coli* isolates and detect the association of integron class 1 with antibiotic resistance (single or multiple drug resistance) that may be transport horizontally to other bacteria. Three hundred one *E. coli* isolates from stools were obtained from healthy individuals (age from 1to 80 years). All isolates were tested for their susceptibility against 16 antimicrobial agents using standard disc diffusion method and for the presence of class 1 integrons by PCR. Integrase genes detected in 112 isolates (37%) out of 301 tested isolate. High resistance observed for most antibiotics especially ampicilln, amoxcillin, lincomycin, cephalexin while resistance to amikacin and gentamicin was less common. Furthermore resistance to cefotaxime, ciprofloxacin, oxytetracyclin, tetracycline and trimethoprim significantly associated ($p < 0.05$) with *IntI1* gene positive isolates. All integrons positive isolates are resist to more than five antibiotics and significantly associated with many resistance patterns. In conclusion**,** increase antibiotic resistance among commensal *E. coli* in this population that companied with increase frequency of integron class 1 that significantly associated with resistance for single or multiple antibiotic agents.

**Key words: Commensal *E. coli*, integron class 1, antibiotic resistance, multiple drug resistance**

## Introduction

The emergence of antibiotic resistance among pathogenic and commonsal bacteria became one of most clear global health challenges of this century **(1).** Resistance to antibiotics has a high predominant in bacterial isolates from developing countries which are result from over and improper use of antimicrobial agents. Commensal *E. coli* inhabit the intestine of many mammals including human and act as a potential reservoir for antimicrobial resistance genes and play an important role in the ecology of antimicrobial resistance of bacterial populations. The prevalence of resistance in commensal *E. coli* is a useful indicator of antibiotic resistance in bacteria in the community **(2,3).** Commensal *E. coli* strains efficiently exchange genetic material with other pathogens such as *Salmonella*, *Shigella*,

*Yersinia* and *Vibrio*, as well as pathogenic *E. coli* **(2,4,5).** Recently, exchange of many different and diverse genes responsible for antibiotic resistance has been linked to genetic structures called integrons, that integrate and mobilize individual gene cassettes encoding antibiotic resistance determinants **(6).** Integron that have resistance gene can be moved to other genetic sites or transferred horizontally to other bacteria mainly by conjugative plasmid. There are many types of integron have been identified and distinguished by their respective integrase (*IntI*) genes **(1).** Class 1 integrons are strongly associated with multi-resistance seen in enterobacteriaceae in the hospital environment **(7).** The present study aimed to indicate the role of commensal *E. coli* as reservoir of single or multiple antibiotic resistance that can be transported by potential transposable elements as integron to pathogenic and commonsal strains in human gut.

## Materials and Methods

**Study design and sample collection**: a cross sectional study that come in agreement with ethics of Al-Diwaniya Teaching Hospital and verbal informed consent was obtained from all healthy participants. Fecal samples collected from healthy workers, officers, students and their families seen in College of Medicine of Al-Qadisiya University and from clinical staff and their family of Al-Diwaniya Teaching Hospital from march 2016 to may 2016.……..

**Isolation and Identification of Microorganisms**: The identification of *E. coli* was done depending on morphological features and the rose pink color of the colonies on MacConkey agar plats that confirmed by subculture on Eosin Methylene Blue agar and incubated for 24 hours at 37˚C, the typical greenish metallic sheen color indicate of *E. coli* **(24)** then the result confirmed by biochemical tests and microscopically examination with Gram's stain. After primary identification of *E. coli* bacterial cell cultured in Nutrient broth for DNA extraction for molecular study and cultured on Muller- Hinton agar for antibiotic susceptibility test.

**Antibiotic Susceptibility Test**: All identified *E. coli* isolates were subjected to sensitivity test by disc-diffusion method **(25).** Antibiotic resistance of 16 antibiotic discs (ampicillin, amoxicillin, amikacin, norfloxacin, streptomycin, trimethoprim, lincomycin, gentamicin, ciprofloxacin, nalidixic acid, chloramphenicol, cefotaxime, ceftriaxone, cephalexin, tetracyclin and oxytetracyclin) was determined according to the guidelines recommended by the CLSI (2013), corresponding to the drugs considered routine testing and reporting on *Enterobacteriaceae* **(25).**

**Molecular study**: Conducted by conventional PCR for amplification primer of conserved region of *IntI1* gene (forward: GGTCAAGG ATCTGGATTTCG and reverse: ACATGCGTGTAAATCATCGTC) **(26).** **Statistical analysis:** Statistical analyses were performed using the odd ratio and $\chi 2$ test or Fisher's exact test. A *P* -value of $<0.05$ was considered to be statistically significant. Statistical analyses were performed by the Statistical Package for Social Sciences version 17 for Windows software and Microsoft Excel 2010.

## Results

**Frequency Of Integron Class 1 and Antibiotic Resistance Among Commonsal *E. coli* Isolates**

Integrase genes detected in 112 isolates (37%) out of 301 tested isolate by PCR amplification (figure 1and 2).

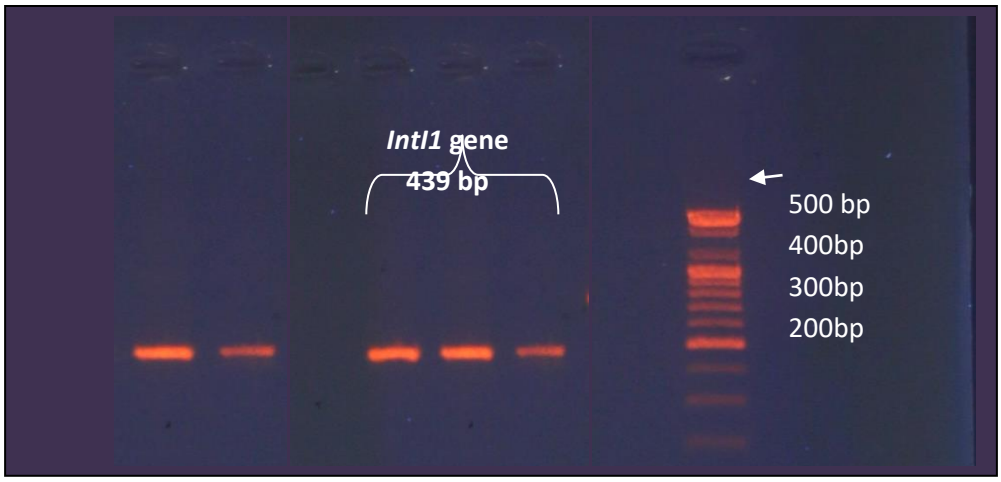


**Figure (1): PCR amplification of *IntI1*gene of *E. coli* flora**

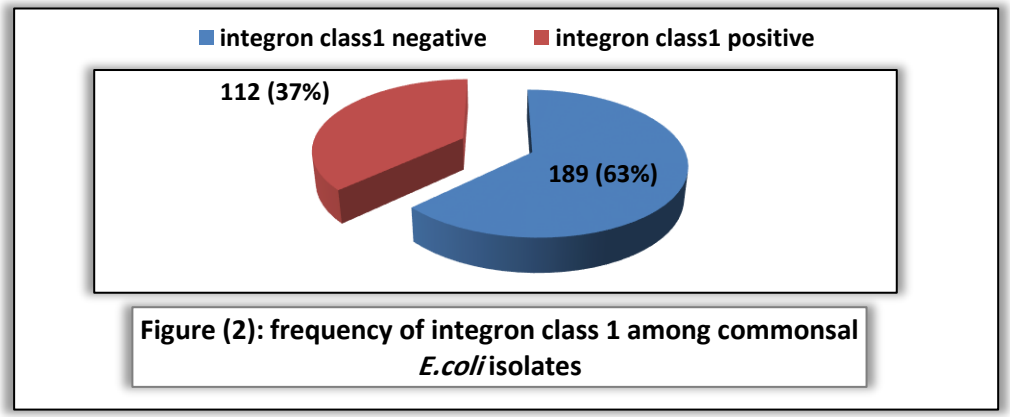


**Figure (2): frequency of integron class 1 among commonsal *E.coli* isolates**

Among the drugs under the study, ampicillin, amoxicillin, cephalexin and lincomycin not have any antimicrobial effect (100% of the total isolates were resistant). In addition, the highest resistance rate was recorded to cefotaxime (93%), tetracyclin (74%), trimethoprim and oxytetracyclin (67%), ceftriaxone (63%), nalidixic acid (56%) and high resistance also investigated for streptomycin (53%). on the other hand low resistance indicated to gentamicin (21%), ciprofloxacin (26%), norfloxacin (31%) and chloramphenicol (35%) while resistance to amikacin was less common and seen in only 14% of the isolates (table 1). Compared resistance antibiotic of integrons positive *E. coli* isolates with integrons- negative *E. coli* isolates in table 1 reflect that resistance to cefotaxime, ciprofloxacin, oxytetracyclin, tetracycline and trimethoprim are significantly associated with *IntI1* gene positive isolates (*P*= 0.0001).

**Table (1): association between antibiotic resistance and integron class 1**

| Antibiotic agents | Total resistance N (%) | *IntI*1 positive isolates (N=112) | $X^2$ | OR | *P* value |
|---|---|---|---|---|---|
| | | R N (%) | | | |
| **Pencillines** | | | | | |
| **Ampicillin** | **301(100)** | **112(100)** | **0.075** | **1.44** | **0.61** |
| **Amoxcillin** | **301(100)** | **112(100)** | **0.075** | **1.44** | **0.61** |
| **Cephalosporin** | | | | | |
| **Cefotaxime** | **280(93)** | **112(100)** | **33.14** | **4.44** | **0.0001** |

| | | | | | |
|---|---|---|---|---|---|
| **Ceftriaxone** | **188(63)** | **77(69)** | **2.60** | **1.02** | **0.111** |
| **Cephalexin** | **301(100)** | **112(100)** | **0.075** | **1.44** | **0.61** |
| **Quinolones** | | | | | |
| **Nalidixic acid** | **168(56)** | **69(62)** | **1.77** | **1.36** | **0.076** |
| **Norfloxacin** | **92(31)** | **35(31)** | **0.033** | **1.17** | **0.822** |
| **Ciprofloxacin** | **77(26)** | **21(19)** | **17.40** | **4.45** | **0.0001** |
| **Aminoglycsides** | | | | | |
| **Amikacin** | **42(14)** | **17(15)** | **0.302** | **1.39** | **0.333** |
| **Gentamicin** | **64(21)** | **26(23)** | **0.109** | **1.25** | **0.544** |
| **Streptomycin** | **161(53)** | **63(56)** | **0.331** | **1.26** | **0.491** |
| **Tetracyclines** | | | | | |
| **Oxytetracyclin** | **201(67)** | **95(85)** | **60.32** | **7.99** | **0.0001** |
| **Tetracyclin** | **223(74)** | **99(88)** | **43.66** | **6.87** | **0.0001** |
| **Phenicol** | | | | | |
| **Chloramphenicol** | **105(35)** | **44(39)** | **2.998** | **1.93** | **0.1310** |
| **Anti -Folate** | | | | | |
| **Trimethoprim** | **202(67)** | **86(77)** | **17.77** | **2.35** | **0.0001** |
| **lincosamid** | | | | | |
| **Lincomycin** | **301(100)** | **112(100)** | **0.075** | **1.44** | **0.61** |
| **Pearson Correlation Coefficient ( r )** | **0.9766** | | | | |

**N = number, R= resistance**

**Association Between Multiple Antibiotic Resistance And Integron Class 1among Commonsal *Escherichia coli***

In present study, all integrons positive isolates are resist to more than five antibiotics (table 2). Among *IntI1* gene positive isolates resist to 6 (9%), 8 (7%), 9 (8%), 10 (16%), 11 (24%), 12 (12%), 13 (12%), 14(9%) and 15 (4%) antibiotics are observed but significantly association ($p < 0.05$) demonstrated to resistance of 10, 11 and 12 antibiotics only.

**Table (2): Comparsion multiple antibiotic resistance between integron positive and integron negative isolates**

| Number of antibiotics | *IntI1* Positive isolates (N=112) | *IntI1* negative isolates (N=189) | P value |
|---|---|---|---|
| | R N(%) | R N(%) | |
| **4** | **0(0)** | **17(9)** | **NS** |
| **5** | **0(0)** | **11(6)** | **NS** |
| **6** | **10(9)** | **44(23)** | **NS** |
| **8** | **8(7)** | **23(12)** | **NS** |
| **9** | **9(8)** | **34(18)** | **NS** |
| **10** | **18(16)** | **0(0)** | **< 0.05** |
| **11** | **27(24)** | **11(6)** | **< 0.05** |
| **12** | **13(12)** | **11(6)** | **< 0.05** |
| **13** | **13(12)** | **23(12)** | **NS** |
| **14** | **10(9)** | **11(6)** | **NS** |
| **15** | **4(4)** | **4(2)** | **NS** |

R= Resist; N = Number; NS= Non-Significant ($P > 0.05$)

nmon
class1
rns in
-C-L-
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CIP-T-C-CRO-S-TE-NOR-TMP (P = 0.077) and AX-CTX-T-C-CRO-S-TE-CL-TMP-AM-L (P= 0.076).

**Table (3): Distribution of MDR among *IntI1* positive isolates**

**Discussion**

Present results show increase dissemination of integrons (37%) among commonsal *E. coli* isolates this may be because of the widespread of horizontal transfer of mobile elements

| Resistance patterns | Integron class 1-positive isolates (N=112) | | Integron class 1-negative isolates (N=189) | | OR | $X^2$ | P value |
|---|---|---|---|---|---|---|---|
| | R | | R | | | | |
| | N | % | N | % | | | |
| AM-AX-L-CL | 112 | 100 | 189 | 100 | 0.0812 | 1.99 | 0.655 |
| AM-AX-L-CL-CTX | 112 | 100 | 157 | 83 | 42.014 | 34.99 | < 0.0001 |
| AM-AX-L-CL-CTX-TE | 99 | 88 | 100 | 53 | 6.22 | 43.811 | < 0.0001 |
| AM-AX-L-CL-CTX-TE-NA | 62 | 55 | 55 | 29 | 3.112 | 19.61 | < 0.0001 |
| AM-AX-TE-T | 95 | 85 | 55 | 29 | 30.61 | 90.16 | < 0.0001 |
| NOR-CIP-NA | 17 | 15 | 45 | 24 | 0.931 | 4.95 | 0.041 |
| C-TMP-L | 39 | 35 | 34 | 18 | 2.653 | 9.191 | 0.006 |
| CTX-CRO-CL | 73 | 65 | 89 | 47 | 3.304 | 12.67 | 0.0021 |
| NOR-CIP-NA-CTX-CRO-CL | 17 | 15 | 23 | 12 | 12.33 | 15.73 | 0.00032 |
| AX-NA-CTX-T-CL-TMP-AM-L | 56 | 50 | 55 | 29 | 1.19 | 14.20 | 0.0001 |
| AX-CTX-T-TE-S-CL-TMP-AM-L | 39 | 35 | 45 | 24 | 1.39 | 4.009 | 0.043 |
| CRO-TE-CL-TMP | 60 | 54 | 55 | 29 | 2.997 | 19.91 | < 0.0001 |
| AX-AM-CTX-CL-TE-T-CIP-NOR-AN-TMP-C-L-AK-CN-CRO | 5 | 4 | 11 | 6 | 0.711 | 0.686 | 0.103 |
| AX-CTX-T-C-CRO-S-TE-CL-TMP-NA-L | 13 | 12 | 11 | 6 | 6.05 | 3.44 | 0.076 |
| AX-CTX-T-CRO-TE-CL-TMP-NA-L | 43 | 38 | 34 | 18 | 2.11 | 11.60 | 0.0004 |
| AX-CTX-T-CRO-TE-CL-TMP-L | 60 | 54 | 34 | 18 | 5.26 | 39.17 | < 0.0001 |
| NA-CTX-CIP-T-C-CRO-S-TE-NOR-TMP | 5 | 4 | 11 | 6 | 0.607 | 3.44 | 0.077 |
| NA-CTX-T-CRO-S-TE-TMP | 26 | 23 | 34 | 18 | 1.35 | 1.033 | 0.0441 |
| CTX-T-CRO-TE-TMP | 60 | 54 | 23 | 12 | 8.94 | 54.03 | < 0.0001 |
| T-TE-TMP | 82 | 73 | 55 | 29 | 7.31 | 56.88 | < 0.0001 |
| AX-CTX-T-CRO-TE-CL-AM-L | 69 | 62 | 34 | 18 | 7.23 | 55.01 | < 0.0001 |
| AX-CTX-T-C-CRO-S-TE-CL-TMP-AM-L | 13 | 12 | 11 | 6 | 6.05 | 3.44 | 0.076 |
| AX-CTX-T-CRO-S-TE-CL-TMP-AM-L | 39 | 35 | 34 | 18 | 2.38 | 8.99 | 0.0026 |

***df* = 1 for each resistance pattern**

***Abbreviations: Number (N), df (degree of freedom), Ampicillin (AM), Amoxicillin (AX), Amikacin (AK), Gentamicin (CN), Streptomycin (S), Trimethoprim (TMP), Lincomycin (L), Norfloxacin (NOR), Ciprofloxacin (CIP), Nalidixic Acid (NA), Chloramphenicol (C), Cefotaxime (CTX), Ceftriaxone (CRO), cephalexin (CL), Tetracyclin (TE) and oxytetracyclin (T).**

such as plasmids and transposones that associated with integons between commensal *E. coli* isolates and *Enterobacteriaceae* **.** In the same line, Lee *et al*. (2006) suggest that class 1 integrons harbored by commensal *E. coli* isolates may be acquired from other pathogenic or commensal bacteria by horizontal transfer of R-plasmids carrying class 1 integrons and that agreement with Sunde (2005), that found rife integrons could be a part of successful plasmids or transposons with a wide, perhaps global dissemination **(1,10).** In agreement with other studies , this study noted that class 1 integrons were common in *E. coli* isolates **(11,12,13,15)**. In additional, present study and many other studies demonstrated high resistance of commonsal *E. coli* to antimicrobials that commonly used for chemotherapy as amoxicillin, ampicillin, cephalexin, cefotaxime and tetracyclines **(2,28).** Current study similar to study in Mexico that showed resistance to Ampicillin is100% of commonsal *E. coli* also like with studies of Marshall *et al*., (2011) and Li *et al.*, (2014) who showed high resistances of gut flora to streptomycin, nalidixic acid, tetracycline and amoxicillin that may be due to the extensive and long-term use of these antibiotics in humans and livestock **(3,9)**. In additional most recent researches suggest increase and spread antibiotics resistance related to horizontal transfer of resistance genes such as integrons among bacterial populations. Transport resistance genes from commonsal *E. coli* to pathogenic bacteria represents a potential risk to public health **(28,29).** Marshall *et al*., (2009) elucidating that transfer of antibiotic resistance genes from commensals to pathogens depends on the density of donor and recipient cells, the availability of a transfer mechanism, nutrition, and selective pressures and regarded the intestinal environment is considered optimal **(28).** Low resistance for aminoglycosides (amikacin and gentamicin) among commonsal *E. coli* isolates recorded in this study agreement with results of Phongpaichit and his coworker (2008) but this results unlike results of Yang *et al.*, (2009) in Taiwan that showed high resistance rates in fecal strains of *E. coli* were observed for streptomycin (52%), ampicillin (50.2%), trimethoprim (47.6%) and chloramphenicol (33.8%) **(2,18).** However the various percentages of resistance in different parts of the world are due to differences in the prevalence of antibiotic consumption in each country **(32).**

Higher percentage of resistance to some antimicrobial agents (aminoglycoside, cephalosporines, quinolones, and beta-lactam agents) were observed among integron-positive strains with respect to integron-negative strains. The fact could be explained by the presence of resistance genes of these antibiotics in the conserved or variable region of integrons, or by the inclusion of resistance genes in the same mobile elements that carry integrons **(17,3).**Significant correlated between *IntI1* gene positive isolates and resistance to cefotaxime, tetracycline and trimethoprim ($P < 0.05$) also determent in results of Li and his coworkers in china that also detected clear association between *IntI1* gene and resistance to nalidixic acid and ampicillin/sulbactam **(3).** Other study conducted in Taiwan has shown that fecal *E. coli* isolates have high levels antibiotic resistance gene cassette containing class 1 integron **(18).**

All isolates in present study are multiple drug resistance isolates and are resist to at least four antibiotics but integron positive isolates are resist to at least six antibiotics this may be related to present many resistance genes associated with integrons or may be due to present more than one integron in the same isolate and each one carry out different resistance genes for different antibiotics. Current results agreement with study of Phongpaichit *et al*., (2008) who found multiple drug resistance was more frequent in integron-positive isolates (89%) than those in integron-negative *E. coli* (57%) **(2).** Moreover study of Dureja and his coworker (2014) showed the class 1 integron positive isolates contain eight different resistance gene cassettes in five different combinations, namely dfrA12-

orfF-aadA2, dfrA1- aadA1, dfrA17-aadA5, dfrA5 and dfrA7 and this similar to those observed earlier by Karczmarczyk *et al* (2011) that remember class 1 and class 2 integrons in the collection were found to contain trimethoprim (*dfr*) and streptomycin (*aad*) resistance-encoding genes, which are frequently reported in *E. coli* isolates recovered from various sources, including human, animal, and environmental samples **(15,20).** The presence of more than one gene cassettes in most integrons positive samples support the literature suggesting that since 1990 there is a prevalence of class 1 integrons carrying multiple resistant gene cassettes **(15,21).**These data indicate that human fecal *E. coli* is a reservoir of antibiotic-resistant genes that poses a significant risk of the spread of microbial resistance in the community. In this study *IntI1* gene positive isolates significantly associated with most detected resistance patterns this may be due to high frequency of integron class 1 that integrated with resistance gene cassettes for multiple antibiotics resistance also class 1 integrons, the 5´ conserved region encodes a site-specific recombinase (integrase, *intI1*) and a strong promoter or promoters that ensure expression of the integrated resistance gene cassettes for multiple antibiotics in the same *IntI* positive isolates **(2).** This high frequency of multidrug resistance among *intI1*-positive isolates supports the hypothesis of an association between the presence of class 1 integrons and emerging multidrug resistance in *E. coli* **(22).**

finally, numerous studies have shown that integrons play a considerable role in the dissemination of MDR in clinical isolates and environmental samples. Fewer studies have aimed at investigating the role of integrons in the propagation and maintenance of MDR phenotypes in non-clinical commensal isolates from humans. There are probably at least two reasons for the lack of studies investigating integrons in commensal isolates from healthy people. One reason may be due to the lack of availability of samples. While bacteria are routinely obtained from hospitalized patients in order to identify and treat infections, healthy people may be far less likely to voluntarily submit to a rectal swab or fecal sample. Perhaps a second reason is that studying bacteria isolated from clinical samples and from individuals with infections can easily seem like a more pressing matter, and seem more vital to understanding the problem of the rapid spread of multidrug-resistance**( 23).**

## Conclusion

High prevalence of integron class 1 and antibiotic resistance that produced significant correlation between multiple drug resistance and integron class 1 among *E. coli* flora that may be act as reservoir of antibiotic-resistant genes that has a significant risk for spread of antibiotic resistance to pathogenic or commonsal bacteria in the community.

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