ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

# Class 1 Integrons, Genetic Factor for the Dissemination of Tetracycline and Chloramphenicol Resistance Genes in *Escherichia coli* isolated from Children with Diarrhea in Rural Burkina Faso

**René Dembélé[1,2]*, Ali Konaté[1], Assèta Kagambèga[1,3], Issiaka Soulama[4], Wendpoulomdé AD Kaboré[5], Oumar Traoré[1,2], Alfred S Traoré[1], Awa Aidara-Kane[6], Amy Gassama Sow[7] and Nicolas Barro[1]**

[1]*Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food (LaBESTA)/Center for Research in Biological, Food and Nutritional Sciences (CRSBAN)/Graduate School of Science and Technology (EDST)/University of Ouaga I, Professor Joseph KI-ZERBO, Burkina Faso*

[2]*Training and Research Unit in Applied Sciences and Technologies (TRU/AST)/University of Dédougou, Burkina Faso*

[3]*Institute of Sciences, Ouagadougou 01, Burkina Faso*

[4]*National Centre for Research and Training on Malaria (NCRTM), Ouagadougou 01, Burkina Faso*

[5]*Training and Research Unit in Health Sciences (TRU/HS)/University of Ouaga I, Professor Joseph KI-ZERBO, Ouagadougou 03, Burkina Faso*

[6]*World Health Organization, WHO-AGISAR, Department of Food Safety and Zoonoses, Switzerland*

[7]*Unit of Experimental Bacteriology, Pasteur Institute of Dakar, Dakar, Senegal*

***Corresponding Author:** Rene Dembele, Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food (LaBESTA), University of Dedougou, Dedougou, Burkina Faso.

**Received:** April 10, 2019; **Published:** May 28, 2019

## Abstract

Antimicrobial resistance has been declared a global threat to public health, as a massive increase in this problem has been observed worldwide. This study aims to investigate the role of class I integrons (*Int1*) in the dissemination of tetracycline and chloramphenicol resistance genes in *Escherichia coli* in rural Burkina Faso. A study was conducted to screen for *E. coli* species from 275 stool samples collected from under five age children suffering from diarrhea, in rural area of Burkina Faso. The antimicrobial resistance determinants were investigated by Polymerase Chain Reaction, checking the presence of class 1, 2, 3 integrons, *tet* and *cat*A1 resistance genes. Class 1 integrons were reported in all the five (5/5) *E. coli* isolates. One tetracycline resistant ($Tet^r$) strain (20%) possessed the *tet* gene and two strains harbored the *cat*A1 gene. There was a coexistence between the *tet* and *cat*A1 genes and class 1 integrons in one atypical EPEC. Class 1 integrons have been playing an important role in the development of multidrug resistance in *E. coli* pathovars. A better understanding of the molecular mechanisms by which antimicrobial resistance emerges and spreads should enable us to design intervention strategies to reduce its progression.

***Keywords:*** *Antibiotic Resistance Genes; Class 1 Integrons; E. coli; Diarrhea; Children*

## Abbreviations

AR: Antibiotic Resistance; CAT: Chloramphenicol Acetyl Transferase; CMA: Centre Médical avec Antenne Chirugicale; MDR: Multidrug Resistant

## Introduction

Antibiotic resistance (AR) continues to pose a great threat to public health in both developed and developing countries [1]. In many parts of the world, the emergence of multidrug-resistant Gram-negative bacteria is a major concern in hospital settings. Infections caused by these pathogens have become significantly challenging over the past two decades, particularly in the developing countries,

and are associated with high morbidity and mortality rates as well as protracted hospital stay [2]. *Enterobacteriaceae* including *Klebsiella pneumoniae*, *Escherichia coli* as well as *Enterobacter* spp. and non-lactose fermenting bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been identified as major cause of multi-drug resistant bacterial infections [3]. Diarrheagenic *Escherichia coli* is a ubiquitous presence in the developing world and is responsible for a range of enteric infections [4]. Antimicrobial resistance occurs through various mechanisms, such as drug efflux pumps [5]. In low-resource countries, the extent and the impact of the phenomenon tend to be even larger than in industrialized countries. In fact, high resistance rates have often been reported in surveillance studies dealing with clinical isolates [6,7] and in prevalence studies of commensal bacteria taken as indicators to estimate spread of acquired resistance [8,9]. Tetracyclines have been used extensively since the late 1940s as broad-spectrum inexpensive antibiotics that are effective against a wide variety of diseases in humans, animals, and plants. However, resistance to tetracycline has increased dramatically since the first appearance of resistance in 1953 in *Shigella dysenteriae* [10]. The transmissibility of resistance in bacterial populations can take place by either clonal spread of particular strains or horizontal transfer of resistance determinants by plasmid- or transposon-mediated conjugation [11]. Most tetracycline resistance determinants, defined as genetic units which contain both structural and regulatory genes involved in resistance [12], have been found on resistance plasmids or transposons, making gene transfer the likely method of acquiring resistance.

One of the best-characterized bacterial antibiotic resistance mechanisms is the synthesis of chloramphenicol acetyl transferase (CAT) [13]. Production of this enzyme, encoded by the cat gene, is the most common means by which bacteria become resistant to chloramphenicol [14], a small bacteriostatic antibiotic that interacts with a peptidyl transferase center [15]. Dissemination of antibiotic resistance genes by horizontal transfer has led to the rapid emergence of antibiotic resistance among clinical isolates of bacteria [16]. In this study, we screened the *tet* and *cat*A1-producing *E. coli* to elucidate the dissemination mechanism. In addition, to the best of our knowledge, this report is the first to investigate the role of class I integrons (*Int1*) in the dissemination of tetracycline and chloramphenicol resistance genes in *E. coli* in rural Burkina Faso.

## Materials and Methods

### *E. coli isolates*

Sampling was done in two "Centre Médical avec Antenne Chirugicale (CMA)" in rural area of Burkina Faso (Boromo and Gourcy: figure 1).

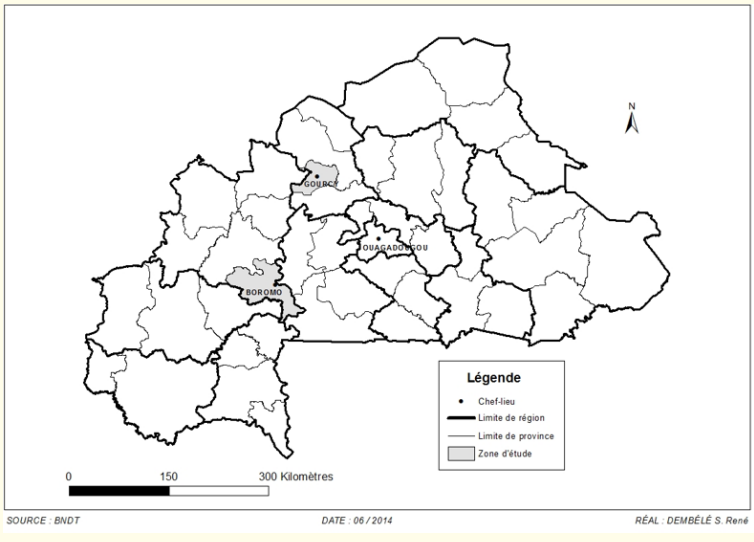


***Figure 1:*** *Map of Burkina Faso. In dark = Gourcy and Boromo where the study was conducted.*

Trained healthcare personnel using sterile stool containers collected stool samples. All the samples were immediately iced and brought to the laboratory for further analysis. Bacterial isolates were identified according to the standard microbiological procedures [17]. The 16-plex PCR was used to detect simultaneously 16 genes from the five main pathogroups of *E. coli* (EHEC, EPEC, EAEC, EIEC, and ETEC) as described by Antikainen., *et al* [18]. The genes investigated and primers used are listed in table 1.

| Pathotype | Target gene | Primer sequence (5' to 3') | Size (bp) | [C] (µM) | Ref. |
|---|---|---|---|---|---|
| Typical EPEC | *bfpB* | MP3-bfpB-F: GACACCTCATTGCTGAAGTCG | 910 | 0.1 | [19] |
| | | MP3-bfpB-R: CCAGAACACCTCCGTTATGC | | 0.1 | |
| EHEC and EPEC | *eaeA* | eae-F: TCAATGCAGTTCCGTTATCAGTT | 482 | 0.1 | [19] |
| | | eae-R: GTAAAGTCCGTTACCCCAACCTG | | 0.1 | |
| | *escV* | MP3-escV-F: ATTCTGGCTCTCTTCTTCTTTATGGCTG | 544 | 0.4 | [19] |
| | | MP3-escV-R: CGTCCCCTTTTACAAACTTCATCGC | | 0.4 | |
| | *Ent* | ent-F: TGGGCTAAAAGAAGACACACTG | 629 | 0.4 | [19] |
| | | ent-R: CAAGCATCCTGATTATCTCACC | | 0.4 | |
| EHEC | EHEC-*hly* | hlyEHEC-F: TTCTGGGAAACAGTGACGCACATA | 688 | 0.1 | [18] |
| | | hlyEHEC-R: TCACCGATCTTCTCATCCCAATG | | 0.1 | |
| | *Stx1* | MP4-stx1A-F: CGATGTTACGGTTTGTTACTGTGACAGC | 244 | 0.2 | [19] |
| | | MP4-stx1A-R: AATGCCACGCTTCCCAGAATTG | | 0.2 | |
| | *Stx2* | MP3-stx2AF: GTTTTGACCATCTTCGTCTGATTATTGAG | 324 | 0.4 | [19] |
| | | MP3-stx2A-R: AGCGTAAGGCTTCTGCTGTGAC | | 0.4 | |
| EAEC | *astA* | MP-astA-F TGCCATCAACACAGTATATCCG | 102 | 0.4 | [19] |
| | | MP2-astA-R ACGGCTTTGTAGTCCTTCCAT | | 0.4 | |
| | *aggR* | MP2-aggR-F: ACGCAGAGTTGCCTGATAAAG | 400 | 0.2 | [19] |
| | | MP2-aggR-R:AATACAGAATCGTCAGCATCAGC | | 0.2 | |
| | *Pic* | MP2-pic-F: AGCCGTTTCCGCAGAAGCC | 1111 | 0.2 | [19] |
| | | MP2-pic-R: AAATGTCAGTGAACCGACGATTGG | | 0.2 | |
| EIEC | *invE* | MP2-invE-F: CGATAGATGGCGAGAAATTATATCCCG | 766 | 0.2 | [19] |
| | | MP2-invE-R: CGATCAAGAATCCCTAACAGAAGAATCAC | | 0.2 | |
| | *ipaH* | ipaH-F: GAAAACCCTCCTGGTCCATCAGG | 437 | 0.1 | [20] |
| | | ipaH-R: GCCGGTCAGCCACCCTCTGAGAGTAC | | 0.1 | |
| ETEC | *elt* | MP2-LT-F: GAACAGGAGGTTTCTGCGTTAGGTG | 655 | 0.1 | [19] |
| | | MP2-LT-R: CTTTCAATGGCTTTTTTTTGGGAGTC | | 0.1 | |
| | *estA* | MP4-STIa-F : CCTCTTTTAGYCAGACARCTGAATCASTTG | 157 | 0.4 | [19] |
| | | MP4-STIa-R: CAGGCAGGATTACAACAAAGTTCACAG | | 0.4 | |
| | *estB* | MP2-STI-F: TGTCTTTTTCACCTTTCGCTC | 171 | 0.2 | [19] |
| | | MP2-STI-R CGGTACAAGCAGGATTACAACAC | | 0.2 | |
| *E. coli* | *uidA* | MP2-uidA-F: ATGCCAGTCCAGCGTTTTTGC | 1487 | 0.2 | [20] |
| | | MP2-uidA-R: AAAGTGTGGGTCAATAATCAGGAAGTG | | 0.2 | |

***Table 1:*** *Oligonucleotides primers used for multiplex PCR reaction.*

***Legend****: EAEC: Enteroaggregative E. coli; EPEC: Enteropathogenic E. coli; EIEC: Enteroinvasive E. coli; EHEC: Enterohemorrhagic E. coli; ETEC: Enteroenterotoxigenic E. coli;* µM*: Micromolaire; [C]: Concentration; pb: "Paire de Base"; Ref.: Reference.*

### Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed using the disk diffusion method on Mueller-Hinton agar as described by the European Committee on Antimicrobial Susceptibility Testing [21]. The following antimicrobials (belonging to 7 different families) were used: amoxicillin (25 µg), amoxicillin-clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefixime (10 µg), piperacillin (75 µg), piperacillin-tazobactam (100 +10 µg), imipenem (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25 ± 23.75 µg), aztreonam (30 µg), colistin sulfate (50 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamycin (15 µg), netilmicin (10 µg), and tobramycin (10 µg) (Bio-Rad, France). EUCAST guidelines were used to interpret results.

### Antimicrobial resistance genes detection

To detect the molecular determinants of resistance, all the multidrug-resistant isolates of *E. coli* were considered and PCR was carried out with specific primers for resistance genes including chloramphenicol (*cat*A1) [22], tetracycline (*tet*) [23] and for integrons (*intI1*, *intI2*, *intI3*) [24] as shown in table 2. Two point five (2.5) µl of supernatant were added to 22.5 µl reaction mixture containing 5U of Taq DNA polymerase (Accu Power, Korea), deoxyribonucleic triphosphate (10 mM), buffer GC (10X), $MgCl_2$ (25 mM), and PCR primers (10 µM). The PCR conditions were as follows: 5 minutes at +94°C, followed by 35 amplification cycles of +94°C for 30s, 59±4 °C for 60s and +72°C for 60s with a final extension of +72°C for 10 minutes on a thermal cycler (Gene Amp 9700, Applied Biosystems). The reaction products were separated by electrophoresis in (1.5% weight/volume) agarose gel, stained with a Redsafe solution (Prolabo, France) and visualized under ultraviolet (UV) light (Gel Logic 200).

| Genetic resistance supports | Genes | Primers sequence (5'to3') | Size (bp) | References |
|---|---|---|---|---|
| Chloramphenicol | *cat*A1 | F : CGC CTG ATG AAT GCT CAT CCG | 456 | [22] |
| | | R : CCT GCC ACT CAT CGC AGT AC | | |
| Tetracycline | *tet* | F : GCA GGC AGA GCA AGT AGA GG | 956 | [23] |
| | | R : GTT TCG GGT TCG GGA TGG TC | | |
| Integrons | *Int1* | F: ATT TCT GTC CTG GCT GGC GA | 600 | [24] |
| | | R: ACA TGT GAT GGC GAC GCA CGA | | |
| | *Int2* | F : CAC GGA TAT GCG ACA AAA AGG T | 806 | |
| | | R : GTA GCA AAC GAC TGA CGA AAT G | | |
| | *Int3* | F: GCC CCG GCA GCG ACT TTC AG | 600 | |
| | | R: ACG GCT CTG CCA AAC CTG ACT | | |

***Table 2:*** *Oligonucleotides primers used for PCR reaction.*

### Ethical considerations

The National Ethical Committee (s) for Health Research in Burkina Faso (N ° 2009-39) approved the study protocol. Informed verbal consent was also obtained from the parents/guardians of every child before sample collection.

## Results and Discussion

### Global prevalence of antibiotic resistance in *E. coli* isolates

Five (5) isolates were confirmed positive for diarrheagenic *E. coli* by 16-plex PCR: three (3) enteroaggregative *E. coli* (EAEC) and two (2) atypical enteropathogenic *E. coli* (EPEC). All of the five isolates (100%) were multidrug resistant (resistant to at least three different classes of antimicrobials) *E. coli* and harbored resistance patterns to tetracycline, amoxicillin-clavulanic acid and amoxicillin. Eighty

percent (80%) of our isolates were resistant to colistin-sulfate, trimethoprim-sulfamethoxazole and piperacillin. Resistance to nalidixic acid, cefotaxime, aztreonam, ceftriaxone, cefepime, piperacillin-Tazobactam and cefixime was detected in three isolates (60%). Two (2) *E. coli* strains showed resistance to imipenem and chloramphenicol.

### Resistance genes and integrons in *E. coli* isolates

One atypical EPEC (043B) that showed resistance to tetracycline and chloramphenicol was simultaneously positive for the presence of *tet* and *cat*A1 genes by PCR. Another atypical EPEC (046B) harbored *cat*A1 gene. Class 1 integrons were reported in all the five *E. coli* strains (Table 3). Class 2 and 3 integrons were not detected in this study.

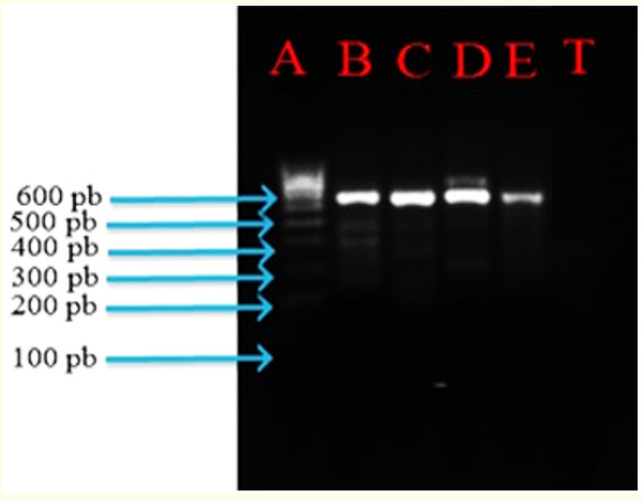


***Figure 2:*** *PCR-amplified class 1 integrons*

*A = Molecular Weight Marker (100 bp), B = Int1 positive control (600pb), C-E = Positive Samples (E. coli) to Int1 detection, T = Negative Control.*

### Coexistence of resistance genes and integrons in the same *E. coli* isolates

Our results showed that *cat*A1 gene and class 1 integrons were simultaneously harbored by one atypical EPEC. Similarly, there was a coexistence between the *tet* and *cat*A1 genes and class 1 integrons in one atypical EPEC (Table 3).

| Codes | Pathovars | Antibiotic-resistance phenotype | Resistance genes | Integrons |
|---|---|---|---|---|
| 025B | EAEC | AMC, AMX, CTX, ATM, CRO, FEP, CFM, TET, SXT, NAL, CST, TZP, PIP | - | *Int1* |
| 039B | EAEC | AMC, AMX, TET, SXT, CST, PIP | - | *Int1* |
| 043B | Atypical EPEC | AMC, AMX, CTX, ATM, IPM, CRO, FEP, CFM, TET, CHL, CST, TZP, PIP, GMI, TMN | *cat*A1, *tet* | *Int1* |
| 044B | EAEC | AMC, AMX, CTX, ATM, IPM, CRO, FEP, CFM, TET, CHL, SXT, NAL, CST, TZP | - | *Int1* |
| 046B | Atypical EPEC | AMC, AMX, TET, SXT, NAL, PIP | *cat*A1 | *Int1* |

***Table 3:*** *Antibiotic resistance phenotypes and genes detected in E. coli isolates from clinical samples*

***Legend :*** *EAEC: Eneteroaggregative E. coli; EPEC: Enteropathogenic E. coli; AMC: Amoxicillin-Clavulanic Acid; AMX: Amoxicillin; CTX: Cefotaxime; ATM: Aztreonam; IPM: Imipenem; CRO: Ceftriaxone; FEP: Cefepime; CFM: Cefixime; TET: Tetracycline; CHL: Chloramphenicol; SXT: Trimethoprim-sulfamethoxazole; NAL: Nalidixic Acid; CST: Colistin sulfate; TZP: Piperacillin-Tazobactam; GMI: Gentamicin; PIP: Piperacillin; TMN: Tobramycin.*

## Discussion

In this study, all isolated strains were resistant to tetracycline while 40% were resistant to chloramphenicol which results are similar to data reported by Wu., *et al.* [25] who found 100% resistant to tetracycline and 20% to chloramphenicol. In addition, all the study strains were multidrug resistant (MDR). Studies showed that MDR Gram-negative bacterial pathogens are a global public health threat [26]. This is the first study to investigate the prevalence and dissemination of *tet* and *cat*A1 genes in *E. coli* strains isolated from children in Burkina Faso. The pattern of antibiotic resistance detected in our isolates is in line with the resistance encountered in different strains of *E. coli* isolated from various clinical sources worldwide [27]. In the last few years, it has been shown that *E. coli* is exhibiting resistance to more antibiotic classes, hence rendering these drugs ineffective in treating its infections [28].

In the present study, one tetracycline resistant ($Tet^r$) strain (20%) possessed the *tet* gene. A study by Lanz., *et al.* [29] showed that the *tet*(A) gene alone was the most prevalent *tet* gene among *E. coli* isolates from pigs with diarrhea or enterotoxemia. The presence of *Int1* was reported in 100% of the study isolates that is higher than 24% of class 1 integrons reported among clinical strains [30]. Integrons are mobile genetic elements thought to play an important role in the dissemination and accumulation of resistance genes in bacteria [31]. The presence of an integron is strongly associated with antimicrobial resistance. Thus, class 1 integrons have been associated with tetracycline resistance genes [32] and have been shown to be selected and maintained by antimicrobial pressure [30]. In addition to their fungible gene cassettes, which often confer resistance to antimicrobial agents, all class 1 integrons contain an independent *sulI* gene encoding sulfonamide resistance. Because the integron system has the ability to create novel combinations of resistance genes, it may be a dynamic force in the evolution of multidrug-resistant (MDR) bacteria [33].

In this study, one atypical EPEC that showed resistance to chloramphenicol was positive for the presence of *cat*A1 gene by PCR. However, one strain (atypical EPEC) which were chloramphenicol sensitive also possessed the *cat*A1 gene as confirmed by PCR. Thus, antibiotic resistance genes *cat*A1 were present in 40% of the study strains. Our founding is similar to data reported by Deekshit., *et al.* 2011 who found the *cat*A1 gene in seafood-associated *Salmonella* Weltevreden (both in a resistant strain and in a susceptible strain) with a *cat*A1 rate equal to 57.52% [34]. Chloramphenicol resistance can due to degradation of the antibiotic by either chloramphenicol acetyl transferase or chloramphenicol efflux mechanism [34]. The *cat*A gene has been reported to be located upon plasmid [34,35]. The gene *cat*A1 reported in the study conducted by Deekshit., *et al.* [34] was found to identical to that reported from *E. coli* (FN554766) and other *Salmonella* serovars. Concerning the strain susceptible to chloramphenicol in which *cat*A1 gene was detected, it is possible that the gene was not expressed in this case. Indeed, Deekshit., *et al.* showed that the reason for the unexpressive characteristic of *cat*A1 gene of *Salmonella* Weltevreden in their report was the deletion of the promoter region which has been confirmed by PCR using cat F2 / R2 primers [34]. Silencing of antibiotic-resistant genes in environments, where the gene product does not confer any selective advantage, may be a phenomenon that has not received much attention and therefore should be adressed in perspectives.

## Conclusion

This study highlights that class 1 integrons have been playing an important role in the development of multidrug resistance in diarrheagenic *E. coli* pathovars. Further research examining integron persistence over time and determining whether class 1 integrons are located in conjugative plasmids to assess the efficiency of integron transfer would improve our understanding of how integron may move among strains. Indeed, a better understanding of the molecular mechanisms by which antimicrobial resistance emerges and spreads should enable us to design intervention strategies to reduce its progression.

## Bibliography

1. WHO. "Global action plan on antimicrobial Resistance" (2015).

2. Singh N and Manchanda V. "Control of multidrug-resistant gram-negative bacteria in low-and middle-income countries-high impact interventions without much resources". *Clinical Microbiology and Infection* 23.4 (2017): 216-218.

3. Oduro-Mensah D., *et al*. "Genetic characterization of TEM-type ESBL associated antibacterial resistance in *Enterobacteriaceae* in a tertiary hospital in Ghana". *Annals of Clinical Microbiology and Antimicrobials* 15 (2016): 29-38.

4. Vila J., *et al*. "*Escherichia coli*: an old friend with new tidings". *FEMS Microbiology Reviews* 40.4 (2016): 437-463.

5. Adewoye L., *et al*. "Antimicrobial drug efflux genes and pumps in bacteria of animal and environmental origin". In: Li XZ; Elkins C; Zgurskaya H. editors. Efflux-mediated antimicrobial resistance in bacteria. Adis, Cham: Springer International Publishing (2016): 561-593.

6. Byarugaba DK. "A view on antimicrobial resistance in developing countries and responsible risk factors". *International Journal Antimicrobial Agents* 24.2 (2004): 105-110.

7. Shears P. "Antibiotic resistance in the tropics. Epidemiology and surveillance of antimicrobial resistance in the tropics". *Transactions of The Royal Society of Tropical Medicine and Hygiene* 95.2 (2001): 127-130.

8. Bartoloni A., *et al*. "High prevalence of acquired antimicrobial resistance unrelated to heavy antimicrobial consumption". *The Journal of Infectious Diseases* 189.7 (2004): 1291-1294.

9. Nys S., *et al*. "Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries". *Journal of Antimicrobial Chemotherapy* 54.5 (2004): 952-955.

10. Chopra I and Roberts M. "Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance". *Microbiology and Molecular Biology Reviews* 65.2 (2001): 232-260.

11. Amabile-Cuevas CF and Chicurel ME. "Bacterial plasmids and gene flux". *Cell* 70.2 (1992): 189-199.

12. Levy SB., *et al*. "Nomenclature for tetracycline resistance determinants". *Antimicrobial Agents Chemotherapy* 33.8 (1989): 1373-1374.

13. Zgurskaya HI and Nikaido H. "Multidrug resistance mechanisms: drug efflux across two membranes". *Molecular Microbiology* 37.2 (2000): 219-225.

14. Zaidenzaig Y., *et al*. "Characterization and comparison of chloramphenicol acetyltransferase variants". *European Journal of Biochemistry* 100.2 (1979): 609-618.

15. Vester B and Garret RA. "The importance of highly conserved nucleotides in the binding region of chloramphenicol at the peptidyl transfer center of *Escherichia coli* 23S ribosomal RNA". *EMBO Journal* 7.11 (1988): 3577-3587.

16. Ploy MC., *et al*. "Integrons: an antibiotic resistance gene capture and expression system". *Clinical Chemistry and Laboratory Medicine* 38.6 (2000): 483-487.

17. Gillespie SH and Hawkey PM. "Principles and practice of clinical Bacteriology". 2nd edition. Chischester: John Wiley & Sons, England (2006): 367-377.

18. Antikainen J., *et al*. "New 16 plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples". *European Journal of Clinical Microbiology and Infectious Diseases* 28.8 (2009): 899-908.

19. Müller D., *et al*. "Identification of unconventional intestinal pathogenic *Escherichia coli* isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR". *Applied and Environmental Microbiology* 73.10 (2007): 3380-3390.

20. Vidal M., *et al*. "Single Multiplex PCR Assay To Identify Simultaneously the Six Categories of Diarrheagenic *Escherichia coli* Associated with Enteric Infections". *Journal of Clinical Microbiology* 43.10 (2005): 5362-5365.

21. European Committee on Antimicrobial Susceptibility Testing (EUCAST). "Recommendation 2017". 1.0 (2017) 1-127.

22. Letchumanan V., *et al*. "Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia". *Frontiers in Microbiology* 6 (2015): 33.

23. Waters SHP., *et al*. "The tetracycline resistance determinants of RP1 and Tnl72l: nucleotide sequence analysis". *Nucleic Acids Research* 11.17 (1983): 6089-6105.

24. Ploy MC., *et al*. "Molecular characterization on integrons in *Acinetobacter baumanii:* description of a hybrid class 2 integron". *Antimicrobial Agents Chemotherapy* 44.10 (2000): 2684-2688.

25. Wu RB., *et al*. "Prevalence and diversity of class 1 integrons and resistance genes in antimicrobial-resistant *Escherichia coli* originating from beef cattle administered subtherapeutic antimicrobials". *Journal of Applied Microbiology* 111.2 (2011): 511-523.

26. Ayukekbong JA., *et al*. "The threat of antimicrobial resistance in developing countries: causes and control strategies". *Antimicrobial Resistance and Infection Control* 6 (2017): 47.

27. Poonia S., *et al*. "Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim". *Indian Journal of Community Medicine* 39.3 (2014): 156-160.

28. Ghanem B and Haddadin RN. "Multiple drug resistance and biocide resistance in *Escherichia coli* environmental isolates from hospital and household settings". *Antimicrobial Resistance and Infection Control* 7 (2018): 47.

29. Lanz R., *et al*. "Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland". *Veterinary Microbiology* 91.1 (2003): 73-84.

30. Dıaz-Mejıa JJ., *et al*. "An analysis of the evolutionary relationships of integron integrases, with emphasis on the prevalence of class 1 integrons in *Escherichia coli* isolates from clinical and environmental origins". *Microbiology* 154.1 (2008): 94-102.

31. Fluit AC and Schmitz FJ. "Resistance integrons and superintegrons". *Clinical Microbiology and Infection* 10.4 (2004): 272-288.

32. Boerlin P., *et al*. "Antimicrobial resistance and virulence genes of *E. coli* isolates from swine in Ontario". *Applied and Environmental Microbiology* 71.11 (2005): 6753-6761.

33. Solberg OD., *et al*. "Origin of Class 1 and 2 Integrons and Gene Cassettes in a Population-Based Sample of Uropathogenic *Escherichia coli*". *Journal of Clinical Microbiology* 44.4 (2006): 1347-1351.

34. Deekshit VK., *et al*. "Detection of class 1 integrons in Salmonella Weltevreden and silent antibiotic resistance genes in some seafood associated nontyphoidal isolates of *Salmonella* in south-west coast of India". *Journal of Applied Microbiology* 112.6 (2012): 1113-1122.

35. Michael GB., *et al*. "Genes and mutations conferring antimicrobial resistance in *Salmonella:* an update". *Microbes and Infection* 8.7 (2006): 1898-1914.

**Volume 15 Issue 6 June 2019**