ejpmr, 2018,5(7), 111-115

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

GENOTYPIC CHARACTERIZATION OF ANTIBIOTICS RESISTANCE INTEGRONS IN EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING STRAINS OF *E. COLI* AND *PROTEUS* SPP. IN OUAGADOUGOU, BURKINA FASO

Dissinviel Stéphane Kpoda[1,3*], Innocent Kouamé Kouadio[5], Nathalie Guessennd[4], Aboubakar Sidiki Ouattara[1], Lassana Sangaré[2] and Alfred S. Traoré[1]

[1]Laboratoire des Sciences Appliquées et Nutritionnelles (LabSAN), Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN), Université Ouaga I Pr Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.

[2]Centre Hospitalier Universitaire Yalgado Ouedraogo (CHU-YO), 03 BP 7022, Ouagadougou 03, Burkina Faso.

[3]Laboratoire National de Santé Publique (LNSP), 09 BP 24, Ouagadougou 09, Burkina Faso.

[4]Département de Bactériologie et de Virologie, Institut Pasteur de Côte d'Ivoire (IPCI), 01 BP 490, Abidjan 01, Côte d'Ivoire.

[5]Laboratoire de Microbiologie et Biologie Moléculaire, Unité de Formation et de Recherches des Sciences et Technologies des aliments, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire.

***Corresponding Author: Dissinviel Stéphane Kpoda**
Laboratoire des Sciences Appliquées et Nutritionnelles (LabSAN), Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN), Université Ouaga I Pr Joseph KI-ZERBO, 03 BP 7021, Ouagadougou 03, Burkina Faso.

Article Received on 07/05/2018 **Article Revised on 28/05/2018** **Article Accepted on 19/06/2018**

ABSTRACT

Introduction: During the 1980s, genetic elements that could acquire or lose genes for antibiotic resistance were identified and referred to as integrons. The aim of this study was to genotypically characterize integron in extended-spectrum beta-lactamase producing *E. coli* and *Proteus* spp. strains. **Materials and methods**: 125 Extended Spectrum Beta Lactamases producing strains isolated from various biological products in 3 hospitals in Ouagadougou were used for this study. Isolates were subjected to antibiotic susceptibility test using the disc diffusion method: Class 1, 2 and 3 integrons investigation was performed by conventional Polymerase Chain Reaction. **Results:** 59.2% (74/125) strains including 61.5% (72/117) *E. coli* and 25% (2/8) *Proteus* spp. ESBL producers carried class 1 integrons. No isolates carried class 2 and / or 3 integrons. Strains carrying the resistance integrons showed 97.6% resistance to amoxicillin, 97.6% to cefotaxime, 98.4% to ceftriaxone, and 93.6% to cefepime, compared to those without integrons. **Conclusion:** This study revealed 59.2% of ESBLs strains which carried class 1 integrons. The link between resistance integrons and high levels of antibiotic resistance found in these strains poses a risk to public health and hence the need for increased surveillance and control of the indiscriminate use of antibiotics.

KEYWORDS: *E. Coli, Proteus* spp., Integrons of Antibiotic Resistance, Ouagadougou.

INTRODUCTION

The level of antibiotic resistance among pathogenic bacteria has steadily increased and has become a global health challenge.[1] High levels of antibiotics resistance in clinical strains of extended-spectrum beta-lactamase (ESBLs) -producing bacteria have been reported in many studies.[2-3-4] However, recent studies on ESBLs genes reveal that ESBLs genes are located on integrons, hence their ease of transferability from one bacteria to another.[5] Integrons are genetic elements that also contribute to the prevalence and horizontal transmission of antibiotics resistance.[6-7-8] They are genetic elements with a site-specific recombination system for capturing, expressing and exchanging gene cassettes.[9] They are characterized by conserved features, namely an intI gene encoding an integrase, a recombination site (attI), a promoter (P) and the ability to integrate gene cassettes comprised of a single open reading frame (orf) and a specific recombination site, attC.[6-10] Gene cassettes exist either in a linear form inserted into an integron or as a free circular cassette that is not dependent on an integrin.[10-11] At least nine classes of integrons have been described and class 1 integrons are associated with antibiotic resistance in clinical isolates.[12] These commonly reported classes are found in a wide variety of hosts and environments, highlighting a high level of horizontal dissemination among bacterial populations and species. In Burkina Faso, there is a few data on integrons in bacteria strains isolated from clinical and environmental settings. There is very little known about the clinical samples reservoir of integron-borne genes. In this study, we investigated the prevalence of classes 1, 2 and 3 integrons, their association with antimicrobial resistance *Escherichia coli* and *Proteus* spp. strains

isolated from clinical samples in 3 hospital centers in Ouagadougou (Burkina Faso).

MATERIALS AND METHODS

Bacterial isolates: A total of 125 Gram negative bacterial isolates were obtained from 3 hospitals in Ouagadougou the capital of Burkina Faso during October 2014-November 2015. All isolates were ESBLs producing strains. The choice of these hospitals was made because they are the biggest health centers in the country. The study was approved by Ministry of Health of Burkina Faso (Authorization N°2014-01632/MS/RCEN/DRSC, December 09, 2014). Informed consent was obtained from all subjects and at least one family relative before collecting bacteria.

Antimicrobial Susceptibility testing

Antimicrobial susceptibility testing for each isolate was performed by the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, and Hampshire, England). The Double Disk synergy Test (DDST) was used to detect ESBL-producing strains on Mueller-Hinton agar by placing the cefepime, cefotaxime and ceftriaxone disks around the amoxicillin + clavulanic disk. Antibiotic disks were placed in media at a distance of 20 to 30 mm from other disks (CLSI, 2005). Test antibiotics were: amoxicillin (20µg), amoxicillin + clavulanic acid (20 µg + 10µg), cefepime (30 µg), cefotaxime (5µg), ceftriaxone (30µg), cefoxitin (30µg), nalidixic acid (30µg), ciprofloxacin (5µg) (BioMérieux, France). ATCC standard strains *E. coli* (ATCC 25922) was used as susceptibility controls. The inoculated plates were incubated at 37˚C for 24 hours. Interpretation of the diameters of the zones of inhibition was done according to EUCAST/CASFM (2016) guidelines.

Detection of Class 1, 2 and 3 Integrons by PCR

Bacterial isolates were grown overnight (24 h) on Nutrient Agar. Detection of class 1, 2 and 3 integrons using the classical PCR method was performed after extraction of the total DNA by boiling method. The primers used are shown in above Table 1. PCR amplification of classes 1, 2 and 3 integrase genes was performed in 50 µL reaction mixtures containing 5 µg DNA template, 30.3 µL ultrapure water (Nuclease-Free Water, Promega, USA), 5 µL of colored buffer (Green GoTaq®, Promega, USA), 5 µL of uncolored buffer (Colorless GoTaq®, Promega, USA), 3 µL of MgCl 2 (25 mM), 0.5 µL of DNTP (10 mM), 0.5 µL of each primer (20 mM) and 0.2 µL of Taq polymerase (GoTaq®, Promega, USA). Amplification was carried out in a thermocycler (Gene Amp PCR System 9700, Applied Biosystems) with amplification conditions shown in Table 2. The PCR products were analyzed by electrophoresis with 1.5% agarose (Invitrogen). *E. coli* DH5/pTrc 99A (Inserm, France) was used as a positive control for class 1, 2 and 3 integron for PCR tests. The reaction mixture without DNA was considered as negative control.

Table. 1: Primers of class 1, 2 and 3 integrons.

Genes		Sequences	Size of amplicon(pb)	Reference
intI1	Forward	CCTCCCGCACGATGATC	280	[29]
	Reverse	TCCACGCATCGTCAGGC		
intI2	Forward	CACGGATATGCGACAAAAAGGT	788	[29]
	Reverse	GTAGCAAACGAGTGACGAAATG		
intI3	Forward	AGTGGGTGGCGAATGAGTG	600	[29]
	Reverse	TGTTCTTGTATCGGCAGGTG		

Legend: intI1: class 1 integron, intI2: class 2 integron, intI3: class 3 integron.

Table 2. PCR amplification conditions of class 1, 2 and 3 integron

Amplification steps	Temperature conditions / duration	
	intI 1	intI 2 et intI 3
Initial denaturation	94ºC / 5 min	94ºC / 5 min
Cyclic denaturation	94ºC / 1 min	94ºC / 1 min
Annealing	50ºC / 1 min	55ºC / 1 min
Cyclic elongation	72ºC / 1 min	72ºC / 1min
Final elongation	72ºC / 7 min	72ºC / 7 min
Number of cycles	30	30

Statistical analysis data: The data were entered and analyzed using Excel and ANOVA one-way GraphPad Prism version 5.01 software

RESULTS

Characteristics of clinical strains: In this study, 125 ESBLs producing *E. coli* and *Proteus* spp. were investigated for class 1, 2 and 3 integrons: Of 125 strains 117 (93.6%) were *E. coli* and 8 (6.4%) were *Proteus* spp. strains.

Three (2.4%) of these total strains were obtained from vaginal swab, 5 (4%) from pleural fluid, 38 (30.4%) from pus, 1 (0.8%) from blood, 3 (2.4%) stool and 75

(60%) were from urine samples. Twenty seven (21.6%) were collected from Centre Hospitalier Universitaire Pédiatrique Charles De Gaulle, 10(8%) were from Saint Camille Hospital of Ouagadougou and 88 (70.4%) were from Centre Hospitalier Universitaire Yalgado Ouedraogo. Fifty eight (46.4%) of strains were collected from female patients and 67 (53.6%) from male.

Antibiotics Susceptibility of ESBLs producing strains: Our results revealed that resistance rate of ESBLs producing *E. coli* and *Proteus* spp. strains tested during the study varied from 73.6 to 98. 4 (Fig 1). We observed also that they are all multidrug resistant to all these antibiotics used (Fig 1).

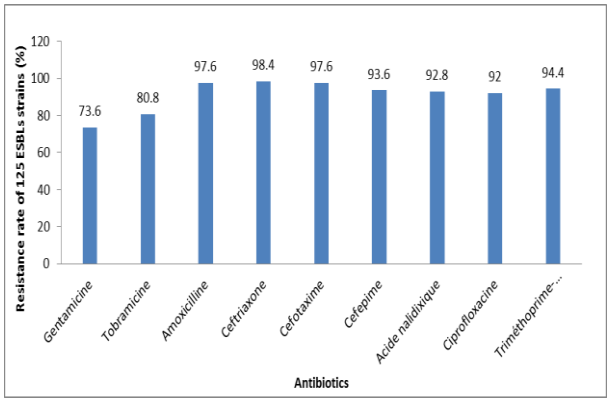

Figure. 1: Resistance level of 125 ESBLs strains tested (n = 125).

Detection of different classes of integrons

All 117 *E. coli* and 8 *Proteus* spp. isolates were examined for resistance gene marker intl1. Seventy two (61.5%) *E.coli* and 2 (25%) *Proteus* spp. were positive for intl1. No isolates carried class 2 and / or 3 integrons (Figure 2). All isolates that were positive for class 1 integrons (Intl1, 280 pb) were isolated from urine samples.

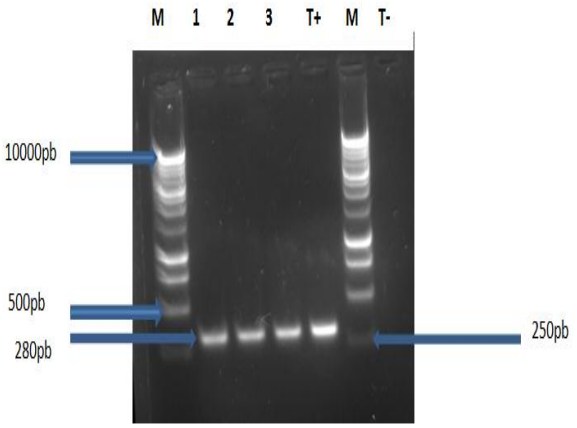

Figure. 2. 1.5% agarose gel electrophoresis showing simplex PCR for the detection of class 1 integrons.

Legend: Line M = DNA Ladder (1kb), Line T+ = Positive control, Line T- = Negative control, Line 1, 2, 3 = Samples.

DISCUSSION

The spread of multidrug resistance in different ecosystems has become a major problem in the treatment of infections caused by pathogenic bacteria[13] including *E. coli* and *Proteus* spp. According to Bao *et al.*[13] and Rangaiahagari *et al.*,[14] *E. coli* strains are the most isolated bacterial species in bacteriology, and the most frequently isolated organism from urine samples which are the most frequent biological products collected in bacteriology unit.[15] Higher prevalence of *E. coli* in our study might be due to its frequent presence in hospital environment from where study cases were selected.[16] The susceptibility test results showed that all the ESBLs producing isolates were resistant to 3rd generation cephalosporin, aminoglycosides, quinolones and sulfonamide antibiotics. This reflects the relationship between ESBLs and 3rd generation cephalosporins. Increased resistance might be due to extensive use of beta-lactam and other drugs.

Antimicrobial resistance rates were high for most antibiotics tested (Tobramycin 80.8%, Amoxicillin 97.6%, Ceftriaxone 98.4, Cefotaxime 97.6% and Trimethropin-sulfomethoxaole 94.4%). These results corroborate those of Murshed *et al.*[17], Rezaee *et al.*[18] and Tahou *et al.*[19] who reported high rates of antibiotics resistance. These multidrug resistances may be due to antibiotics overuse and irrational use in the treatment of infectious diseases. Resistance to Trimethropin-sulfomethoxaole is so high because this antibiotic is used in HIV patients' opportunist treatment in Burkina Faso. In Burkina Faso as in many other African countries, the lack of antibiotic surveillance system, unfavorable hygiene conditions in hospitals, may be attributed to the spread of ESBLs and multidrug resistance.[20]

Gene coding ESBLs are usually located on conjugative plasmids although many of the most recently described ESBLs genes are frequently found within integron like structure.[21-22] This study examined integrons genes content in clinical *E. coli* and *Proteus* spp. strains. Integrons are gene exchange systems and are known to play a significant role in the acquisition and dissemination of antimicrobial resistance genes and it is selected by antimicrobial pressure.[9] Class 1 integrons are the most commonly prevalent in hospital and community bacterial strains. ESBLs genes located on integrons- like structures are being increasingly reported worldwide.[23] Class 1 integrons of *E. coli* is closely associated with human related environments that are more likely affected by antibiotic selective pressures. In this study, 59.2% (74/125) ESBLs producing clinical strains harbored class 1 integrons but no other class of integrons (Class 2 and 3) found. In previous studies, class 1 integrons have been found in clinical strains. In Burkina Faso, Bagré *et al.*[24] found 8 of 10 diarrheic *E. coli* strains harboring class 1 integrons. No classes 2 and 3 integrons were detected in their study.

In a study conducted by Tahou *et al.*,[19] in Côte d'Ivoire, they reported the presence of class 1 integrons (47.25%) of 91 clinical multidrug *Klebsiella pneumoniae* strains. Mushed *et al.*[17] detected the gene marker intl1 (Class 1 integrons) in 32(67%) *E. coli* isolates collected from wound infections in Dhaka, Bangladesh. In Southwest Nigeria, Odumosu *et al.*[25] also reported an incidence rate of class 1 integrons of 57% (31) of 54 clinical *Pseudomonas aeruginosa* strains.

Our findings are similar to those of Nigeria (57%) and Bangladesh (67%) and higher than that of Côte d'Ivoire (47.25%). Furthermore, other authors in Nigeria detected class 1 and 2 integrons in *E. coli* of faecal origin.[26]

The spread of resistance genes involving integrons is a major problem that is not limited to hospitals. Kouadio *et al.*[27] detected class 1 integrons in 25 (71%) of 35 ESBLs producing *E. coli* strains isolated from piglets faecal samples in the village of Abidjan Doumé close to Abidjan (Côte d'Ivoire). Several studies have underlined environmental resistome as a source of resistance genes and environmental hot spots of integron dissemination, including hospital effluents.[28] Class 1, 2 and 3 integrons are considered to be involved in the dissemination of antibiotic resistance in both clinical settings and the environment, and could participate in the exchange of antibiotic-resistance genes between these two ecosystems.

CONCLUSION

Recent investigations suggest that integrons are common among multidrug-resistant (MDR) isolates and they can be used as a marker for the identification of MDR isolates. In our study we found 59.2% ESBLs producing strains harboring class 1 integrons. This could lead to a serious threat of an outbreak of antimicrobial resistance development which could complicate treatment of infections in the future. Therefore precautionary measures must be adopted to prevent the spread of these integrons.

AKNOWLEDGEMENTS

France Embassy in Burkina Faso ; Institut Pasteur de Côte d'Ivoire ; Laboratoire national de santé publique, Burkina Faso ; Centre Hospitalier Universitaire Yalgado Ouedraogo, Burkina Faso ; Centre Hospitalier Universitaire Pédiatrique Charles De Gaulles, Burkina Faso ; Hôpital Saint Camille de Ouagadougou, Burkina Faso.

REFERENCES

1. World Health Organization (WHO) first global report on antibiotic resistance reveals serious, worldwide threat to public health; 2014. http://www.who.int/mediacentre/news/releases/2014 /amr- report/en/. Cited 28 May 2018.
2. Lavigne JP, Marchandin H, Delmas J, Bouziges N, Lecaillon E, Cavalie L, Jean-Pierre H, Bonnet R, Sotto A. QnrA in CTX-M-producing Escherichia coli isolates from France. Antimicrob Agents Chemother, 2006; 50(12): 4224-4228
3. Jiang Y, Zhou Z, Qian Y, Wei Z, Yu Y, Hu S, Li L. Plasmid-mediated quinolone resistance determinants qnr and aac (6′)-Ib-cr in extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in China. J Antimicrob Chemother, 2008; 61(5): 1003-1006.
4. Fody AM, Boubou L, Moussa A, Bawa HI, Konaté A, Yaou C, Zongo C, Salaou C, Daouda A, Sidikou R, Traoré AS, Barro N. Phenotypic detection of extented spectrum beta-lactamase in multidrug-resistant Escherichia coli from clinical isolates in Niamey, Niger. Afr J Microbiol Res., 2017; 11: 712-717.
5. Machado E, Cantón R, Baquero F, Galán JC, Rollán A, Peixe L, Coque TM. 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): 1823-9.
6. Domingues S, Harms K, Fricke FW, Johnsen PJ, da Silva GJ, Nielsen KM. Natural Transformation Facilitates Transfer of Transposons, Integrons and Gene Cassettes between Bacterial Species. PLoS Pathog, 2012; 8(8): 1002837. https://doi.org/10.1371/journal.ppat.1002837 PMID: 22876180.
7. Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, Miao J, Chen D, Bian H, Li Y, Yu G. Resistance integrons: class 1, 2 and 3 integrons. Ann Clin Microbiol Antimicrob, 2015; 14: 45. https://doi.org/10.1186/s12941-015-0100-6 PMID: 26487554.
8. Singha P, Chanda DD, Maurya AP, Paul D, Chakravarty A, Bhattacharjee A. Distribution of Class II integrons and their contribution to antibiotic resistance within Enterobacteriaceae family in India. Indian J Med Microbiol, 2016; 34: 303–7 https://doi.org/10.4103/0255-0857.188319 PMID: 27514951.
9. Mazel D. Integrons: agents of bacterial evolution. Nat Rev Microbiol, 2006; 4(8): 608–20.
10. Partridge SR, Tsafnat G, Coiera E, Iredell JR. Gene cassettes and cassette arrays in mobile resistance integrons. FEMS Microbiol Rev, 2009; 33(4): 757–84. https://doi.org/10.1111/j.1574-6976.2009. 00175.x PMID: 19416365.
11. Boucher Y, Labbate M, Koenig JE, Stokes HW. Integrons: mobilizable platforms that promote genetic diversity in bacteria. Trends Microbiol, 2007; 15(7): 301–9. https://doi.org/10.1016/j.tim.2007.05.004 PMID: 17566739.
12. Kang HY, Jeong YS, Oh JY, Tae SH, Choi CH, Moon DC, Lee WK, Lee YC, Seol SY, Cho DT, Lee JC. Characterization of antimicrobial resistance and class 1 integrons found in Escherichia coli isolates from humans and animals in Korea. J Antimicrob

Chemother, 2005; 55: 639–644. https://doi.org/10.1093/jac/dki076 PMID: 15761064
13. Bao L, Peng R, Ren X, Ma R, Li J, Wang Y. Analysis of some common pathogens and their drug resistance to antibiotics. Pak J Med Sci, 2013; 29(1): 135-139.
14. Rangaiahagari A, Uwizeyimana JP, Nyirabanzi J, Ngoga E and Wane J. Antibiotic sensitivity patterns of Enterobacteriaceae isolated at king Faisal hospital, Kigali a three years study. Rwand Med J, 2013; 70: 11-4.
15. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis, 2004; 38: 1150–8.
16. Zafar A. Prevalent nosocomial gram negative aerobic bacilli and their antimicrobial susceptibility pattern in intensive care unit. J Pak Med assoc, 1999; 49(7): 169-172.
17. Murshed M, Shahnaz S and Abdul Malek M. Detection of resistance gene marker intl1 and antimicrobial resistance pattern of E. coli isolated from surgical site wound infection in holy family Red Crescent Medical College Hospital. Bangladesh J Med Microbiol, 2010; 4(2): 19-23.
18. Rezaee MA, Sheikhalizadeh V, Hasani A. Firt report of class1 and class 2 integron in multdrug- resistant (MDR) Escherichia coli strains isolated from clinical specimens in northern west of Iran. Jpn J Infect Dis, 2012; 65(3): 256-259.
19. Tahou EJ, Guessennd KN, Gbonon V, Gba KMK, Konan F, Toty A, Tiekoura KB, Diplo T, N'guetta ASP. First detection of class 1 integrons isolated from clinical strains of Klebsiella pneumoniae producing broad-spectrum beta-lactamases in Abidjan (Cote d'Ivoire). Intern J Rec Sc Res., 2018; 9(1): 22867-22870.
20. Kpoda DS, Guessennd N, Bonkoungou JI, Ouattara MB, Konan F, Ajayi A, Simpore J, Ouedraogo R, Drabo KM, Sangare L, Dosso M, Traore A. Prevalence and resistance profile of extended-spectrum β-lactamases-producing Enterobacteriaceae in Ouagadougou, Burkina Faso. Afr J Microbiol Res., 2017; 11(27): 1120-1126. DOI: 10.5897/AJMR2017.8598.
21. Canton R, Coque TM, Baqueero F. Multi resistance gram- negative bacilli: from epidemics to endemics. Curr Open Infect. Dis., 2003; 16: 315-325.
22. Bonnnet R. Growing group of extended spectrum Beta-lactamases: The CTX-M enzymes. Antimicrob Agents Chemother, 2004; 48: 1-14.
23. Rowe-Magnus DA, Mazel D. The role of integrons in antibiotic resistance gene capture. Int J Med Microbiol, 2002; 292: 115-125.
24. Bagré TS, Sambe-Ba B, Bawa HI, Tchamba GB, Dembélé R, Wane AA, Bako E, Traoré AS, Barro N and Gassama-Sow A. Isolation and characterization of enteropathogenic and enterotoxinogenic Escherichia coli from dairy products consumed in Burkina Faso. Afr J Microbiol Res., 2017; 11(13): 537-545. DOI: 10.5897/AJMR2017.8485.
25. Odumosu BT, Adeniyi A, Chandra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant Pseudomonas aeruginosa from Southwest Nigeria. Clin Microbiol and Antimicrob, 2013; 12: 29.
26. Odetoyin BW, Labar AS, Lamikanra A, Aboderin AO, Okeke. Classes 1 and 2 integrons in faecal Escherichia coli strains isolated from mother-child pairs in Nigeria. PLoS ONE, 2017; 12(8): 0183383. https://doi.org/10.1371/journal. pone.0183383.
27. Kouadio IK, Guessennd N, Dadié A, Gbonon V, Tiekoura B, Tahou E, Kpoda S, Dosso M. Detection of Resistance Integron in Escherichia coli of Porcine Origin Producing Extended-spectrum Beta-lactamase in Abidjan, Côte d'Ivoire. J Adv Microbiol, 2018; 8(3): 1-7.
28. Stalder T, Barraud O, Casellas M, Dagot C, Ploy MC. Integron involvement in environmental spread of antibiotic resistance. Front Microbiol, 2012; 3: 119.
29. Goldstein C, Lee M, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers A. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. J Antimicrob Chemother, 2001; 45: 723–726.