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Detection of Resistance Integron in *Escherichia coli* of Porcine Origin Producing Extended-spectrum Beta-lactamase in Abidjan, Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author IKK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NG, AD, ET and SK managed the analyses of the study. Authors VG and BT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Aim of the study was to detect resistance integrons involved in multidrug resistance phenotypes in *Escherichia coli* strains of porcine origin producing extended-spectrum beta-lactamases (ESBL).

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Study Design: Genotypic study.
Place and Duration of Study: National Reference Center for Antibiotics and Molecular Biology Platform of Pasteur Institute of Côte d'Ivoire, between June 2017 at July 2017.
Methodology: Thirty-five (35) *Escherichia coli* strains of porcine origin producing extended-spectrum beta-lactamases were collected for study. The strains were analyzed using an antibiotic susceptibility test according to the diffusion method in agar medium. The research of class 1, 2 and 3 resistance integrons was performed using the conventional PCR method.
Results: 25 strains (71%) of *E. coli* producing ESBL harboured class 1 resistance integrons. None of the isolates carried class 2 and 3 resistance integrons. The strains harbouring resistance integrons were more resistant to amoxicillin, ceftriaxone, cefotaxime, tetracycline with a much higher rate of resistance (71%) compared to integron negative isolates (31%). The resistance to kanamycin and cotrimoxazole were 60% in integron positive isolates. Concerning integron negative isolates, the resistance to kanamycin was 11% and cotrimoxazole 20%.
Conclusion: The integron positive isolates is one of the major causes of resistance gene dissemination. This represents a risk for public health that must challenge the public authorities on the reasoned use of antibiotics in animal production chains.

Keywords: E. coli; extended-spectrum beta-lactamase (ESBL); antibiotic; piglets; integron resistance.

1. INTRODUCTION

Escherichia coli is a gram-negative bacteria, commensal to the intestines of animals that, through these sources, can enter the food chain. *E. coli* is considered an opportunist pathogen but some enteropathogenic strains have properties that can cause infections [1,2]. Among the many properties, acquired antibiotic resistance through the production of extended-spectrum beta-lactamases (ESBLs) is observed in *E. coli* strains. Animal and human origins of *E. coli* are responsible for many antibiotic failures [3]. The genes coding for ESBL resistance is located on mobile genetic elements [4]. These mobile genetic elements such as plasmids carry genetic units called integrons [5]. Integrons, major supports for multidrug resistance, may contain cassettes [6]. For class 1, 2 and 3 integrons, it was shown that the cassettes were predominantly composed of resistance genes to beta-lactams, aminoglycosides (*aad* genes) and trimethoprim (*dfr* genes) [7,8]. Livestock is an important reservoir of resistance where prevalence observed from commensal *E. coli* in cattle, pigs, and chickens are 52% [9]. A prevalence of 30% of class 1 resistance integrons was detected in more of 300 *E. coli* strains isolated from cattle, swine and poultry [10]. The most important figures concern pigs because the use of antibiotics in the swine sector accounts for nearly 60% of the world's consumption of antibiotics in the animal sector [11-13]. Digestives portages in resistant integrons of healthy pigs are generally found at very high levels, whether from commensal *Salmonella* with 45% of class 1 resistance integrons or from commensals *E. coli* with 23% [14]. Other more recent studies found resistance integron levels of more than 60% from *E. coli* isolated from chicken and turkey meat [15,16]. Resistance integrons can be a source of resistance gene dissemination to consumers [17]. Given the probable public health risks linked to the transfer of antibiotic resistance genes by the presence of resistance integrons in strains of animal origin, it was considered important by the present study to characterize the genetic support of resistance involved in multidrug resistance phenotypes in *E. coli* of porcine origin.

2. MATERIALS AND METHODS

2.1 Bacterial Isolate

Thirty-five isolates of *E. coli* were collected from piglets in the village of Abidjan Doumé in the Abidjan region of Côte d'Ivoire in from June to July 2017. The ATCC standard strains *E. coli* (ATCC 25922) was used as susceptibility controls. *E. coli* DH5/pTrc 99A (Inserm, France) was used as a positive control for class 1, 2 and 3 integron PCR tests.

2.2 Antimicrobial Susceptibility

In vitro antimicrobial susceptibility of ESBL producing isolates was determined to 14 antibiotics bracket (Bio-rad, Marne-la-coquette, France) according to CASFM criteria (CASFM, 2014). The antimicrobial discs were : Amoxicillin (25 µg), Amoxicillin/clavulanic acid (20/10 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg),

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

Genes		Sequences	Size of amplicon (bp)	Reference
***intI*1**	Forward	CCTCCCGCACGATGATC	280	[18]
	Reverse	TCCACGCATCGTCAGGC		
***intI*2**	Forward	CACGGATATGCGACAAAAAGGT	788	[19]
	Reverse	GTAGCAAACGAGTGACGAAATG		
***intI*3**	Forward	AGTGGGTGGCGAATGAGTG	600	[20]
	Reverse	TGTTCTTGTATCGGCAGGTG		

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

Cefoxitin (30 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Tetracycline (30 µg), Cotrimoxazole (1,25/23,75 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Kanamycin (30 µg), Streptomycin (10 µg), Colistin (50 µg).

2.3 Detection of Class 1, 2 and 3 Integrons by PCR

Isolates were grown overnight (24 h) in Nutrient Agar (Liofilchem, Italy). 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 aboveTable 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). The reaction mixture without DNA was considered negative control.

3. RESULTS

3.1 Resistance Integrons Detected

The results of the PCR showed that 25 strains (71%) on 35 ESBL producing *E. coli* harboured class 1 resistance integrons with 280 base pair sizes (Fig. 1). None of the isolates carried class 2 and 3 resistance integrons.

3.2 Association between Antimicrobial Susceptibility and Integron Carriage in 35 ESBL Producing Isolates of *E. coli*

The comparison showed that the strains harbouring resistance integrons were more resistant to amoxicillin, ceftriaxone, cefotaxime, tetracycline with a much higher rate of resistance (71%) compared to integron negative isolates (31%). These higher rates of resistance were also observed to kanamycin and cotrimoxazole (60%) in integron positive isolates. On the other hand, in the negative integron isolates the rates were low. The resistance rate to kanamycin was 11% and resistance rate to cotrimoxazole was 20% (Fig. 2).

4. DISCUSSION

During the study, the results showed that 71% of *E. coli* strains were ESBL-producing harboured

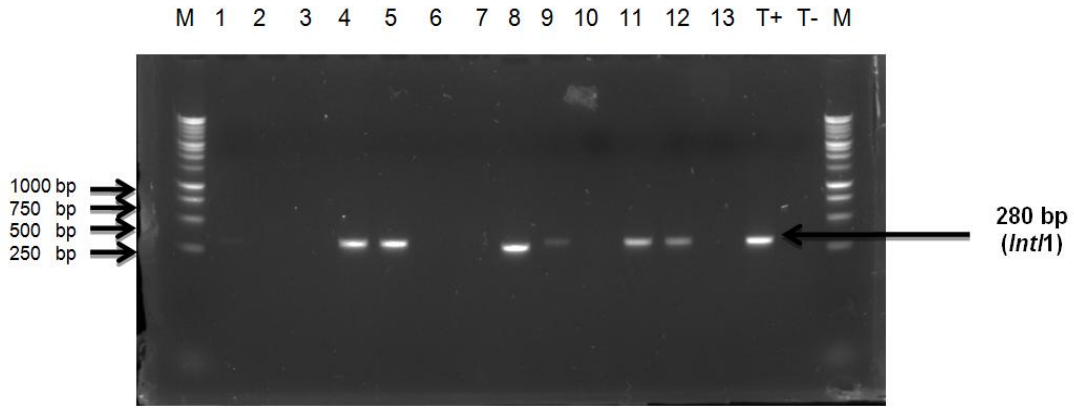

Fig. 1. Electrophoretic profile of class 1 integron amplification product
Line M: Molecular weight marker (Benchtop, 1 kb DNA Ladder, USA)
Line 4, 5, 8, 9, 11 and 12: reference of strains analyzed harboring class 1 integron
Line T+: Positive control strain
Line T- : Negative control strain

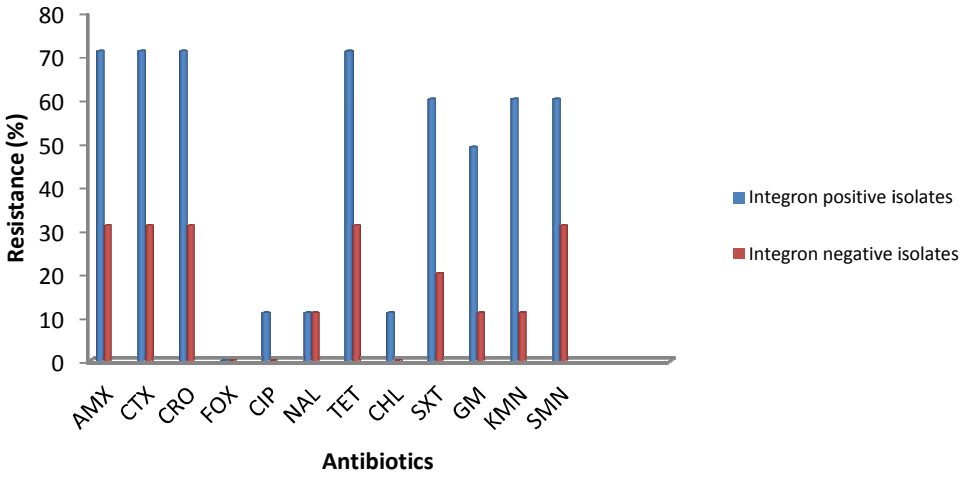

Fig. 2. Association between antimicrobial susceptibility and integron carriage in ESBL producing isolates of *E. coli*
AMX : Amoxicillin, CTX: Cefotaxim, CRO: Ceftriaxone, FOX: Cefoxitn, CIP: Ciprofloxacin, NAL: Nalidixic Acid, TET: Tetracycline, CHL: Chloramphenicol, SXT: Cotrimoxazole, GM: Gentamicin, KMN: Kanamycin, SMN: Streptomycin

class 1 integron versus 0% for class 2 and 3 integrons. This predominance of class 1 resistance integrons relative to class 2 and 3 resistance integrons is reported by several authors in the world. In the United States, results from a study showed the dominance of class 1 resistance integrons which was 86% compared to class 2 and 3 resistance integrons that were 0% in *E. coli* strains of porcine origin [20]. In Germany, a study conducted in 2005 showed a prevalence of 66% for class 1 integrons, 10% for class 2 integrons and 0% for class 3 integrons [21]. A study in China described a prevalence rate in pigs with a prevalence of 64.2% in class 1 resistance integrons from *E. coli* isolated from diarrheal stools [14] and 66% in another study of Enterobacteriaceae with ESBL phenotype [22]. But several other studies have reported contrary

results with lower class 1 resistance integron levels compared to the high prevalence detected in our study and other studies mentioned above. The prevalence of 5% in ampicillin-resistant *E. coli* isolated from bovine faeces in a Scottish farm [23]. A Norwegian study on porcine-derived products also showed a prevalence of 18% resistance integrons from multiresistant *E. coli* strains [15]. It has been reported a rate of 22% of class 1 resistance integrons from commensal *E. coli* of animal origin [9]. Another reports a prevalence of 30% of class 1 resistance integrons on 300 strains of *E. coli* isolated from cattle, swine and poultry [10]. Other studies have also reported a presence of class 2, but restricted, resistance integrons in the family of Enterobacteriaceae (*E. coli, Salmonella, Enterobacter*) of animal origin [8]. A study conducted in the United States reported a rate of 23% in class 2 integrons in cattle and 17% in reptilian faeces, a rate almost equivalent to that of class 1 integrons. In our study, 71% of ESBL producing *E. coli* were carriers of class 1 resistance integrons [20]. This high rate of this multidrug resistance detected could be related to the presence of these resistance integrons. Many studies have demonstrated the link between resistance integron and bacterial resistance, particularly with regard to Enterobacteriaceae [24,25]. The presence of resistance integrons and higher resistance levels to beta-lactamases (amoxicillin, cefotaxime, ceftriaxone with 71%), aminoglycosides (kanamycin and streptomycin with 60%) and sulfonamide (cotrimoxazole 60%) were found to be related.

These results are in agreement with a study in Iran where he found a link between the presence of resistance integrons and higher levels of resistance to beta-lactamases and aminoglycosides in strains of ESBL-producing *Klebsiella pneumoniae* patients [26]. In 1998, the study indicated the association between resistance integrons and antibiotic resistance of 163 isolated bacterial strains from 14 different European hospitals [24].Those with a resistance integron (43% prevalence) were more resistant to aminoglycosides (streptomycin and spectinomycin), beta-lactamases (penicillins and beta-lactamase inhibitors) and even fluoroquinolones. Similarly, a study showed nearly 900 enterobacteria of the hospital and community origin that there was a significant relationship between resistance integrons and resistance to at least 2 antibiotics (71% of Gram-negative bacilli) with 2 resistance containing class 1 resistance integrons, whatever the species considered or its origin [27]. A recent study in China has shown on nearly 100 strains of *Acinetobacter baumanii*, two-thirds of which harboured a class 1 integron resistance, the resistance levels of the positive integron isolates were higher than those of the negative integron isolate [28].

The predictive character of the presence of resistance integron towards a particular resistance to antibiotic seems therefore difficult to bring, except perhaps with respect to cotrimoxazole whose resistance is strongly associated with the presence of class 1 resistance integrons due to the frequent presence of a *far* cassette and the *sul1* gene of the 3 'region. This link has been highlighted by several studies for both *E. coli* [29,30] and other enterobacteria [31].

5. CONCLUSION

The presence of resistance integrons in the digestive portage in farm animals is a crucial problem for animal health and public health. In fact, they are mobile genetic elements capable of promoting the acquisition and dissemination of resistance genes. In this study, the digestive portage into resistance integrons of strain ESBL-producing *E.coli* porcine commensals was revealed important class 1 resistance. The strong link detected between the resistance integrons and antibacterial resistance must challenge the public authorities on a good use of antibiotics in animal production chains.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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