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EC MICROBIOLOGY
Research Article

Detection of Quinolone Resistance Genes in Salmonella spp. Isolated from Livestock in Aabidjan District (Côte d'ivoire)

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Received: October 18, 2019; **Published:** November 13, 2019

Abstract

Salmonella is a zoonotic pathogen and one of the causes of gastroenteritis in developed and developing countries. Because of the susceptibility of these bacteria to the Quinolone's family, theses antibiotics are considered to be as drugs of choice for the treatment of salmonellosis during a long time. The appearance, the spread and the increase in *Salmonella* resistance to these antibiotics is an additional difficulty in controlling infections caused by it in a context of poverty. The objective of this study was to detect from molecular markers the level of quinolone resistance in *Salmonella* spp. strains isolated in cattle faeces in the Abidjan district. Thus, out of a total of 84 *Salmonella* strains isolated in cattle faeces, all *Salmonella* strains resistant to at least one antibiotic (26 strains) were used for the detection of quinolone resistance genes (*qnrA*, *qnrB* and *qnrS*). The *qnrB* and *qnrS* genes were detected with frequencies of 30.8% and 7.7% respectively. No *qnrA* genes were detected. Quinolones and fluoroquinolones are antibiotics of choice in human and veterinary medicine. Thus, monitoring and tracking the spread of resistance genes is important for controlling *Salmonella* resistance to quinolones, thereby limiting the increased risk to public health at the level of the country.

Keywords: *Salmonella; Quinolone; Resistance; Cattle; Abidjan; Côte d'Ivoire*

Introduction

Salmonella is one of the zoonotic pathogens that pose a significant threat to public health worldwide [1]. This bacterium has long been the main cause of many epidemics and infections worldwide. It is considered to be one of the main causes of human gastroenteritis. There are an estimated 93.8 million cases of *Salmonella* gastroenteritis in the world, of which more than 85% are food-borne [2], particularly animal foods such as beef, poultry, eggs and dairy products, as well as raw fruits and vegetables [3].

The emergence and spread of multi-resistant antibiotic-resistant strains of *Salmonella* adds another important dimension to the challenge of controlling *Salmonella* [4]. This situation can compromise the ability to treat human infections, which is a particularly important problem in the case of infections. Antibiotics are used effectively to treat and control certain diseases in cattle [5].

Some antibiotics such as quinolones and fluoroquinolones are used to treat diseases in both cattle and humans. These antibiotics are currently constituting the groups of antibiotics with increased importance in livestock farming. Their interest is related to their low toxicity and their speed in killing enteric and other pathogenic bacteria [6]. Resistance can sometimes be of chromosomal origin and this through a mutation at the gene level, causing a modification of the antibiotic binding site, or by active efflux, but more often than not, they are of plasmid origin, therefore transferable horizontally between bacteria of the same species, or even bacteria from distant species [7]. Although highly quinolone-resistant *Salmonella* strains are still rarely isolated [8], decreased sensitivity should be considered an alert, since quinolones are antibiotics of last resort. However, there is very limited data on the mechanism of quinolone resistance in *Salmonella* spp. isolated from cattle in Côte d'Ivoire.

Objective of the Study

The objective of this study was to determine the prevalence of quinolone resistance in cattle faeces in the Abidjan district.

Materials and Methods

Collection of strains of Salmonella spp.

All strains of *Salmonella* used in this study were isolated from cattle feces over a six-month period from April to September 2016. A total of 84 strains of *Salmonella* were isolated using the conventional *Salmonella* detection method. spp according to ISO 6579: 2002 (E) and then confirm with MALDI-TOF MS (BioMérieux, France). These strains come from five communes of Abidjan district (Côte d'Ivoire) and are divided as follows: 5 strains of *Salmonella* come from Abobo, 30 strains come from Adjamé, 4 strains of Yopougon, 4 strains of Bingerville and 41 strains from Port-Bouet.

Susceptibility to antibiotics

The resistance profile of *Salmonella* strain to quinolone was obtained by diffusion tests of the disks in an agar medium using a panel of antibiotic discs (Bio-Rad France) for human and veterinary therapeutic use, such as ampicillin. (10 µg), amoxicillin + clavulanic acid (30 µg), cefalotine (30 µg), cefepime (30 µg), aztreonam (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefuroxime (30 µg), imipenem (10 µg), tetracycline (30 µg), minocycline (30 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), nalidixic acid (30 µg), norfloxacin (5 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), colistin (50 µg) and trimethoprim/sulfamethoxazole (25 µg). The results were interpreted according to the standard of the Antibiogram Committee of the French Microbiology Society (EUCAST / CA-SFM, 2016). The reference strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were used for internal quality control. In order to determine the resistance mechanism, a molecular study of these strains was performed by gene amplification (PCR).

Detection of quinolone resistance genes by PCR

The study of quinolone and fluoroquinolone resistance genes was performed by PCR amplification on all *Salmonella* strains phenotypically resistant to at least one antibiotic using specific primer sequences (Table 1). Extraction of the bacterial DNA was performed by the heat shock method [9] on 24-hour colonies followed by phenol-chloroform-alcohol-isoamyl purification (v/v/v) [10]. The *qnrA, qnrB* and *qnrS* genes were amplified by PCR simplex. The reference strains provided by the National Food Institute (DTU Food) were used as positive controls for PCR (Table 2) and a reaction mixture without DNA extract served as a negative control. The genomic amplification was carried out in a final reaction volume of 50 µl and containing a 5 × color buffer (Promega, USA), a non-stained 5X buffer (Promega, USA), MgCl 2; 25 mM (Promega, USA), of each dNTPs; 10 mM (Bio-Rad, France), from Go taq DNA polymerase; 5 U/µl (Promega, USA) and specific primers; 10 µM. The amplification conditions are shown in table 3. The amplification products were analyzed by electrophoresis on a 1.5% agarose gel prepared from a 10X TAE buffer (Tri-Acetate-EDTA) and 5 µL of a solution of EZ-vision® (Inqaba biotec, West Africa) at 120 volts/cm for 1 hour.

Results

Of the 84 strains of *Salmonella* identified, 26 strains showed resistance to at least one antibiotic, 31%. In addition, among these 3.6% strains showed resistance to the family of quinolones and fluoroquinolones (Table 4).

Target	Primers	Sequences (5'-3')	Fragment size (pb)	References
qnrA	QnrA fw	GGATGCCAGTTTCGAGGA	492	[11]
	QnrA rw	TGCCAGGCACAGATCTTG		
qnrB	qnrB (1-6)F	GGMATHGAAATTCGCCACTG	264	[11]
	qnrB (1-6)R	TTTGCYGYYCGCCAGTCGAA		
qnrS	qnrS (1-2)F	TCGACGTGCTAACTTGCG	466	[11]
	qnrS (1-2)R	GATCTAAACCGTCGAGTTCGG		

Table 1: *Primers used for PCR.*

Identity	Strains	Genes	Origin
03-577	*Enterobacter cloacae*	*qnrA*	DTU
KP15	*Klebsiella pneumoniae*	*qnrB*	DTU
pHC19	*Escherichia coli*	*qnrS*	DTU
ATCC25922	*E coli*	-	DTU
ATCC 29213	*Staphylococcus aureus*	-	IPCI

Table 2: *Reference strains.*

DUT: Technical University of Denmark, IPCI: Pasteur Institute of Côte d'Ivoire.

Amplification step	Temperature C°/ Time/ Cycle
Initial denaturation	95°C/5min
Cyclic denaturation	95°C/30s
Hybridization	60°C/30s
Cyclic elongation	72°C/1min
Final elongation	72°C/10min
Number of cycles	35

Table 3: *Amplification condition.*

Antibiotics	Resistance rate (%)
Quinolones/Fluoroquinolones	R (n = 84)
Nalidixic acid (NAL)	3,6
Ciprofloxacin (CIP)	3,6
Norfloxacin (NOR)	3,6

Table 4: *Resistance profile of Salmonella to quinolones.*

The *qnr* gene was detected in 8 strains of *Salmonella* on the 26 strains tested (Figure 1). Among the positive strains, the *qnrB* and *qnrS* genes were detected with respective frequencies of 30.8% and 7.7%. None of the strains had the *qnrA* gene (Table 5). Analysis of the genotypic profiles of these strains showed the presence of *qnr* genes expressed either singly or in combination. Individually, the frequency of these genes was 23.1% (*qnrB*). The association of *qnrB*/*qnrS* genes was found with a frequency of 7.7% (Table 6).

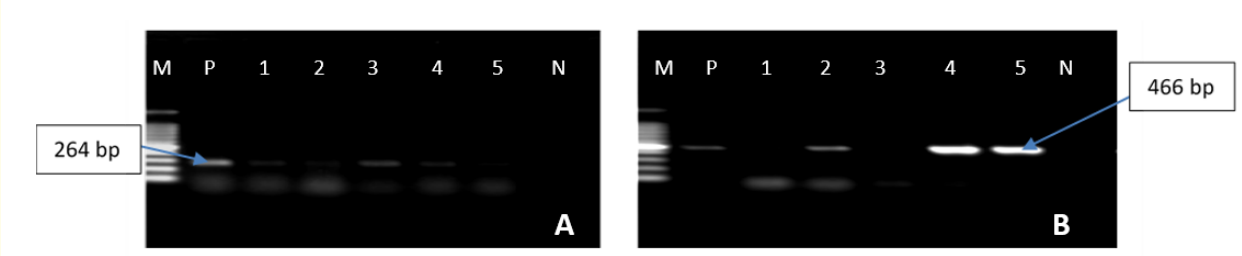

Figure 1: *Electrophoretic profile of quinolone resistance genes.*
A: Detection of the qnr B gene (264 bp); B: Detection of the qnrS gene (466 bp); M: Molecular Weight Marker (100 bp DNA Ladder); Positive control; Number 1 to 5: Bacterial strain; N: Negative Control.

Number of genes			
Strains	**qnrA**	**qnrB**	**qnrS**
*Salmonell*a (N = 26)	0	8	2
Frequency (%)	0%	30,8%	7,7%

Table 5: *Detection rate of quinolone resistance genes.*

Genotypic profiles	qnr genes				
	qnrA	**qnrB**	**qnrS**	**Souches**	**N (%)**
P1	-	+	-	*Salmonella*	6 (23,1)
P2	-	+	+	*Salmonella*	2 (7,7)

Table 6: *Genotypic profiles of quinolone resistance.*

Discussion

Salmonella resistance to commonly used antibiotics is increasing in both veterinary and human medicine and has become a public health problem. This phenomenon is generally attributed to poor management of livestock practices. This leads to a strong emergence of resistance bacteria such as the one observed in our study. The results showed that cattle dung generally contained a significant proportion of antibiotic-resistant *Salmonella*, which amounted to 31%. These results support those of [13] who showed similar resistance in cattle. These results therefore support the hypothesis that cattle dung could be a reservoir of resistant bacteria and the chances of these resistant bacteria being transmitted to humans are more likely [14]. This study showed that *Salmonella* strains are still largely sensitive to quinolones. Similar results have been reported by Gorman and Adley [15] in the Republic of Ireland and India [16]. Lower rates compared to those obtained in our study were reported by Smith., *et al* [5]. In addition, high resistance rates have been reported in Kenya [17], India

[18], Romania [19]. The emergence of fluoroquinolone resistance is alarming because they are the antibiotics of choice used in veterinary medicine to treat invasive salmonellosis. However, the low rate observed in our study could be explained by the fact that fluoroquinolones are not widely used in cattle breeding in Côte d'Ivoire. In this study, quinolone resistance genes were detected with varying proportions. In Côte d'Ivoire, the detection of *qnr* genes has already been reported by Guessennd., *et al*. [20] on beta-lactamase-producing enterobacteriaceae in human biological products. These authors reported in their study the presence of the *qnrA* and *qnrB* genes with respective proportions of 9% and 14.6% and only the *qnrS* gene was not detected in any strain. Also, another study by Baguy., *et al.* [21] on multi-resistant enterobacteriaceae isolated from different ecosystems revealed the presence of the *qnrS* gene with a prevalence of 95%. However, the *qnrA* and *qnrB* genes were not detected. In this study, only the *qnrA* gene was not identified in *Salmonella* strains. On the other hand, the *qnrB* and *qnrS* genes were detected with frequencies of 30.8% and 7.7% respectively. Our results support those of Sanjukta., *et al*. [22] who detected *qnrB* and *qnrS* genes during their studies, while the *qnrA* gene was not detected. These results indicate that the *qnrB* gene is mainly involved in the quinolone resistance observed in *Salmonella* strains. In addition, these results also showed that 2 strains of *Salmonella* had both the *qnrB* and *qnrS* genes. Genotypic analyses also revealed considerable levels of *qnr* genes while phenotypic tests revealed a low level of resistance to quinolones. This result could have been due to a mutation in the gene that caused the non-expression of the *qnr* gene present.

Conclusion

This study showed that cattle can act as reservoirs for the spread of quinolone-resistant *Salmonella* strains. Although quinolones and fluoroquinolones are antibiotics of choice in human and veterinary medicine, several studies have confirmed the emergence and increasing prevalence of *Salmonella* resistance to this antibiotic family. Monitoring the use of quinolones and monitoring the spread of quinolone resistance genes is therefore important for the control of antibiotic resistance in *Salmonella* strains.

Acknowledgements

Our sincerest thanks go to the University Felix Houphouet Boigny, Pasteur Institute of Côte d'Ivoire, for hosting this research. Special thanks to the Technical University of Denmark (DUT) for providing the PCR reference strains. We are also indebted to all those who participated in this study.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Volume 15 Issue 12 December 2019