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Prevalence and antimicrobial resistance of *Salmonella* and *Yersinia* in the feces of hunted wildlife in Abeokuta, Nigeria

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Keywords

Salmonella, *Yersinia*, antimicrobial resistance, game, zoonoses, Nigeria

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Summary

Meat from wildlife contributes significantly to food security and income generation in many African communities. Salmonellae and yersiniae are important causes of foodborne infections. This study investigated the presence and antimicrobial resistance of salmonellae and yersiniae in the fecal contents of hunted wild rodents and ruminants at a wildlife meat-processing center in Abeokuta, Nigeria. Bacteria were isolated and identified by selective culture methods and biochemical characterization. Antimicrobial susceptibility was determined by the Kirby Bauer disk diffusion method. Salmonellae were isolated from 15 (9.8%) and yersiniae from 11 (7.2%) samples out of 153. Salmonellae were detected in nine cane rats (*Thryonomys swinderianus*), five royal antelopes (*Neotragus pygmaeus*) and one African giant rat (*Cricetomys gambianus*). Yersiniae were detected in eight cane rats, two royal antelopes and one waterbuck (*Kobus ellipsiprymnus*). The levels of resistance in *Salmonella* isolates were 100% for ampicillin and ceftiofur, 93.3% for tetracycline, 33.3% for cefotaxime, 26.7% for ceftazidime, 13.3% for amoxicillin/clavulanic acid, nalidixic acid and sulfamethoxazole/trimethoprim, and 6.7% for gentamicin, streptomycin and norfloxacin. The levels of resistance in yersiniae isolates were 81.8% for ampicillin, 72.7% for ceftiofur, 63.6% for nalidixic acid, 54.5% for cefotaxime, ceftazidime and sulfamethoxazole/trimethoprim, 36.4% for tetracycline, 27.3% for amoxicillin/clavulanic acid and streptomycin, 18.2% for ciprofloxacin, and 9.1% for chloramphenicol and gentamicin. All the isolates showed multiresistance to antimicrobials from at least three different classes. The detection of antimicrobial resistant salmonellae and yersiniae in wildlife is of veterinary and public health significance as these organisms can be transmitted to domestic animals and humans.

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■ INTRODUCTION

Hunted wildlife is a major source of animal protein to support families in many rural African communities. In some rural communities, game is people's main source of meat. In addition, game meats are considered special delicacies in the diet of people of all social statuses across Africa. Hunters also sell game to generate income. Therefore, wildlife hunting contributes to the food security and socioeconomic viability of many countries in Africa (Schulte-Herbrüggen et al., 2013).

Processing and consuming game meat can expose people to a similar risk of foodborne infection to that originating from processed livestock meat. Game meat consumption may pose a higher risk of disease transmission because processing of hunted game is not subjected to routine meat inspection. *Salmonella* spp. and *Yersinia* spp. are important human pathogens transmissible directly or indirectly from animal sources (Saraka et al., 2017; Uche et al., 2017). *Salmonella* in particular is a leading cause of foodborne infection all over the world

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(Majowicz et al., 2010). An earlier study has reported a higher prevalence of *Salmonella enterica* serovars (2.9%) than *Escherichia coli* O157:H7 (0.2%) in samples from wild rodents (Kilonzo et al., 2013). Contact with animals and consumption of food of animal origin are risk factors in the transmission of salmonellae and yersiniae. Infections are characterized by gastroenteritis, septicemia and other complications that can sometimes be fatal (Uche et al., 2017). In order to limit the fatal consequences of infections, physicians prescribe antimicrobial agents. However, the involvement of antimicrobial resistant strains could lead to therapy failure with protracted illness, high medical care costs, increased spread of infection, and high fatality rates, among other problems. Multidrug resistant non-typhoidal *Salmonella* poses a threat in sub-Saharan Africa with the possibility of zoonotic transmission from animal reservoirs (Balasubramanian et al., 2018).

The challenge of antimicrobial resistance is worldwide. The increasing report of antimicrobial resistance in commensal and pathogenic bacteria has reached an alarming state indicating that the end of the antibiotic era is probably nearer than envisaged (Harrison and Svec, 1998a; 1998b). Although every form of antimicrobial usage could lead to the selection of resistant strains, overdependence on antimicrobial agents for therapeutic, prophylactic and growth promotion purposes in livestock production favors the accelerated emergence and widespread dissemination of resistance traits in bacteria. Food animals are recognized as major sources for the distribution of antimicrobial resistant bacteria that could be transmitted to humans through the food chain. Similarly, the presence of antimicrobial resistant bacterium strains has been reported in wildlife (Literak et al., 2010). Therefore, the role of wildlife in the dissemination of antimicrobial resistance in the environment cannot be ignored (Furness et al., 2017). The present study investigated the presence and antimicrobial resistance of salmonellae and yersiniae in the feces of hunted wildlife, while being processed for public consumption in Abeokuta, Nigeria.

■ MATERIALS AND METHODS

Sample collection

Fecal contents were collected from the rectum of hunted wildlife during evisceration at a processing center in Abeokuta, Nigeria. Abeokuta is the capital and largest city of Ogun State with a population of about 450,000 inhabitants. It is located at 7º 10' N, 3º 21' E, about 77 kilometers north of Lagos. The processing center is a point of collection, dressing and sale of game meat. Processing involved evisceration, singeing for hair removal and thorough washing of the carcasses. Sometimes, the carcasses were roasted before sale. There was no form of meat inspection of the game before, during and after processing. Carcasses of hunted game were brought to the processing center very early in the morning (4:00–6:00) after overnight hunting. This corresponded to about four to eight hours after the death of the animals. Carcasses were transported in sacks, bowls or hand-woven baskets. There was no official documentation on the activities of game meat processing in the center. During the period of sample collection, it was observed that at least 20 carcasses were processed daily. All animals included in the study were fresh carcasses without obvious signs of autolysis. The animals were hunted in the vegetation of immediate rural communities around Abeokuta. However, the specific origin of individual animals could not be ascertained.

A total of 153 samples were collected that represented four categories of game: 108 cane rats (*Thryonomys swinderianus*), 40 royal antelopes (*Neotragus pygmaeus*), 3 African giant rats (*Cricetomys gambianus*), and 2 waterbucks (*Kobus ellipsiprymnus*). Visits were made to the processing center once a week between January and May 2013. The samples were labeled, preserved in icepacks and transported to the Veterinary Microbiology Laboratory of the Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Nigeria, for microbiological analysis. They were analyzed within 24 hours of collection.

Isolation and identification of salmonellae

Ten grams of each fecal content was homogenized and inoculated into 90 ml of buffered peptone water (BPW CM0509, Oxoid, Basingstoke, UK) for pre-enrichment. They were incubated at 37°C for 18–24 hours. Subsequently, 0.1 ml of the pre-enrichment culture was transferred onto modified semisolid Rappaport Vassiliadis broth (MSRV CM0910, Oxoid) with novobiocin supplement (Oxoid SR0161) for enhanced selective isolation of *Salmonella* at 42°C. After 24 hours of incubation, the MSRV culture was observed for growth of motile *Salmonella* as indicated by the presence of migrating opaque halo larger than 20-mm circumference around the point of inoculation. Subcultures were made from the periphery of the spreading growth on MRSV onto xylose-lysine desoxycholate agar (XLD CM0469, Oxoid) and modified brilliant green agar (mBGA CM0329, Oxoid) containing sulphamandelate supplement (Oxoid SR0087). Both XLD and mBGA cultures were incubated at 37°C for 24 hours. Suspected *Salmonella* colonies (pink colonies with black centers on XLD, and red to pink-white opaque colonies surrounded by red zones on mBGA) were selected for biochemical characterization using a biochemical test kit (Oxoid Microbact GNB 24E). Isolates identified as *Salmonella* were tested serologically for the detection of *Salmonella* somatic (O) and flagellum (H) antigens by slide agglutination using *Salmonella* polyvalent O (BD Difco Salmonella O Antiserum) and H (Oxoid Salmonella Test Kit, DR1108) antisera, according to the manufacturer's instruction.

Isolation and identification of yersiniae

Ten grams of each fecal content was inoculated into 90 ml of buffered peptone water and kept in the refrigerator at 4°C for three weeks for cold enrichment. Subcultures were made weekly from BPW onto yersinia selective agar base (Oxoid CM0653) containing yersinia selective supplement (Oxoid SR0109: cefsulodin, irgasan and novobiocin). The subcultures were incubated at 35°C for 24 hours. They were examined for colonies with deep red centers and transparent margins (bull's eye). Presumptive *Yersinia* colonies were identified by biochemical characterization using a test kit for the identification of Gram-negative, oxidase-negative bacilli (Oxoid Microbact GNB 24E). The results of biochemical reactions were interpreted according to the manufacturer's instruction using the accompanying computer software package (Oxoid Microbact 2000 vers. 2.03).

Antimicrobial susceptibility testing

All identified isolates were tested for susceptibility to ampicillin (10 µg), amoxicillin/clavulanic acid (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftiofur (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), gentamicin (30 µg), streptomycin (10 µg), sulfamethoxazole/trimethoprim (25 µg) and tetracycline (30 µg) by the Kirby Bauer disk diffusion method. Results were interpreted according to the guideline of the Clinical and Laboratory Standards Institute (CLSI, 2012) (Table I). *Escherichia coli* ATCC25922 was tested along with the isolates for quality control.

■ RESULTS

Salmonellae were isolated from 15 (9.8%) samples out of 153 (Table II). The positive samples were from cane rats (9/108, 8.3%), royal antelopes (5/40, 12.5%) and an African giant rat (1/3, 33.3%).

Table I

Interpretative breakpoints according to CLSI (2012) for the determination of antimicrobial susceptibility of salmonellae and yersiniae isolated from hunted wildlife in Abeokuta, Nigeria

Antimicrobial agent	Zone diameter breakpoints for disk diffusion test (mm)		
	Susceptible	Intermediate	Resistant
Ampicillin	≥ 17	14–16	≤ 13
Amoxicillin / clavulanic acid	≥ 18	14–17	≤ 13
Cefotaxime	≥ 26	23–25	≤ 22
Ceftiofur	≥ 21	18–20	≤ 17
Ceftazidime	≥ 21	18–20	≤ 17
Chloramphenicol	≥ 18	13–17	≤ 12
Ciprofloxacin	≥ 21	16–20	≤ 15
Gentamicin	≥ 15	13–14	≤ 12
Nalidixic acid	≥ 19	14–18	≤ 13
Norfloxacin	≥ 17	13–16	≤ 12
Streptomycin	≥ 15	12–14	≤ 11
Sulfamethoxazole/ trimethoprim	≥ 16	11–15	≤ 10
Tetracycline	≥ 15	12–14	≤ 11

Yersiniae were isolated from 11 (7.2%) samples out of 153. *Yersinia pseudotuberculosis* was isolated from nine (5.9%) samples out of 153, whereas *Y. aldovae* was isolated from two (1.3%) samples. *Y. pseudotuberculosis* was detected in cane rats (8/108, 7.4%) and a royal antelope (1/40, 2.5%). *Y. aldovae* was isolated from a royal antelope (1/40, 2.5%) and a waterbuck (1/2, 50%).

Salmonella isolates showed complete (100%) resistance to ampicillin and ceftiofur, and 93.3% to tetracycline (Table III). Other

Table II

Occurrence of salmonellae and yersiniae in hunted wildlife in Abeokuta, Nigeria

Wildlife species (n)	Number (%) of bacterial species		
	Salmonella	*Yersinia pseudotuberculosis*	*Y. aldovae*
Cane rat (108)	9 (8.3)	8 (7.4)	0
Royal antelope (40)	5 (12.5)	1 (2.5)	1 (2.5)
African giant rat (3)	1 (33.3)	0	0
Waterbuck (2)	0	0	1 (50.0)
Total (153)	15 (9.8)	9 (5.9)	2 (1.3)

antimicrobial resistance of *Salmonella* ranged from 33.3% for cefotaxime to zero for chloramphenicol and ciprofloxacin. *Yersinia* isolates showed 81.8% resistance to ampicillin, 72.7% to ceftiofur, 63.6% to nalidixic acid, and 54.5% to cefotaxime, ceftazidime and sulfamethoxazole/trimethoprim (Table III). Other antimicrobial resistance of *Yersinia* ranged from 45.5% for cephalexin to zero for norfloxacin. All the isolates in this study showed multiple antimicrobial resistance with resistance to antimicrobials from at least three different classes.

■ DISCUSSION

Oboegbulem and Okoronkwo (1990) reported a *Salmonella* prevalence of 32% from the intestine, spleen and liver of hunted greater cane rats (*Thryonomys swinderianus*) in Nssuka, Nigeria, which was higher than that of 8.3% observed in our study from the fecal contents of hunted games. To our knowledge, there is no previous report on the detection of *Salmonella* in the waterbuck and the African giant rat in Nigeria. The detection of *Salmonella* from these wildlife sources shows that wildlife species should be considered as important reservoirs of *Salmonella* that could be transmitted to domestic

Table III

Antimicrobial susceptibility profile (%) of salmonellae and yersiniae isolated from hunted wildlife in Abeokuta, Nigeria

Antimicrobial agent	Salmonellae			Yersiniae		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Ampicillin	100	0	0	81.8	18.2	0
Amoxicillin / clavulanic acid	13.3	46.7	40	27.3	36.4	36.3
Cefotaxime	33.3	66.7	0	54.5	36.4	9.1
Ceftiofur	100	0	0	72.7	18.2	9.1
Ceftazidime	26.7	46.7	26.6	54.5	45.5	0
Cephalexin	53.3	20	26.7	45.5	9.1	45.4
Chloramphenicol	0	60	40	9.1	72.7	18.2
Gentamicin	6.7	0	93.3	9.1	18.2	72.7
Ciprofloxacin	0	20	80	18.2	54.5	27.3
Nalidixic acid	13.3	86.7	0	63.6	36.4	0
Norfloxacin	6.7	0	93.3	0	63.6	36.4
Streptomycin	6.7	66.7	26.6	27.3	27.3	45.4
Sulfamethoxazole/ trimethoprim	13.3	26.7	60	54.5	18.2	27.3
Tetracycline	93.3	6.7	0	36.4	54.5	9.1

animals and humans through direct and indirect contacts as well as through consumption of contaminated foods (including the meat of these animals) and water. Improper disposal of intestinal contents of hunted game especially during processing can also lead to environmental contamination and widespread dispersal of potentially zoonotic pathogens leading to possible disease outbreaks or sporadic infections. Hunters and processors of game meat are particularly at risk of acquiring *Salmonella* infections from hunted game. Wild animals may also be an important source of *Salmonella* transmission to domestic animals through contamination of farm environment as well as via fecal contamination of feed and water sources. The nomadic system of ruminant management in West Africa, whereby pastoralists and their livestock migrate from place to place through grasslands and forests, can increase livestock infectious contacts with wildlife and their fecal contaminants, thereby increasing the possibility of exchange of pathogenic microorganisms between wildlife and livestock.

In the present study, *Y. pseudotuberculosis* was isolated from cane rats and royal antelopes. Previous studies have established the reservoirs of *Y. pseudotuberculosis* in wildlife and its zoonotic transmission to humans (Fukushima et al., 1988; Welsh et al., 1992). *Y. pseudotuberculosis* is a zoonotic bacterial pathogen associated with acute gastroenteritis and mesenteric lymphadenitis (Long et al., 2010). The involvement of *Y. pseudotuberculosis* in outbreaks of foodborne infections has been documented (Jalava et al., 2004). Unfortunately, *Y. pseudotuberculosis* is often overlooked leading to misdiagnosis and underestimation of its role as an etiology of gastrointestinal infections (Long et al., 2010). *Y. aldovae* was isolated from a royal antelope and a waterbuck. It is usually isolated from aquatic environments and from fish (Bercovier et al., 1984) but it rarely causes clinical infection. The animals that harbored *Y. aldovae* in this study could have acquired the organism from drinking contaminated water.

There is a dearth of information on the prevalence and antimicrobial resistance of bacteria in Nigerian wildlife populations. Most epidemiological surveillance and antimicrobial monitoring programs do not much address the possible role of wildlife as reservoirs in the emergence, environmental dissemination and food contamination of antimicrobial resistant pathogens. Nevertheless, high rates of antimicrobial resistance have been reported among bacteria isolated from food animal species.

In our study, salmonellae and yersiniae from hunted wildlife showed resistance to a wide range of antimicrobial agents from different classes including the β-lactams (cefotaxime, ceftazidime, ceftiofur), aminoglycosides (streptomycin, gentamicin), quinolones (nalidixic acid, ciprofloxacin, norfloxacin), phenicols (chloramphenicol), and folic acid inhibitors (sulfamethoxazole/trimethoprim combination), and tetracycline. In our study salmonellae and yersiniae also showed a very high degree of resistance to ampicillin and tetracycline, which are the most commonly used antimicrobial agents in veterinary and human medical practices (Ojo et al., 2017). This is similar to other reports where *Salmonella* isolates from chickens and Japanese quails showed a high level of resistance to ampicillin (80.0% to 100%) and tetracycline (35% to 100%) (Ojo et al., 2012; Omoshaba et al., 2017). The rates of resistance to gentamicin (6.7% in *Salmonella* and 9.1% in *Yersinia*) observed in this study were lower than that of 21.4% reported in *Salmonella* in Japanese quails (Omoshaba et al., 2017). Similarly, *Salmonella* and *Yersinia* isolates from this study showed a lower level of resistance to streptomycin (6.7% and 27.3, respectively) than the 57.2% resistance reported in *Salmonella* in Japanese quails (Omoshaba et al., 2017). *Salmonella* and *Yersinia* isolates showed a low resistance below 20.0% to fluoroquinolones (ciprofloxacin and norfloxacin) similar to those reported by Ojo et al. (2012) and Omoshaba et al. (2017). The 26.7% resistance to ceftazidime among *Salmonella* isolates in this study was lower than the 78.6% reported by Omoshaba et al. (2017). In the present study, *Salmonella* showed 100% and *Yersinia* 72.7% resistance to ceftiofur. Previous studies on antimicrobial resistance in *Salmonella* of livestock origin in Nigeria did not include ceftiofur among the antimicrobial agents tested. However, the ceftiofur resistance rates observed here were higher than the 6.0% to 62.0% rates reported in *S. enterica* serovar Heidelberg from chicken meat and humans in Canada (Dutil et al., 2010), 18.1% in *S.* Typhimurium from poultry in Brazil (Biffi et al., 2014), and 30.7% to 36.7% in *Salmonella* from swine and cattle in Minnesota, United States (Hong et al., 2016).

Differences in antimicrobial resistance profiles of bacterium strains between countries may be caused by differences in antimicrobial usage and related practices. Resistance to third-generation cephalosporins and fluoroquinolones is particularly worrisome because of the importance of these drugs as last-resort antimicrobial agents in the treatment of infections that are refractory to treatment by the older generation of antimicrobial agents (Chen et al., 2013; Lunguya et al., 2013). The fluoroquinolones are particularly useful in the treatment of human salmonellosis (Chen et al., 2013). Salmonellae showing resistance to fluoroquinolones are probably more invasive and capable of causing more fulminating infections in humans (Kariuki et al., 2015).

Exposure to antimicrobial agents is a major factor in the emergence, persistence and spread of antimicrobial resistant bacteria. Unlike livestock that are usually exposed to antimicrobial agents through therapeutic, prophylactic and growth promotion applications, wildlife is mainly exposed to antimicrobial agents through environmental pollution (Radhouani et al., 2014; Singer et al., 2016). Improper disposal of antimicrobial packages, migration of wildlife (searching for food) to dwelling areas and farms, discharge of animal effluents from commercial livestock farms and abattoirs into water bodies, discharge of antimicrobial waste from pharmaceutical factories into water bodies are among the various factors that influence exposure of wildlife to antimicrobial residues as well as acquisition of resistant bacteria or resistance genes (Kümmerer, 2004; Radhouani et al., 2014). Antimicrobial usage is a very common practice in Nigerian food animal production (Ojo et al., 2016; 2017). Farmers depend heavily on antimicrobials for growth promotion as well as for the prevention and treatment of infections. Ogun State is the largest producer of poultry. It has clusters of large, medium and small-scale commercial poultry farms. Wastes from these farms and from abattoirs are washed into Ogun River, which serves as the main source of drinking water for wildlife and nomadic livestock during the dry season.

■ CONCLUSION

The emergence of multidrug-resistant bacteria in wildlife observed in the present study is of public health significance. Avoiding contact with wildlife may prevent the transmission of pathogens from these animals to humans. Adherence to the principles of hygiene during processing and marketing of wildlife meat could help limit meat contamination, and cooking meat before consumption may destroy foodborne pathogens. However, cooking may not destroy the antimicrobial resistance genes. Responsible antimicrobial stewardship as well as proper treatment of human and animal wastes before disposal may limit contamination of the environment and thus the transfer of resistance from humans and livestock to wildlife. This study is a preliminary assessment of the role of wildlife as reservoirs of antimicrobial resistant foodborne pathogens. Further studies on antimicrobial resistance in wildlife involving larger collections of samples across wider geographical locations are needed for better understanding of the role of wildlife in the emergence and spread of antimicrobial resistant pathogens.

Author contributions statement

OEO and MAO conceptualized and designed the study. OEO and OOO were responsible for sample collection and laboratory works. OEO, OOO and MAO participated in data analysis and result interpretation. OEO and OOO prepared the draft manuscript. MAO, MAD and EBO critically reviewed the manuscript.

REFERENCES

Balasubramanian R., Im J., Lee J.-S., Jeon H.J., Mogeni O.D., Kim J.H., Rakotozandrindrainy R., et al., 2019. The global burden and epidemiology of invasive non-typhoidal *Salmonella* infections. *Hum. Vaccines Immunother.*, **15** (6), 1421-1426, doi: 10.1080/21645515.2018.1504717

Bercovier H., Steigerwalt A.G., Guiyoule A., Huntley-Carter G., Brenner D.J., 1984. *Yersinia aldovae* (formerly *Yersinia enterocolitica*-like group X2): a new species of *Enterobacteriaceae* isolated from aquatic ecosystems. *Int. J. Syst. Evol. Bacteriol.*, **34** (2): 166-172, doi: 10.1099/00207713-34-2-166

Biffi C.P., Stefani L.M., Miletti L.C., Matiello C.A., Backe R.G., Almeida J.M., Neves G.B., 2014. Phenotypic and genotypic resistance profile of *Salmonella* Typhimurium to antimicrobials commonly used in poultry. *Rev. Bras. Cienc. Avic.*, 16 (2): 93-96, doi: 10.1590/1516-635x160293-96

Chen H.-M., Wang Y., Su L.-H., Chiu C.-H., 2013. Nontyphoid *Salmonella* infection: microbiology, clinical features, and antimicrobial therapy. *Pediatr. Neonatol.,* **54**: 147-152, doi: 10.1016/j.pedneo.2013.01.010

CLSI, 2012. Performance standards for antimicrobial susceptibility testing: 25th informational suppl. M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA, USA

Dutil L., Irwin R., Finley R., Ng L.K., Avery B., Boerlin P., Bourgault A.-M., et al., 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans. *Emerg. Infect. Dis.,* **16** (1): 48-54, doi: 10.3201/eid1601.090729

Fukushima H., Gomyoda M., Shiozawa K., Kaneko S., Tsubokura M., 1988. *Yersinia pseudotuberculosis* infection contracted through water contaminated by a wild animal. *J. Clin. Microbiol.,* **26** (2): 584-585

Furness L.E., Campbell A., Zhang L., Gaze W.H., McDonald R.A., 2017. Wild small mammals as sentinels for the environmental transmission of antimicrobial resistance. *Environ. Res.,* **154**: 28-34, doi: 10.1016/j.envres.2016.12.014

Harrison J.W., Svec T.A., 1998a. The beginning of the end of the antibiotic era? Part I. The problem: Abuse of the "miracle drugs". *Quintessence Int.,* **29** (3): 151-162

Harrison J.W., Svec T.A., 1998b. The beginning of the end of the antibiotic era? Part II. Proposed solutions to antibiotic abuse. *Quintessence Int.,* **29** (4): 223-229

Hong S., Rovira A., Davies P., Ahlstrom C., Muellner P., Rendahl A., Olsen K., et al., 2016. Serotypes and antimicrobial resistance in *Salmonella enterica* recovered from clinical samples from cattle and swine in Minnesota, 2006 to 2015. *Plos One*, **11** (12): e0168016, doi: 10.1371/journal.pone.0168016

Jalava K., Hallanvuo S., Nakari U.-M., Ruutu P., Kela E., Heinäsmäki T., Siitonen A., et al., 2004. Multiple outbreaks of *Yersinia pseudotuberculosis* infections in Finland. *J. Clin. Microbiol.*, **42** (6): 2789-2791, doi: 10.1128/JCM.42.6.2789–2791.2004

Kariuki S., Gordon M.A., Feasey N., Parry C.M., 2015. Antimicrobial resistance and management of invasive *Salmonella* disease. *Vaccine*, **33** (3): C21-C29, doi: 10.1016/j.vaccine.2015.03.102

Kilonzo C., Li X., Vivas E.J., Jay-Russell M.T., Fernandez K.L., Atwilla E.R., 2013. Fecal shedding of zoonotic foodborne pathogens by wild rodents in a major agricultural region of the Central California Coast. *Appl. Environ. Microbiol.*, **79** (20): 6337-6344, doi: 10.1128/AEM.01503-13

Kümmerer K., 2004. Resistance in the environment. *J. Antimicrob. Chemother.*, **54** (2): 311-320, doi: 10.1093/jac/dkh325

Literak I., Dolejska M., Radimersky T., Klimes J., Friedman M., Aarestrup F.M., Hasman H., et al., 2010. Antimicrobial-resistant faecal *Escherichia coli* in wild mammals in central Europe: multiresistant *Escherichia coli* producing extended-spectrum beta-lactamases in wild boars. *J. Appl. Microbiol.,* **108**: 1702-1711, doi: 10.1111/j.1365-2672.2009.04572.x

Long C., Jones T.F., Vugia D.J., Scheftel J., Strockbine N.A., Ryan P., Shiferaw B., et al., 2010. *Yersinia pseudotuberculosis* and *Y. enterocolitica* infections, FoodNet, 1996–2007. *Emerg. Infect. Dis.*, **16** (3): 566-567, doi: 10.3201/eid1603.091106

Lunguya O., Lejon V., Phoba M.-F., Bertrand S., Vanhoof R., Glupczynski Y., Verhaegen J., et al., 2013. Antimicrobial resistance in invasive non-typhoid *Salmonella* from the Democratic Republic of the Congo: emergence of decreased fluoroquinolone susceptibility and extended-spectrum beta-lactamases. *PLoS Negl. Trop. Dis.,* **7** (3): e2103, doi: 10.1371/journal.pntd.0002103

Majowicz S.E., Musto J., Scallan E., Angulo F.J., Kirk M., O'Brien O.J., Jones T.F., et al., 2010. The global burden of nontyphoidal salmonella gastroenteritis. *Clin. Infect. Dis.,* **50**: 882-889, doi: 10.1086/650733

Oboegbulem S.I., Okoronkwo I., 1990. Salmonellae in the African great cane rat (*Thryonomys swinderianus*). *J. Wildl. Dis.,* **26** (1): 119-121, doi: 10.7589/0090-3558-26.1.119

Ojo O.E., Awoyomi O.J., Fabusoro E., Dipeolu M.A., 2017. Activities and influence of veterinary drug marketers on antimicrobial usage in livestock production in Oyo and Kaduna States, Nigeria. *J. Agric. Rural Dev. Trop. Subtrop.,* **118** (2): 207-216

Ojo O.E., Fabusoro E., Majasan A.A., Dipeolu M.A., 2016. Antimicrobials in animal production: usage and practices among livestock farmers in Oyo and Kaduna states of Nigeria. *Trop. Anim. Health Prod.,* **48**: 189-197, doi: 10.1007/s11250-015-0939-8

Ojo O.E., Ogunyinka O.G., Agbaje M., Okuboye J.O., Kehinde O.O., Oyekunle M.A., 2012. Antibiogram of *Enterobacteriaceae* isolated from free-range chickens in Abeokuta, Nigeria. *Vet. Arhiv.,* **82** (6): 577-589

Omoshaba E.O., Olufemi F.O., Ojo O.E., Sonibare A.O., Agbaje M., 2017. Multidrug-resistant salmonellae isolated in Japanese quails reared in Abeokuta, Nigeria. *Trop. Anim. Health Prod.,* **49**: 1455-1460, doi: 10.1007/s11250-017-1347-z

Radhouani H., Silva N., Poeta P., Torres C., Correia S., Igrejas, G., 2014. Potential impact of antimicrobial resistance in wildlife, environment, and human health. *Front. Microbiol.,* **5**: 23, doi: 10.3389/fmicb.2014.00023

Saraka D., Savin C., Kouassi S., Cissé B., Koffi E., Cabanel N., Brémont S., et al., 2017. *Yersinia enterocolitica*, a neglected cause of human enteric infections in Côte d'Ivoire. *PLoS Negl. Trop. Dis.,* **11** (1): e0005216, doi: 10.1371/journal. pntd.0005216

Schulte-Herbrüggen B., Cowlishaw G., Homewood K., Rowcliffe J.M., 2013. The importance of bushmeat in the livelihoods of West African cash-crop farmers living in a faunally-depleted landscape. *PLoS One,* **8** (8): e72807, doi: 10.1371/journal.pone.0072807

Singer A.C., Shaw H., Rhodes V., Hart A., 2016. Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Front. Microbiol.*, **7**: 1728, doi: 10.3389/fmicb.2016.01728

Uche I.V., MacLennan C.A., Saul A., 2017. A systematic review of the incidence, risk factors and case fatality rates of invasive nontyphoidal *Salmonella* (iNTS) disease in Africa (1966 to 2014). *PLoS Negl. Trop. Dis.,* **11** (1): e0005118, doi: 10.1371/journal.pntd.0005118

Welsh R.D., Ely R.W., Holland R.J., 1992. Epizootic of *Yersinia pseudotuberculosis* in a wildlife park. *J. Am. Vet. Med. Assoc.,* **201** (1): 142-144

Résumé

Ojo O.E., Ogunjobi O.O., Oyekunle M.A., Dipeolu M.A., Otesile E.B. Prévalence et résistance antimicrobienne de salmonelles et de yersinias dans les fèces d'animaux sauvages issus de la chasse à Abeokuta, Nigeria

La viande de brousse contribue de manière significative à la sécurité alimentaire et à la génération de revenus de nombreuses communautés africaines. Les salmonelles et les yersinias sont des causes importantes d'infections alimentaires. Cette étude a porté sur la présence et la résistance antimicrobienne de salmonelles et de yersinias dans les fèces de rongeurs et de ruminants sauvages issus de la chasse dans un centre de traitement de viande de brousse à Abeokuta, au Nigeria. Les bactéries ont été isolées et identifiées par culture sélective et caractérisation biochimique. La sensibilité aux antimicrobiens a été déterminée par la méthode de diffusion sur disque Kirby Bauer. Des salmonelles ont été isolées dans 15 (9,8 %) échantillons sur 153 et des yersinias dans 11 (7,2 %). Des salmonelles ont été détectées chez neuf aulacodes (*Thryonomys swinderianus*), cinq antilopes royales (*Neotragus pygmaeus*) et un cricétome des savanes (*Cricetomys gambianus*). Des yersinias ont été détectées chez huit aulacodes, deux antilopes royales et un cobe (*Kobus ellipsiprymnus*). Les isolats de salmonelles ont présenté des taux de résistance de 100 % à l'ampicilline et au ceftiofur, de 93,3 % à la tétracycline, de 33,3 % à la céfotaxime, de 26,7 % à la ceftazidime, de 13,3 % à l'amoxicilline / acide clavulanique, à l'acide nalidixique et au sulfaméthoxazole/triméthoprime, et de 6,7 % à la gentamicine, à la streptomycine et à la norfloxacine. Les isolats de yersinias ont révélé des taux de résistance de 81,8 % à l'ampicilline, de 72,7 % au ceftiofur, de 63,6 % à l'acide nalidixique, de 54,5 % à la céfotaxime, à la ceftazidime et au sulfaméthoxazole/triméthoprime, de 36,4 % à la tétracycline, de 27,3 % à l'amoxicilline / acide clavulanique et à la streptomycine, de 18,2 % à la ciprofloxacine, et de 9,1 % au chloramphénicol et à la gentamicine. Tous les isolats ont présenté une multirésistance à au moins trois classes différentes d'antimicrobiens. La détection de salmonelles et de yersinias résistantes aux antimicrobiens chez les animaux sauvages révèle un problème de santé publique et vétérinaire, car ces organismes peuvent être transmis aux humains et aux animaux domestiques.

Mots-clés : *Salmonella*, *Yersinia*, résistance aux antimicrobiens, gibier, zoonose, Nigeria

Resumen

Ojo O.E., Ogunjobi O.O., Oyekunle M.A., Dipeolu M.A., Otesile E.B. Prevalencia y resistencia antimicrobiana a *Salmonella* y *Yersinia* en las heces de fauna cazada en Abeokuta, Nigeria

La carne de fauna silvestre contribuye significativamente a la seguridad alimenticia y a la generación de ingresos en muchas comunidades africanas. *Salmonella* y *Yersinia* son causas importantes de infecciones alimenticias. El presente estudio investigó la presencia y resistencia antimicrobiana a *Salmonella* y *Yersinia* en los contenidos fecales de roedores y rumiantes cazados, en una planta procesadora de carne de fauna silvestre en Abeokuta, Nigeria. Se aislaron e identificaron bacterias mediante medios de cultura selectivos y caracterización bioquímica. La susceptibilidad antimicrobiana se determinó mediante el método de difusión en disco de Kirby Bauer. *Salmonella* se aisló en 15 (9,8%) y *Yersinia* en 11 (7,2%) de las 153 muestras. *Salmonella* se detectó en nueve ratas de caña (*Thryonomys swinderianus*), cinco antílopes reales (*Neotragus pygmaeus*) y una rata gigante africana (*Cricetomys gambianus*). *Yersinia* se detectó en ocho ratas de caña, dos antílopes reales y un antílope acuático (*Kobus ellipsiprymnus*). Los niveles de resistencia en los aislamientos de *Salmonella* fueron de 100% para ampicilina y ceftiofur, 93,3% para tetraciclina, 33,3% para cefotaxima, 26,7% para ceftazidima, 13,3% para amoxicilina / ácido clavulánico, ácido nalidíxico y sulfametoxazol trimetroprima, y 6,7% para gentamicina, estrepnomicina y norfloxacina. Los niveles de resistencia en aislamientos de *Yersinia* fueron de 81,8% para ampicilina, 72,7% para ceftiofur, 63,6% para ácido nalidíxico, 54,5% para cefotaxima, ceftazidima y sulfametoxazol trimetroprima, 36,4% para tetraciclina, 27,3% para amoxicilina / ácido clavulánico y estrepnomicina, 18,2% para ciprofloxacina, y 9,1% para cloranfenicol y gentamicina. Todos los aislamientos mostraron resistencia múltiple a por lo menos tres clases diferentes de antimicrobianos. La detección de la resistencia antimicrobiana a *Salmonella* y *Yersinia* en fauna silvestre tiene un significado en salud pública y veterinaria, ya que estos organismos pueden transmitirse a animales domésticos y humanos.

Palabras clave: *Salmonella*, *Yersinia*, resistencia a los antimicrobianos, animales de caza, zoonosis, Nigeria

Antimicrobial use and detection of cefotaxime-resistant Enterobacteriaceae in the pig production chain, Ogun State, Nigeria

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Keywords

Swine, *Escherichia coli, Klebsiella pneumoniae*, Enterobacteriaceae, antimicrobial resistance, cefotaxime, Nigeria

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Summary

Cefotaxime belongs to the third-generation cephalosporin group of antimicrobials, which are classified as critical for the treatment of infections in humans. The upsurge in the incidence of cefotaxime-resistant (C-R) bacteria from animal sources is of global public health importance. This study investigated the presence of C-R Enterobacteriaceae in the pig production chain in Ogun State, Nigeria, and examined C-R isolates for production of extended-spectrum β-lactamase (ESBL). The knowledge, attitude and practices of pig farmers regarding antimicrobial usage were also investigated. C-R bacteria were detected in 54 (17.8%) out of 303 samples. C-R isolates were identified as *Escherichia coli* (n = 22), *Klebsiella* spp. (n = 17), *Enterobacter aerogenes* (n = 10) and *Citrobacter freundii* (n = 5). The organisms were present in feces from on-farm pigs (15/109; 13.7%), fresh pork at slaughterhouses (19/40; 47.5%), frozen pork at retail shops (7/28; 25.0%), cutting surfaces of butchers' tools (7/52; 13.5%), and abattoir effluent water (6/41; 14.6%). No C-R bacteria were detected in ready-to-eat pork. Three isolates of *Es. coli* and one of *K. pneumoniae* were ESBL-producers and possessed $bla_{CTX\text{-}M\text{-}15}$ ESBL gene variant. ESBL-producing *Es. coli* belonged to phylogenetic group A. All C-R isolates were resistant to more than three antimicrobials from different classes of antimicrobials. Tetracycline, ampicillin, amoxicillin, ciprofloxacin and enrofloxacin were among the commonly used antimicrobials in pig production, whereas cephalosporins were rarely used. Farmers knew that pigs could serve as reservoirs of pathogenic bacteria transmissible to humans. However, they were not aware that the use of antimicrobials in pig production could lead to the development and proliferation of antimicrobial-resistant bacteria in pigs. Efforts should be made to improve awareness among farmers on the roles of antimicrobial use in the emergence and dissemination of antimicrobial-resistant bacteria in animal production.

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■ INTRODUCTION

Cefotaxime is a broad-spectrum third-generation cephalosporin (Klein and Cunha, 1995). It has been used extensively for the treatment of infections caused by bacteria that are refractory to treatment with the older generation cephalosporins and penicillins (Neu, 1982). Subsequent to the first report of transferrable resistance to cefotaxime and other third and fourth generation cephalosporins among clinical bacterial isolates (Knothe et al., 1983; Sirot et al., 1987), there have been increasing reports of global emergence and widespread dissemination of resistant bacterial strains that harbor extended-spectrum cefotaximase from human, animal and environmental sources (Rossolini et al., 2008; Bevan et al., 2017).

Extensive use of cephalosporins might have contributed to the selection and proliferation of plasmids encoding extended-spectrum beta-lactamases (ESBLs) (Medeiros, 1997). It is also worrisome that such plasmids may additionally harbor genes encoding resistance to other classes of antimicrobials thereby conferring multidrug resistance traits on cefotaxime-resistant (C-R) bacteria (Canton and Coque, 2006). It is important to note that cefotaxime remains a preferred choice for the

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treatment of many complicated and uncomplicated infections such as urinary-tract infection and meningitis in humans. Indeed, the World Health Organisation listed cefotaxime among the critically important drugs (WHO, 2017). As such, the preservation of the continued efficacy of this drug is highly desirable. There are very limited options for the elimination of C-R bacteria in clinical infections.

Pig production and pork consumption contribute to the economic and nutritional well-being of many people in Nigeria (Obayelu et al., 2017). Nigeria is not self-sufficient in the supply of animal protein and pig production has enormous potential to fill the gaps. Pig has been described as the most important domestic animal in Nigeria in terms of the number of farmers engaged in its production, economic values, potentials for the supply of animal protein and role in the transmission of zoonotic infections (Ugbomoiko et al., 2008; Obayelu et al., 2017). Antimicrobials including cephalosporins are routinely used as a major input to boost animal production in Nigeria (Ojo et al., 2016a). The extensive use of third- and fourth-generation cephalosporins (3GC and 4GC) especially ceftiofur and cefquinone in food animals might have greatly facilitated the rapid development of 3GC-resistant bacteria in food animals and the spread of such resistant strains along the production and marketing chain (Cavaco et al., 2008; Wittum, 2012). There is evidence that the use of ceftiofur, cefquinone and related antimicrobials exerts selective pressure that promotes the development, proliferation and transmission of 3GC-resistant bacteria among farm animal populations and in farm environment (Cavaco et al.; 2008, Wittum, 2012). Thus, farm animals may serve as reservoirs of 3GC-resistant bacteria transmissible to humans through direct and indirect contact, contaminated environments and foods of animal origin.

Production of ESBLs is one of the major mechanisms of bacterial resistance to β-lactam antimicrobials. It confers resistance to a broad range of β-lactam antimicrobials including penicillin, cephalosporins (including 3GC) and monobactam (aztreonam) (Bradford, 2001). ESBL production can be acquired by plasmid transfer which is very common among Enterobacteriaceae. ESBL-producing bacteria are often multidrug resistant because of the colocation of resistance genes that confer resistance to other classes of antimicrobials on the ESBL-carrying plasmid. Thus, ESBL-producing bacteria play a significant role in the widespread dissemination of antimicrobial resistance among farm animals (Okpara et al., 2018). The use of third- or fourth-generation cephalosporin has been associated with increased detection of ESBL-producing *Escherichia coli* in pigs (Hammerum et al., 2014).

The present study investigated the occurrence of C-R Enterobacteriaceae in sources along the pig production chain from farms, slaughterhouses and markets in Ogun State, Nigeria. Cefotaxime-resistance bacteria were further examined for the production of ESBLs. Data were also collected on the knowledge, attitude and practices of pig farmers regarding antimicrobial usage in pig production.

■ MATERIALS AND METHODS

Sample collection

Samples were collected along the pig-production, pork-processing and marketing chain (hereafter called the pig-production chain) for the detection of C-R Enterobacteriaceae in six locations (Ifo, Sagamu, Oke-Aro, Abeokuta, Atan and Ijebu-Ode) in Ogun State. Six types of samples were collected (Table I): feces of on-farm pigs, fresh pork at slaughterhouses, swabs of cutting surfaces (knives and tables) at slaughterhouses, abattoir effluent water at points of discharge into natural flowing water bodies (streams and rivers), frozen pork at meat retail shops, and ready-to-eat pork from hawkers in open markets. One hundred and nine pooled fecal samples from pigs were collected irrespective of age, sex and breed in 60 farms. Two pooled fecal samples were collected from each of 49 farms whereas one pooled fecal sample was collected from each of 11 farms. Each pooled sample represented rectal swabs of 10 individual pigs from the same farm. Ten pigs were randomly sampled from every 50 pigs on each farm visited. Forty fresh and 28 frozen pork samples (5 g each) were collected from meat vendors at slaughterhouses and retail shops, respectively. Only one sample was collected from an individual vendor. Swabs of cutting surfaces were collected from 52 individual butchers at slaughterhouses. A single sterile swab was used for the knife and table of each butcher. Forty-one effluent water samples (5 ml each) from slaughterhouses (exclusively for pigs) were collected at the various points of discharge into natural flowing water bodies. Thirty-three ready-to-eat fried pork samples (5 g each) were collected from meat hawkers in open markets. Only one sample was collected from each hawker. Samples were collected aseptically (avoiding cross contamination), labeled appropriately and transported in icepacks to the laboratory for immediate microbiological analysis.

Isolation and identification of cefotaxime-resistant Enterobacteriaceae

Each sample was inoculated into buffer peptone water (BPW, Oxoid, Basingstoke, UK) and incubated at 37°C overnight for pre-enrichment. A loopful of the pre-enrichment broth culture was streaked onto MacConkey agar supplemented with ampicillin at 100 g/L ($MAC_{AMP-100}$) for the isolation of ampicillin-resistant bacteria. This was incubated at 37°C for 24 hours. Five colonies from each $MAC_{AMP-100}$ plate were inoculated onto separate MacConkey agar supplemented with cefotaxime at 1 g/L (MAC_{CTX-1}) and incubated at 37°C for 24 hours for the isolation of C-R strains. One distinct colony of C-R isolate was selected from each MAC_{CTX-1} plate. Selected isolates were preserved on nutrient agar slants for further analysis. Each selected C-R isolate was subjected to biochemical characterization for identification of Enterobacteriaceae using a commercially available biochemical kit (Oxoid Microbact GNB 24E) and interpreted using available computer software.

Phenotypic detection of ESBL-producing Enterobacteriaceae

All C-R isolates were tested for production of ESBL using the combination disk kit (Oxoid, Basingstoke) containing cefpodoxime (CPD, 10 µg) and cefpodoxime-clavulanic acid (CD 01, 10/1 µg). A fresh culture of the test organism on nutrient agar was emulsified in normal saline and adjusted to an optical density corresponding to 0.5 McFarland standard. This was spread evenly on Mueller Hinton agar (MHA) and the disks introduced firmly on the agar. The inoculated MHA was incubated at 35 ± 2°C for 16 hours. The difference in the zones of inhibition around the two disks was determined. Isolates that produced

Table I

Distribution of samples collected for the detection of cefotaxime-resistance Enterobacteriaceae in the pig production chain, Ogun State, Nigeria

Location	Feces	Fresh pork	Frozen pork	Effluent water	Ready-to-eat pork	Swab of cutting surface	Total
Ifo	12	7	4	2	5	3	32
Sagamu	17	7	6	3	5	8	43
Okearo	53	6	3	3	5	4	75
Abeokuta	22	5	5	4	7	2	40
Atan	5	5	4	11	6	8	35
Ijebu-ode	0	10	6	18	5	27	78
Total	109	40	28	41	33	52	303

differences equal to or greater than five millimeters in the diameter of zone of inhibition between cefpodoxime and cefpodoxime/clavulanic acid disks were identified as phenotypic ESBL producers (CLSI, 2018).

ESBL-gene detection and determination of phylogenetic groups of ESBL-producing Es. coli

The presence of ESBL-associated genes bla_{CTX}, bla_{SHV} and bla_{TEM} was investigated in phenotypic ESBL-producing isolates using polymerase chain reaction (PCR) assays, nucleotide sequencing and sequence analysis. Genomic deoxyribonucleic acid (DNA) was extracted from overnight tryptic soy broth culture of phenotypic ESBL-producing isolates by thermolysis according to Ojo et al. (2016b). DNA was quantified by nanospectrophotometry and adjusted to a final working concentration of 100 ng/µl. A multiplex PCR assay was used for the amplification and detection of ESBL-associated genes: $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}9}$, bla_{TEM} and bla_{SHV}. The PCR reaction mix and amplification conditions were as previously described (Gröbner et al., 2009; Cullik et al., 2010). The amplicons were electrophoresed in agarose gels stained with midori-green direct and visualized under ultraviolet transilluminator. Positive samples with targeted genes were further subjected to another round of PCR assays for sequencing of the whole $bla_{CTX\text{-}M\text{-}group1}$ and/or $bla_{CTX\text{-}M\text{-}group9}$ genes (LGC Genomic, Berlin, Germany). The nucleotide sequences were analyzed with bioinformatics software Geneious 10.1.3 (Biomatters, New Zealand). The ESBL gene variants were determined from analyzed sequences by comparing the nucleotide sequences with reference sequences from Lahey Clinic (www.lahey.org) and those deposited at the National Center for Biotechnology Information website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Furthermore, the phylogenetic groups of ESBL-producing *Es. coli* isolates were determined by a previously described PCR-based method (Clermont et al., 2013) for assigning *Es. coli* to one of seven *Es. coli sensu stricto* phylogenetic groups (A, B1, B2, C, D, E, and F).

Antimicrobial susceptibility testing

C-R isolates were tested for susceptibility to selected antimicrobials with the Kirby Bauer disk diffusion method according to the guideline of the Clinical and Laboratory Standards Institute (CLSI, 2018). The following antimicrobials were included: amikacin (AMK, 30 µg), ampicillin (AMP, 10 µg), amoxicillin/clavulanic acid (AMX, 30 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg), ceftazidime (CAZ, 30 µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NAL, 30 µg), gentamicin (GEN, 30 µg), kanamycin (KAN, 30 µg), streptomycin (STR, 10 µg), sulfamethoxazole (SXT, 25 µg), trimethoprim (TMP, 5 µg) and tetracycline (TET, 30 µg). A suspension of test organisms was prepared in normal saline and adjusted to a turbidity level of 0.5 McFarland standard (approximately 1×10^6 colony forming units/ml). The suspension was spread evenly on Mueller Hinton agar using a sterile cotton swab. The antimicrobial disks were placed firmly on the inoculated agar and incubated at 35 ± 2°C for 16 hours. The diameter of the zones of inhibition around each disk was measured and interpreted according to CLSI (2018). *Es. coli* ATCC 25922 was also tested for quality control.

Assessment of antimicrobial usage on pig farms

A structured questionnaire and in-depth interviews were used to collect data on knowledge, attitude and practices regarding antimicrobial usage on pig farms. Sixty farm managers/farmers or their representatives from 60 pig farms where fecal samples were collected were interviewed. Information was obtained on farm characteristics, commonly used antimicrobials, factors influencing the use of antimicrobials as well as practices regarding their administration. Farm characteristics related to farm size, management system, form of operation (breeding or fattening), year of establishment and presence of other animal species. Farmers were also asked to provide a complete list of antimicrobials routinely used on the farm. When an unfamiliar trade name of an antimicrobial was cited, the farmer was requested to provide its package in order to get the generic name of the antimicrobial(s). Questions were also asked on disease occurrence, diagnosis, management practices, and the reasons (prophylaxis, therapeutic and growth promotion) for antimicrobial use. Information was obtained on routes and duration of drug administration as well as the involvement of farm attendants in drug administration. A typical interview section lasted for about one hour.

Statistical analysis

Data were presented in absolute values and percentages. Data from different sample categories were compared with the Chi square test (significant values at $p < 0.05$).

■ RESULTS

Cefotaxime-resistant Enterobacteriaceae from the pig-production chain

Fifty-four (17.8%) of 303 samples yielded C-R isolates as follows: 15 (13.7%) from fecal samples, 19 (47.5%) from fresh pork, 7 (25.0%) from frozen pork, 6 (14.6%) from water discharge, and 7 (13.5%) from cutting surfaces (Table II). No C-R bacteria were detected in ready-to-eat pork. The rates of detection of C-R bacteria were significantly higher in fresh and frozen pork than in other sample types ($p < 0.05$). C-R isolates were identified as *Escherichia coli* (n = 22), *Klebsiella pneumoniae* (n = 17), *Enterobacter aerogenes* (n = 10), *K. oxytoca* (n = 2) and *Citrobacter freundii* (n = 5) (Table II). C-R isolates were

Table II

Cefotaxime-resistant Enterobacteriaceae isolated from sample sources along the pig-production chain, Ogun State, Nigeria

Sample source (n)	Number (%) of cefotaxime-resistant Entrobacteriaceae				
	Escherichia coli	*Klebsiella* spp.	*Enterobacter aerogenes*	*Citrobacter freundii*	All isolates
Feces (109)	7 (6.4)	4 (3.7)	2 (1.8)	2 (1.8)	15 (13.7)
Fresh pork (40)	8 (20.0)	6 (15.0)	3 (7.5)	2 (5.0)	19 (47.5)
Frozen pork (28)	3 (10.7)	1 (3.6)	2 (7.1)	1 (3.6)	7 (25.0)
Effluent water (41)	1 (2.4)	3 (7.3)	2 (4.9)	–	6 (14.6)
Ready-to-eat pork (33)	–	–	–	–	–
Swab of cutting surface (52)	3 (5.8)	3 (5.8)	1 (1.9)	–	7 (13.5)
Total (303)	22 (7.3)	17 (5.6)	10 (3.3)	5 (1.7)	54 (17.8)

detected in fecal samples from six (10.0%) out of 60 farms. The six positive farms were distributed across four out of six towns included in this study. The fresh pork samples positive for C-R isolates were obtained from 19 (47.5%) out of 40 meat vendors. The vendors with positive samples were from five out of six slaughterhouses investigated. Similarly, seven (25.0%) out of 28 frozen meat retail shops yielded positive isolates. The positive meat retail shops were from four towns. Effluent water from two slaughterhouses yielded C-R isolates. Swab samples of cutting surfaces used by seven (13.5%) out of 52 butchers yielded C-R isolates. The positive butchers' samples were from three slaughterhouses.

Four (7.4%) out of 54 C-R isolates were positive for phenotypic ESBL production. The detection rate of ESBL-producing bacteria in all 303 samples was 1.3%. The ESBL-producing isolates were *Es. coli* (three isolates) and *K. pneumoniae* (one isolate). They all harbored $bla_{\text{C-TX-M-15}}$ ESBL gene variant as well as $bla_{\text{TEM-1}}$ penicillinase gene. In addition, the *K. pneumoniae* isolate possessed the bla_{SHV} gene but the ESBL status of this gene was not determined. The ESBL-producing isolates originated from the fecal samples collected from three different farms located in two cities: two farms in Ifo with one ESBL-producing *Es. coli* each, and one farm in Sagamu with one ESBL-producing *Es. coli* and one *K. pneumoniae*. All three ESBL-producing *Es. coli* isolates belonged to phylogenetic group A.

Antimicrobial susceptibility testing showed that C-R isolates were highly resistant to many of the antimicrobials tested. Among the 54 C-R isolates, 100% were resistant to trimethoprim, 96.3% to cefoxitin and streptomycin, 94.4% to kanamycin, 92.6% to amoxicillin/clavulanic acid and nalidixic acid, 87.0% to chloramphenicol, 81.5% to sulfamethoxazole, 77.8% to ceftazidime and tetracycline, 75.9% to amikacin, 74.1% to ciprofloxacin, and 37.0% to gentamicin (Table III). All the isolates displayed multidrug resistance with resistance to at least one representative of each of the seven tested classes of antimicrobials (β-lactam, aminoglycosides, phenicol, fluoro/quinolone, sulfonamide, folate inhibitor and tetracycline).

Knowledge, attitude and practices of pig farmers on antimicrobial usage

All the pig farms investigated reported to use regularly antimicrobials in pig production. Forty-eight (80%) administered them on a monthly basis. Tetracycline was the most frequently used and was administered in the injectable form as oxytetracycline Long Acting (200 mg/ml). It was also frequently administered in oral form in combination with multivitamins as anti-stress agent. Other antimicrobials administered were ampicillin (35% of farmers), amoxicillin (33%), ciprofloxacin (20%), and streptomycin and penicillin (18%) (Table IV). The cephalosporins were among the least administered antimicrobials (3.3%). Most farmers (91.7%) administered antimicrobials mainly for the prevention of infections. Only five (8.3%) administered them principally for growth promotion. Antimicrobials such as chloramphenicol, gentamicin, ciprofloxacin, enrofloxacin and the cephalosporins were used purposefully for the treatment of infections. Only 18 (30.0%) engaged the services of veterinarians in disease management.

According to the farmers, the most commonly encountered diseases were parasitism (96.7%), diarrhea (83.3%), respiratory tract infections (70.0%), nutritional deficiencies and malnutrition (70.0%), and skin infections (66.7%). Only very few farmers (13.3%) practiced routine vaccination for disease prevention. Many farmers (63.3%) reported that they would discontinue the use of an antimicrobial without completing the recommended course once the clinical signs had resolved. Forty-four (73.3%) would increase the dose of an antimicrobial above the recommended dose if symptoms failed to abate within three days. Forty-one (68.3%) would change the type of antimicrobial or combine different antimicrobials in the presence of a refractory infection. All the farmers used antimicrobials without microbiological diagnosis nor antimicrobial susceptibility testing. Most of them (88.3%) were aware of the importance of observing a withdrawal period following antimicrobial therapy before slaughter of pigs. However, only 16 (26.7%) observed a withdrawal period before slaughtering pigs for human consumption. In case of antimicrobial resistant infection, most farmers (73.3%) disregarded observing the withdrawal period and slaughtered or sold sick pigs that were on antimicrobial treatment in order to minimize loss. All of them reported that they had ready access to antimicrobial and had never been asked to provide a prescription before purchasing them. Many of them (65.0%) agreed that pigs and pig products could serve as reservoirs and vehicles for the transmission of zoonotic pathogens including antimicrobial-resistant bacteria to humans. However, only 29 (48.3%) believed that

Table III

Antimicrobial resistance rate of cefotaxime-resistant Enterobacteriaceae isolated along the pig-production chain, Ogun State, Nigeria

Antimicrobial	*Escherichia coli* (n = 22)	*Klebsiella* spp. (n = 17)	*Enterobacter aerogenes* (n = 10)	*Citrobacter freundii* (n = 5)	All isolates (n = 54)
Chloramphenicol	86.4	76.5	100	100	87.0
Ciprofloxacin	59.1	82.5	80.0	100	74.1
Ceftazidime	68.2	94.1	60.0	100	77.8
Amoxicillin/clavulanic	100	94.1	80.0	80.0	92.6
Cefoxitin	100	94.1	100	80.0	96.3
Gentamycin	40.9	17.6	50.0	60.0	37.0
Tetracycline	77.3	76.5	80.0	80.0	77.8
Streptomycin	100	100	90.0	80.0	96.3
Amikacin	72.7	70.6	90.0	80.0	75.9
Nalidixic acid	100	82.5	100	80.0	92.6
Kanamycin	100	82.5	100	100	94.4
Trimethoprim	100	100	100	100	100
Sulfamethoxazole	72.7	76.5	100	100	81.5

Table IV

Commonly used antimicrobials in farms, Ogun State, Nigeria

Antimicrobial	Farm (%)	Antimicrobial	Farm (%)
Tetracycline	52 (86.7)	Sulfamethoxazole/ trimethoprim	5 (8.3)
Ampicillin	21 (35.0)	Chloramphenicol	3 (5.0)
Amoxicillin	20 (33.3)	Kanamycin	2 (3.3)
Ciprofloxacin	12 (20.0)	Ceftiofur	2 (3.3)
Enrofloxacin	12 (20.0)	Cefotaxime	2 (3.3)
Penicillin	11 (18.3)	Cefoxitin	2 (3.3)
Streptomycin	11 (18.3)	Amikacin	0 (0.0)
Gentamicin	6 (10.0)	Nalidixic acid	0 (0.0)

administration of antimicrobials to their pigs could facilitate the development of antimicrobial-resistance bacteria in these animals.

■ DISCUSSION

Cefotaxime-resistant Enterobacteriaceae were detected in all sample categories along the pig-production chain except in ready-to-eat (fried) pork. The rate of detection of these organisms was particularly high in fresh and frozen pork. C-R organisms survived in frozen meat but were destroyed by frying. Thus, pig production may be important in the dissemination of C-R Enterobacteriaceae in the study area, and fresh and frozen pork represent major vehicles for possible transmission of the organisms to humans. The detection of C-R organisms in swabs of cutting surfaces of knives and butchers' tables during slaughter suggests that these tools may play important roles in the contamination of meat thereby aiding the spread of the microbes from original animal host through meat at slaughterhouses and markets. The higher detection of the organisms in pork across slaughterhouses and retail shops than in on-farm pigs showed that contamination during processing at slaughterhouses and during marketing is critical for the dissemination and possible zoonotic transmission. Pigs from a small number of isolated farms in remote regions may harbor C-R bacteria but these bacteria may reach many people within and outside the originating farms through contaminated meat due to unhygienic practices during slaughtering, processing and marketing. It is important to note that the C-R bacteria identified in meat and cutting surfaces may not necessarily originate from pigs but could be from environmental and possibly human sources during handling.

This study also showed that different species of Enterobacteriaceae possess C-R traits. This is in agreement with previous reports where many different species of Enterobacteriaceae were found to be resistant to cefotaxime (Jean et al., 2002). Although not all the Enterobacteriaceae species encountered in this study are considered major pathogens, *Es. coli* and *K. pneumoniae* are known to cause significant morbidity in humans (Jean et al., 2002; Harris et al., 2015). *En. aerogenes* and *C. freundii* are also associated with opportunistic infections with various forms of complications (Gajdács and Urbán, 2019). Cefotaxime resistance could be due to the production of ESBL $_{CTX-M}$ type enzymes that hydrolyze and inactive third and fourth generation cephalosporins (Bauernfeind et al., 1990; Zhao and Hu, 2013). In this study, only four out of 54 C-R isolates were ESBL producers. These four isolates possessed $bla_{CTX-M-15}$ ESBL gene variant which encodes a very potent extended-spectrum cefotaximase. Moreover, only fecal samples from live pigs were positive for ESBL-producing bacteria. Thus, this study showed that the rate of detection of ESBL-producing bacteria was low in pigs in the area. Nevertheless, the detection of ESBL-producing bacteria in pigs is of public health significance. It shows that pigs are reservoirs and potential sources of ESBL-producing bacteria that could be transmitted to humans. The non-detection of ESBL-producing bacteria in other sample types could be a reflection of the low incidence in pigs in such a way that the chances of contamination from pig sources and subsequent detection in pork and other sources along the pig-production chain are limited.

The 1.3% ESBL-detection rate observed in our study is similar to that of 2.0% detected in pigs from Nsukka, Nigeria (Chah et al., 2018), but far lower than that of 51.1% detected in pigs from Thailand (Nuangmek et al., 2018). High detection of ESBL-producing bacteria has been linked with the administration of third and fourth generation cephalosporins in farm animals (Hammerum et al., 2014). However, third and fourth generation cephalosporins are rarely used in livestock production (including pig production) in Nigeria (Ojo et al., 2016a). The $bla_{CTX-M-15}$ is the predominant ESBL-gene variant in Nigeria and has been reported from different animal sources (Ojo et al., 2016b; Okpara et al., 2018) and from clinical isolates in humans (Aibinu et al., 2012). The $bla_{CTX-M-15}$ has global spread and is the most commonly identified ESBL-gene variant in human clinical conditions. The non-ESBL C-R bacteria encountered in this study may possess resistance mechanisms other than ESBL production. Overexpression of inducible chromosomally expressed AmpC β-lactamase could lead to cefotaxime resistance in Enterobacteriaceae (Jacoby, 2009). Studies have also shown that alteration of the outer membrane protein in Enterobacteriaceae could be responsible for resistance to β-lactams and other antimicrobials (Ojo et al., 2016b). This study did not investigate the other possible resistance mechanisms in the non-ESBL C-R isolates. ESBL genes are often located in transmissible mobile genetic elements especially plasmids, which could be shared among enteric bacteria by horizontally gene transfer thereby widening the niche of ESBL production in pathogenic and commensal bacteria populations. All ESBL-producing *Es. coli* isolates in our study belonged to phylogenetic group A. In an earlier study in the study area, most of ESBL-producing *Es. coli* that harbored $bla_{CTX-M-15}$ belonged to phylogenetic group A (Okpara et al., 2018). *Es. coli* strains in phylogroup A are predominantly commensal (Duriez et al., 2001). However, members of phylogenetic group A have been associated with extraintestinal infections in humans (Chakraborty et al., 2015).

The present study showed that C-R isolates were also resistant to many of the tested antimicrobials. The isolates were resistant to representative members of the following antimicrobial classes: β-lactams, aminoglycosides, phenicols, fluoro/quinolones, sulfonamides, folate inhibitors and tetracyclines. The high level of multidrug resistance observed is similar to the reports of other authors where C-R bacteria were shown to be multidrug resistant (Okpara et al., 2018; Fukuda et al., 2018). Earlier studies have reported a high level of multidrug-resistant Enterobacteriaceae in various animal species from the study area and elsewhere in Nigeria (Ojo et al., 2012; Oloso et al., 2018). The use of antimicrobials in animal production can select for resistant strains and facilitate their proliferation while eliminating competing susceptible bacteria. The selection and proliferation of antimicrobial-resistant bacteria in the presence of antimicrobial usage aided with unhygienic practices on farms and in slaughterhouses promote widespread dissemination of these bacteria in farm animals, environment and animal foods. The present study showed that cephalosporins including cefotaxime and ceftiofur were used in pig production albeit at a lower level compared to other antimicrobials. Cephalosporins were among the least used antimicrobials in pig production in the study area, whereas tetracycline was the most commonly used, as reported in other studies in Nigeria (Ojo et al. 2016a). Notwithstanding the low level of cephalosporins usage in pigs,

exposure of gut microflora to other antimicrobials could coselect for cefotaxime resistance in the exposed bacteria. Pigs can also acquire C-R bacteria from other sources including feed and water sources. ESBL-producing Enterobacteriaceae were detected in the two farms that reported the use of cephalosporins and in another farm where cephalosporins were not used. The emergence of ESBL-producing bacteria in humans and animals has been linked with the use of cephalosporins (Greko et al., 2009). Farmers in this study knew that pigs could serve as reservoirs of pathogens transmissible to humans but were ignorant of the fact that the use of antimicrobials in their animals could promote the emergence of antimicrobial resistant strains. This signifies an inadequate level of awareness among pig farmers on factors that contribute to the emergence of antimicrobial resistance. Poor regulation of the sale of antimicrobials may also encourage the use of antimicrobials in pigs because farmers reported that they were never asked to provide a prescription before their purchase. Earlier studies also report the poor sale regulation and easy access to antimicrobials by animal producers in Nigeria (Ojo et al., 2017).

■ CONCLUSION

This study revealed a widespread distribution of C-R Enterobacteriaceae (of multidrug-resistance status) in the pig-production chain in Ogun State, Nigeria. Slaughterhouses and meat represent major points of contamination and vehicles for the spread of C-R bacteria. This study also showed that pigs harbor ESBL-producing *Es. coli* and *K. pneumoniae* that possess the $bla_{\text{CTX-M-15}}$ gene. There is a need to raise the level of awareness on the role of antimicrobial usage in the development of antimicrobial resistance because most pig farmers did not know that the use of antimicrobials contributes to the emergence of antimicrobial resistance. Strict monitoring of antimicrobial sales and compliance with existing regulatory policies guiding the use and distribution of antimicrobials could reduce overdependence on antimicrobials in animal production. These are desirable steps in the preservation of the efficacy and benefits of antimicrobials in medical and veterinary practices.

Author contributions statement

OEO, MAD and EAA conceptualized and designed the study. OEO and AMI participated in samples collection and microbiological analyses. OEO was responsible for the molecular characterization of isolates. OEO and AMI participated in data analyses and preparation of the manuscript. JOH, MAD and EAA critically reviewed the manuscript.

Conflicts of interest

The study was carried out without any conflict of interest.

REFERENCES

Aibinu I., Odugbemi T., Koenig W., Ghebremedhin B., 2012. Sequence Type ST131 and ST10 Complex (ST617) predominant among CTX-M-15-producing *Escherichia coli* isolates from Nigeria. *Clin. Microbiol. Infect.*, **18** (3) E49–E51, doi: 10.1111/j.1469-0691.2011.03730.x.

Bauernfeind A., Grimm H., Schweighart S., 1990. A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. *Infection*, **18**: 294-298, doi: 10.1007/BF01647010

Bevan E.R., Jones A.M., Hawkey P.M., 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *J. Antimicrob. Chemother.*, **72**: 2145–2155, doi: 10.1093/jac/dkx146

Bradford, P.A., 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.*, **14** (4): 933–951, doi: 10.1128/CMR.14.4.933–951.2001

Canton R., Coque T.M., 2006. The CTX-M beta-lactamase pandemic. *Curr. Opin. Microbiol.*, **9**: 466–475, doi: 10.1016/j.mib.2006.08.011

Cavaco L.M., Abatih E., Aarestrup F.M., Guardabassi L., 2008. Selection and persistence of CTX-M producing Escherichia coli in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. *Antimicrob. Agents Chemother.*, **52**: 3612–3616, doi: 10.1128/AAC.00354-08

Chah K.F., Ugwu I.C., Okpala A., Adamu K.Y., Andrea A., Ceballos S., Nwanta J.N., et al., 2018. Detection and molecular characterization of extended-spectrum β-lactamase-producing enteric bacteria from pigs and chickens in Nsukka, *Nigeria. J. Glob. Antimicrob. Resist.,* **15**: 36 – 40, doi: 10.1016/j.jgar.2018.06.002

Chakraborty A., Saralaya V., Adhikari P., Shenoy S., Baliga S., Hegde A., 2015. Characterization of *Escherichia coli* phylogenetic groups associated with extraintestinal infections in South Indian population. *Ann. Med Health Sci. Res.,* **5** (4): 241–246, doi: 10.4103/2141-9248.160192

Clermont O., Christenson J.K., Denamur E., Gordon D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Rep.*, **5** (1): 58–65, doi: 10.1111/1758-2229.12019

CLSI, 2018. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Fourth edition. CLSI supplement VET08 (ISBN 978-1-68440-010-2). Clinical and Laboratory Standards Institute, Wayne, PA, USA

Cullik A., Pfeifer Y., Prager R., von Baum H., Witte W., 2010. A novel IS26 structure surrounds $bla_{\text{CTX-M}}$ genes in different plasmids from German clinical *Escherichia coli* isolates. *J. Med. Microbiol.*, **59:** 580–587, doi: 10.1099/jmm.0.016188-0

Duriez P., Clermont O., Bonacorsi S., Bingen E., Chaventré A., Elion J., Picard B., et al., 2001. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. *Microbiol.,* **147 (6)**: 1671-1676, doi: 10.1099/00221287-147-6-1671

Fukuda A., Usui M., Okubo T., Tagaki C., Sukpanyatham N., Tamura Y., 2018. Co-harboring of cephalosporin (bla)/colistin (mcr) resistance genes among Enterobacteriaceae from flies in Thailand. *FEMS Microbiol. Lett.*, **365** (16). fny178. doi: 10.1093/femsle/fny178

Gajdács M., Urbán E., 2019. Resistance trends and epidemiology of *Citrobacter-Enterobacter-Serratia* in urinary tract infections of inpatients and outpatients (RECESUTI): A 10-year survey. *Medicina,* **55** (6): 285, doi:10.3390/medicina55060285

Greko C., Badiola J.I., Catry B., van Duijkeren E., Moreno M.A., Matias Ferreira Pomba M.C., Pyörälä S., et al, 2009. Reflection paper on the use of third and fourth generation cephalosporins in food producing animals in the European Union: development of resistance and impact on human and animal health. *J. Vet. Pharmacol. Therap.*, **32**: 515–533, doi: 10.1111/j.1365-2885.2009.01075.x

Gröbner S., Linke D., Schütz W., Fladerer C., Madlung J., Autenrieth I.B., Witte W., et al., 2009. Emergence of carbapenem-non-susceptible extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates at the University Hospital of Tübingen, Germany. *J. Med. Microbiol.*, **58**: 912–922, doi: 10.1099/jmm.0.005850-0

Hammerum A.M., Larsen J., Andersen V.D., Lester C.H., Skytte T.S.S., Hansen F., Olsen S.S., et al., 2014. Characterization of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. *J. Antimicrob. Chemother,* **69** (10): 2650– 2657, doi: 10.1093/jac/dku180

Harris P.N., Yin M., Jureen R., Chew J., Ali J., Paynter S., Paterson D.L., et al., 2015. Comparable outcomes for β-lactam/β-lactamase inhibitor combinations and carbapenems in definitive treatment of bloodstream infections caused by cefotaxime-resistant *Escherichia coli* or *Klebsiella pneumoniae*. *Antimicrob. Resist. Infect. Control,* **4**:14, doi: 10.1186/s13756-015-0055-6

Jacoby G.A., 2009. AmpC beta-lactamases. *Clin. Microbiol. Rev.*, **22** (1): 161–182, doi:10.1128/CMR.00036-08

Jean S.S., Teng L-J., Hsueh P.R., Ho S.-W., Luh K.T., 2002. Antimicrobial susceptibilities among clinical isolates of extended-spectrum cephalosporin resistant Gram-negative bacteria in a Taiwanese University Hospital. *J. Antimicrob. Chemother.,* **49**: 69–76, doi: 10.1093/jac/49.1.69

Klein N.C., Cunha B.A., 1995. Third-generation cephalosporins. *Med. Clin. North Am.*, **79** (4): 709 – 719, doi: 10.1016/S0025-7125(16)30034-7

Knothe H., Shah P., Krcmery V., Antal M., Mitsuhashi S., 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection,* **11** (6): 315 – 317, doi: 10.1007/BF01641355

Medeiros A.A., 1997. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clin. Infect. Dis*., **24** (Suppl. 1): S19–S45, doi: 10.1093/clinids/24.Supplement_1.S19

Neu H.C., 1982. The new beta lactamase stable cephalosporins. *Ann. Intern. Med*., **97**: 408 – 419. doi: 10.7326/0003-4819-97-3-408

Nuangmek A., Rojanasthien S., Chotinun S., Yamsakul P, Tadee P., Thamlikitkul V., Tansakul N., 2018. Antimicrobial resistance in ESBL-producing *Escherichia coli* isolated from layer and pig farms in Thailand. *Acta Scientiae Veterinariae,* **46**: 1538, doi: 10.22456/1679-9216.81823

Obayelu A.E., Ogunmola O.O., Sowande,O.K. 2017. Economic Analysis and the Determinants of Pig Production in Ogun State, Nigeria. *Agric. Trop. Subtrop.,* **50** (2): 61–70, doi: 10.1515/ats20170007

Ojo O.E., Ogunyinka O.G., Agbaje M., Okuboye J.O., Kehinde O.O., Oyekunle M.A., 2012. Antibiogram of *Enterobacteriaceae* isolated from free-range chickens in Abeokuta, *Nigeria. Vet. Archiv*., **82** (6), 577-589

Ojo O.E., Awoyomi O.J., Fabusoro E., Dipeolu M.A., 2017. Activities and influence of veterinary drug marketers on antimicrobial usage in livestock production in Oyo and Kaduna States, Nigeria. *J. Agric. Rural Dev. Trop. Subtrop*., **118** (2): 207–216

Ojo O.E., Fabusoro E., Majasan A.A., Dipeolu M. A., 2016a. Antimicrobials in animal production: usage and practices among livestock farmers in Oyo and Kaduna States of Nigeria. *Trop. Anim. Health Prod*., **48**; 189–197, doi: 10.1007/s11250-015-0939-8

Ojo O.E., Schwarz S., Michael G.B., 2016b. Detection and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* from chicken production chains in Nigeria. *Vet. Microbiol*., **194**: 62–68, doi: 10.1016/j.vetmic.2016.04.02

Okpara E.O., Ojo O.E., Awoyomi O.J., Dipeolu M.A., Oyekunle M.A., Schwarz S. 2018. Antimicrobial usage and presence of extended-spectrum β-lactamase producing Enterobacteriaceae in animal-rearing households of selected rural and peri-urban communities. *Vet. Microbiol*., **218**: 31-39, doi: 10.1016/j.vetmic.2018.03.013

Oloso N.O., Fagbo S., Garbati M., Olonitola S.O., Awosanya E.J., Aworh M.K., Adamu H., et al., 2018. Antimicrobial resistance in food animals and the environment in Nigeria: A review. *Int. J. Environ. Res. Public Health,* **15** (6):1284, doi: 10.3390/ijerph15061284

Rossolini G.M., D'Andrea, M.M., Mugnaiol C., 2008. The spread of CTX-M-type extended-spectrum β-lactamases. *Clin. Microbiol. Infect*., 14 (Suppl. 1): 33–41, doi: 10.1111/j.1469-0691.2007.01867.x

Sirot D., Sirot J., Labia R., Morand A., Courvalin P., Darfeuille-Michaud A., Perroux R., et al., 1987. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. *J. Antimicrob. Chemother,* **20**: 323-334, doi: 10.1093/jac/20.3.323

Ugbomoiko U.S., Ariza L., Heukelbach J., 2008. Pigs are the most important animal reservoir of *Tunga penetrans* (jigger flea) in a rural Nigeria. *Trop. Doct*., **38** (4): 226 – 227, doi: 10.1258/td.2007.070352

WHO (World Health Organization), 2017. Critically important antimicrobials for human medicine – 5th rev. Geneva: World Health Organization, Geneva, Switzerland

Wittum T.E., 2012. The challenge of regulating agricultural ceftiofur use to slow the emergence of resistance to extended-spectrum cephalosporins. *Appl. Environ. Microbiol*., **78** (22): 7819–7821, doi: 10.1128/AEM.01967-12

Zhao W.H., Hu Z.Q., 2013. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Crit. Rev. Microbiol.,* **39** (1): 79–101, doi: 10.3109/1040841X.2012.691460

Résumé

Ojo O.E., Iledare A.M., Amosun E.A., Hassan J.O., Dipeolu M.A. Usage d'antimicrobiens et détection d'entérobactéries résistantes à la céfotaxime dans la chaîne de production porcine, Etat d'Ogun, Nigeria

Le céfotaxime appartient au groupe des céphalosporines de troisième génération (3GC) qui sont classées comme des agents antimicrobiens d'importance critique pour le traitement des infections chez l'homme. La recrudescence de l'incidence des bactéries résistantes à la céfotaxime d'origine animale est importante pour la santé publique mondiale. Cette étude a porté sur la présence d'entérobactéries résistantes à la céfotaxime dans la chaîne de production porcine de l'état d'Ogun, au Nigeria, et a examiné des isolats résistants à la céfotaxime pour la production de β-lactamase (ESBL) à spectre étendu. Les connaissances, l'attitude et les pratiques des éleveurs de porcs concernant l'utilisation des antimicrobiens ont également été étudiées. Des bactéries résistantes à la céfotaxime ont été détectées dans 54 (17,8 %) des 303 échantillons. Les isolats résistants à la céfotaxime ont été identifiés comme étant *Escherichia coli* (n = 22), *Klebsiella* spp. (n = 17), *Enterobacter aerogenes* (n = 10) et *Citrobacter freundii* (n = 5). Les organismes étaient présents dans les fèces des porcs d'élevage (15/109 ; 13,7%), la viande de porc fraîche dans les abattoirs (19/40 ; 47,5%), la viande de porc congelée dans les magasins

Resumen

Ojo O.E., Iledare A.M., Amosun E.A., Hassan J.O., Dipeolu M.A. Uso de antimicrobianos y detección de Enterobacteriaceae resistente a la cefatoxima en la cadena de producción del cerdo, estado Ogun, Nigeria

La cefotaxima pertenece al grupo de antimicrobianos de las cefalosporinas de tercera generación, que están clasificados como críticos para el tratamiento de infecciones en humanos. El aumento en la incidencia de bacterias resistentes a la cefotaxima (C-R) con origen animal es importante para la salud pública mundial. Este estudio investigó la presencia de Enterobacteriacea C-R en la cadena de producción porcina en el estado de Ogun, Nigeria, y examinó los aislamientos de C-R para la producción de β-lactamasa de espectro extendido (BLEE). Se estudiaron también los conocimientos, la actitud y las prácticas de los criadores de cerdos, referentes al uso de antimicrobianos. Se detectaron bacterias C-R en 54 (17,8%) de 303 muestras. Los aislamientos de C-R se identificaron como *Escherichia coli* (n = 22), *Klebsiella* spp. (n = 17), *Enterobacter aerogenes* (n = 10) y *Citrobacter freundii* (n = 5). Los organismos estaban presentes en las heces de cerdos de finca (15/109; 13,7%), carne de cerdo fresca en mataderos (19/40; 47,5%), carne de cerdo congelada en tiendas minoristas (7/28; 25,0%), superficies de corte de utensilios de carnicero (7/52; 13,5%) y agua efluente de mataderos (6/41; 14,6%). No

de détail (7/28 ; 25,0%), les surfaces de coupe des outils de boucherie (7/52 ; 13,5%) et les eaux usées des abattoirs (6/41 ; 14,6%). Aucune bactérie résistante à la céfotaxime n'a été détectée dans la viande de porc prête à consommer. Trois isolats de *Es. coli* et un de *K. pneumoniae* étaient producteurs d'ESBL et possédaient la variante du gène ESBL $bla_{CTX\text{-}M\text{-}15}$. Les *Es. coli* producteurs d'ESBL appartenaient au groupe phylogénétique A. Tous les isolats résistants à la céfotaxime étaient multirésistants aux médicaments et résistants à plus de trois agents antimicrobiens de différentes classes d'antimicrobiens. La tétracycline, l'ampicilline, l'amoxicilline, la ciprofloxacine et l'enrofloxacine faisaient partie des agents antimicrobiens couramment utilisés dans la production porcine, alors que les céphalosporines étaient rarement utilisées. Les éleveurs savaient que les porcs pouvaient servir de réservoirs de bactéries pathogènes transmissibles à l'homme. Cependant, ils ne savaient pas que l'utilisation d'antimicrobiens dans la production porcine pouvait entraîner le développement et la prolifération de bactéries résistantes aux antimicrobiens chez les porcs. Des efforts devraient être faits pour améliorer la sensibilisation des éleveurs sur le rôle de l'utilisation des antimicrobiens dans l'émergence et la diffusion des bactéries résistantes aux antimicrobiens dans la production animale.

Mots-clés: porcin, *Escherichia coli, Klebsiella pneumoniae*, Enterobacteriaceae, résistance aux antimicrobiens, céfotaxime, Nigeria

se detectó bacteria C-R en carne de cerdo lista para el consumo. Tres aislamientos de *Es. coli* y uno de *K. pneumoniae* fueron productores de BLEE y presentaban la variante del gen $bla_{CTX\text{-}M\text{-}15}$ BLEE. La *Es. coli* productora de BLEE perteneció al grupo filogenético A. Todos los aislamientos C-R fueron resistentes a más de tres antimicrobianos de diferentes clases de antimicrobianos. La tetraciclina, ampicilina, amoxicilina, ciprofloxacina y enrofloxacina se encontraron entre los antimicrobianos comúnmente utilizados en la producción porcina, mientras que las cefalosporinas se usaron rara vez. Los finqueros sabían que los cerdos podían servir como reservorios de bacterias patógenas transmisibles a los humanos. Sin embargo, no sabían que el uso de antimicrobianos en la producción porcina podría conducir al desarrollo y proliferación de bacterias resistentes a los antimicrobianos en los cerdos. Deben realizarse esfuerzos para mejorar la conciencia de los agricultores sobre el papel del uso de antimicrobianos en la aparición y diseminación de bacterias resistentes a los antimicrobianos en la producción animal.

Palabras clave: cerdo, *Escherichia coli*, *Klebsiella pneumoniae*, Enterobacteriaceae, resistencia a los antimicrobianos, cefotaxime, Nigeria

Prévalence et déterminants du portage d'entérobactéries résistantes aux céphalosporines de troisième génération chez *Rattus* sp. à la Réunion et à Mayotte

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Mots-clés

Rat, résistance aux antibiotiques, Enterobacteriaceae, épidémiologie, Réunion, Mayotte

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Résumé

Les entérobactéries résistantes aux céphalosporines de troisième génération (ERC3G) constituent un fardeau sanitaire majeur pour les humains et les animaux dans l'océan Indien. Les rats, au mode de vie synanthrope, en sont des réservoirs avérés. Nous avons utilisé les rats comme des bioindicateurs environnementaux de l'occurrence d'ERC3G. L'objectif principal de cette étude exploratoire était de générer des hypothèses concernant la contamination environnementale par les ERC3G dans les deux territoires français de l'océan Indien. Cet objectif a été poursuivi à travers a) l'estimation de la prévalence des ERC3G et des entérobactéries productrices de bêta-lactamases à spectre étendu (EBLSE) chez les rats des deux territoires en 2013-2014, et b) l'identification des déterminants de ce portage chez les rats (traits d'histoire de vie et occupation du sol). En 2013-2014, des rats ont été échantillonnés selon un gradient altitudinal à la Réunion et à Mayotte sur plusieurs sites peu anthropisés. Sur un échantillon de convenance de 198 et 138 rats, respectivement à la Réunion et à Mayotte, la prévalence des ERC3G s'élevait à 5,1 % et 8,7 %, et celle des EBLSE à 0,5 % et 0,8 %. La masse, la longueur de la queue et la proportion de terrains agricoles dans le domaine vital du rat étaient des déterminants du portage d'ERC3G à la Réunion. A Mayotte, les déterminants de ce portage étaient une masse faible et le site de capture du rat avec un regroupement de cas positifs dans une localité spécifique. Finalement, les résultats obtenus semblent indiquer une faible contamination de l'environnement par les ERC3G à la Réunion et à Mayotte en 2013-2014. A la Réunion l'hypothèse d'une contamination de l'environnement par l'épandage de lisier a été soulevée nécessitant des investigations complémentaires.

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Cet article est issu des travaux de thèse de N. Gay : Homme, animal, environnement : quel est le principal réservoir d'Entérobactéries productrices de bêta-lactamases à spectre étendu dans le Sud-Ouest de l'océan Indien ? Médecine humaine et pathologie, 2019, Université de la Réunion, France.

■ INTRODUCTION

Depuis les années 1990, les professionnels de santé doivent faire face à l'émergence d'entérobactéries multirésistantes aux antibiotiques, d'abord en milieu hospitalier puis progressivement en milieu communautaire (médecine de ville). Ces bactéries multirésistantes aux antibiotiques (BMR) sont caractérisées par leur résistance à au moins trois familles d'antibiotiques (Magiorakos et al., 2012). Parmi elles, les entérobactéries résistantes aux céphalosporines de troisième génération (ERC3G) possèdent plusieurs mécanismes de résistance dont les plus communs sont la production de bêta-lactamases à spectre étendu (EBLSE) (gènes de résistance aux antibiotiques à localisation

plasmidique) et la présence du gène AmpC (gène de résistance aux antibiotiques à localisation chromosomique).

Les infections aux ERC3G constituent un enjeu sanitaire mondial en raison de la difficulté à trouver des thérapies antibiotiques efficaces. La propagation d'ERC3G entraîne l'augmentation de la consommation d'antibiotiques de dernière ligne thérapeutique, notamment les carbapénèmes, et la multiplication d'échecs thérapeutiques (Zahar et al., 2009). En outre, les entérobactéries sont des bactéries commensales du tube digestif qui peuvent devenir pathogènes pour leur hôte par opportunisme. Le portage digestif d'ERC3G par les humains et les animaux participe à leur succès de propagation. En effet, les fèces libérées dans l'environnement permettent de contaminer de nouveaux hôtes (par exemple contamination de l'eau, de la nourriture ou juste de l'environnement direct) (Hawkey, 2008).

Dans le sud-ouest de l'océan Indien, le fardeau sanitaire des ERC3G, dont les EBLSE, est généralisé à tous les territoires pour les humains et les animaux (Gay et al., 2017). En 2016, la surveillance des BMR en milieu hospitalier a permis d'identifier la Réunion comme étant la quatrième région française en termes d'incidence d'EBLSE ; aucune estimation hospitalière n'est disponible en 2019 à Mayotte. De nombreuses inconnues persistent dans ces deux départements français de l'océan Indien, d'une part, sur la prévalence des BMR des humains en communauté (ville) et, d'autre part, sur leur distribution dans l'environnement (appréhendant indirectement l'exposition des populations animales et humaines).

Les rats ont été identifiés comme étant des réservoirs d'entérobactéries multirésistantes aux antibiotiques (Guenther et al., 2013). Ce sont des omnivores opportunistes pouvant utiliser toutes les ressources alimentaires disponibles (Soubeyran et al., 2011). Ils sont présents dans tout type d'habitat que ce soit en milieu urbain, agricole ou peu perturbé (Himsworth et al., 2014). Ces mammifères ont été utilisés comme indicateurs de la contamination environnementale par les ERC3G notamment en milieu urbain en Allemagne (Guenther et al., 2013), au Canada (Himsworth et al., 2015) et en Guinée (Schaufler et al., 2018).

En nous basant sur les résultats de ces études, les rats ont été utilisés comme des indicateurs des niveaux de résistance aux antibiotiques dans l'environnement dans les territoires français de l'océan Indien. L'objectif principal de cette étude exploratoire était de générer des hypothèses concernant la contamination environnementale par les ERC3G dans ces deux territoires. Les objectifs secondaires étaient a) d'estimer la prévalence globale d'entérobactéries résistantes aux C3G et d'EBLSE chez les rats à l'échelle des territoires de la Réunion et à Mayotte en 2013-2014, et b) d'identifier les déterminants (traits d'histoire de vie et occupation du sol) du portage d'entérobactéries résistantes aux C3G par les rats à la Réunion et à Mayotte.

■ MATERIEL ET METHODES

Plan d'échantillonnage

Dans le cadre du projet LeptOI, des rats ont été capturés entre février 2011 et février 2014 selon un gradient altitudinal à la Réunion et dans des zones peu perturbées à Mayotte. Au total 858 rats (*Rattus rattus* et *R. norvegicus)* ont été capturés à la Réunion (Guernier et al., 2016) et 289 rats (*R. rattus*) à Mayotte (Lagadec et al., 2016). Seuls les intestins de 138 rats provenant des zones peu perturbées de Mayotte étaient disponibles en biobanque. Pour les deux territoires, une taille d'échantillon de 196 rats a été calculée à partir d'une prévalence attendue de 15,0 % comme estimée chez les rats urbains de Berlin (Guenther et al., 2013), avec un risque d'erreur de 5,0 % et une précision absolue de 5,0 %. Les animaux échantillonnés dans le cadre de cette étude ont été euthanasiés en accord avec les directives européennes concernant la protection animale (2010/63/EU). Le Comité d'éthique du CYROI n° 11 a approuvé le protocole, de même que le ministère de l'Enseignement supérieur et de la Recherche sous l'accréditation 03387 (LeptOI) et 03584 (BatMan).

Analyses de laboratoire

Après dissection, différents organes des rats étaient prélevés et conservés en biobanque à -80 °C. Les intestins de rats étaient broyés puis placés à 37 °C (+/- 1 °C) pendant 18 h (+/- 2 h) dans 50 ml d'eau peptonée (Jazmati et al., 2016). L'ensemencement était ensuite réalisé sur une gélose ChromID ESBL (bioMérieux) puis les boîtes incubées à 37 °C (+/- 1 °C) pendant 24 h (+/- 3h). Un test Oxydase était réalisé sur les colonies présentes, puis les colonies Oxydases négatives étaient identifiées au niveau spécifique par spectrométrie de masse (MALDI-TOF, Bruker). Un ou deux antibiogrammes étaient réalisés sur chaque espèce d'entérobactérie identifiée selon les recommandations de l'EUCAST (EUCAST, 2015) afin de distinguer l'hyperproduction de céphalosporinases d'une production de BLSE.

Analyses spatiales et statistiques

Afin de caractériser l'environnement dans un rayon de 200 mètres autour du point de capture de l'animal, des cartes d'occupation du sol SPOT5 ont été utilisées (Antenne SEAS-OI, 2014). Ce périmètre correspond au domaine vital du rat (Rahelinirina et al., 2010). Des zones tampons de 200 mètres ont été réalisées à l'aide du logiciel Qgis Version 2.18.13.

Des régressions logistiques binaires (fonction de lien Logit) ont été réalisées entre la variable réponse « présence/absence de bactéries résistantes aux céphalosporines » et les variables explicatives pour identifier les déterminants du portage. Ces analyses univariées ont été réalisées pour chaque territoire sur les données de trait d'histoire de vie des rats collectées sur les individus échantillonnés et d'occupation du sol extrait des cartes Raster SPOT5 (Antenne SEAS-OI, 2014). Il s'agissait de caractéristiques biologiques telles que le sexe, l'espèce, la maturité de l'animal, la longueur de la queue (proxy de l'âge de l'animal et identification de l'espèce), du corps, la masse de l'animal, et de caractéristiques liées aux types d'occupation du sol. Dans un second temps, ces régressions logistiques binaires ont été réalisées sur l'ensemble des données des deux territoires en excluant les données d'occupation du sol, trop disparates entre les deux territoires. Le logiciel R version 3.4.2 a été utilisé pour réaliser ces analyses.

■ RESULTATS ET DISCUSSION

Caractéristiques des entérobactéries résistantes aux C3G et des EBLSE isolées

Parmi les rats porteurs d'ERC3G de la Réunion et de Mayotte en 2013-2014, cinq espèces bactériennes ont été identifiées (tableau I). Pour les deux territoires confondus, la majorité (52,2 %) des entérobactéries identifiées appartenaient à l'espèce *Citrobacter freundii* et 30,4 % à l'espèce *Enterobacter cloacae*. *Escherichia coli* a été identifiée chez un rat de Mayotte.

Parmi les ERC3G, des profils de résistance acquise à la ticarcilline ont été observés majoritairement mais une sensibilité conservée aux aminosides (gentamicine, amikacine) et à l'acide nalidixique. Les profils de résistances des deux entérobactéries identifiées comme productrices de BLSE ne différaient pas des autres entérobactéries hyperproductrices de céphalosporinases.

Bien que les effectifs d'ERC3G aient été réduits, les profils de résistance montraient des différences entre les deux territoires. Plus de 40,0 % des souches de *C. freundii* et *En. cloacae* isolées chez les rats

de Mayotte présentaient des phénotypes résistants ou intermédiaires à l'ertapénème (ERM), un antibiotique de dernière ligne thérapeutique. En outre, la résistance à la tétracycline a été observée uniquement à Mayotte (*Es. coli* et *En. cloacae*). Des phénotypes d'*Es. coli* résistants à la tétracycline ont été rapportés chez des rats au Vietnam en 2012 (Nhung et al., 2015). En conséquence, les pressions de sélections antibiotiques sur les entérobactéries de Mayotte pourraient être supérieures à celles exercées à la Réunion. L'identification de résistance aux carbapénèmes chez les rats à Mayotte soulève l'hypothèse d'une diffusion de bactéries résistantes à ces traitements de dernière ligne thérapeutique hors du milieu hospitalier. Les mécanismes de résistance observée à l'ERM, notamment chez *C. freundii,* devraient être enquêtés pour éventuellement confirmer l'acquisition d'un plasmide conférant une production de carbapénèmases.

Prévalence des ERC3G et des EBLSE

Une prévalence de 5,1 % [2,0%-8,1 %] d'ERC3G a été estimée chez les rats de la Réunion, contre 8,7 % [4,0%-13,4 %] à Mayotte en 2013-2014. La prévalence d'ERC3G observée à Mayotte n'était pas supérieure à celle observée à la Réunion (p = 0,3). A la Réunion en 2013-2014, un rat était porteur d'une EBLSE, soit une prévalence de 0,5 % [0,0%-1,5 %]. A Mayotte en 2014, un rat était porteur d'une EBLSE, soit une prévalence de 0,8 % [0,0%-2,1 %]. Aucune différence de la prévalence d'EBLSE n'a été observée entre les deux territoires (p = 1,0).

Les prévalences d'ERC3G et d'EBLSE obtenues dans les deux territoires étaient inférieures à la prévalence de 16,0 % obtenue à Berlin en 2010 (Guenther et al., 2013). Par conséquent, la prévalence attendue, utilisée a priori dans le calcul de la taille d'échantillon de cette étude était surestimée. Cette inexactitude réduit la précision de la prévalence obtenue à la Réunion. Malgré cette limite, la comparaison de la prévalence d'ERC3G estimée avec les données de la littérature reste pertinente et suggère une contamination environnementale limitée à la Réunion en comparaison avec d'autres territoires. A Berlin en 2010, sur 56 *R. norvegicus* capturés, une prévalence de 16,0 % d'*Es. coli* productrices de bêta-lactamases à spectre étendu a été observée (Guenther et al., 2013). A Hong-Kong, de 2008 à 2013, une prévalence d'EBLSE de 13,4 % a été estimée chez *R. rattus* et *R. norvegicus* (n = 491) (Ho et al., 2015).

Finalement, la sous-représentation des rats provenant de milieux urbains dans les échantillons de la Réunion et Mayotte par rapport aux études réalisées à Hong-Kong et à Berlin pourrait expliquer une part des différences de prévalence d'ERC3G estimées entre ces territoires. En effet, le portage des ERC3G par les rats en milieux urbains pourrait être influencé par la présence et l'activité des humains, et notamment la présence de déchets contaminés constituant une source de nourriture pour ces mammifères (Himsworth et al., 2014). En France, des bactéries résistantes aux antibiotiques de dernière génération comme les céphalosporines de dernières générations et les carbapénèmes ont été détectées dans les eaux à proximité d'hôpitaux ou en zone périurbaine (Almakki, 2017).

L'urbanisation de Mayotte en 2014 et de la Réunion en 2013-2014 reste limitée à l'échelle du territoire et pourrait expliquer les niveaux de prévalence faibles observés. Ainsi, l'habitat du rat semble jouer un rôle central dans le portage de BMR comme l'avaient proposé d'autres auteurs (Guenther et al., 2013) qu'il est nécessaire de considérer.

Occupation du sol et déterminants du portage

L'usage de cartes d'occupation du sol a permis d'identifier le milieu agricole comme un déterminant du portage d'ERC3G des rats à la Réunion (figure 1 ; tableau II). Ce portage était associé à l'augmentation de la proportion de terrains agricoles dans un rayon de 200 mètres autour du point de capture de l'animal. Cette observation pourrait indiquer une contamination du rat par l'épandage de lisier de porc dans les champs à la Réunion. Le portage d'ERC3G était significativement supérieur pour les petits mammifères capturés à proximité d'élevages porcins au Canada (Kozak et al., 2009). Dans le même sens, la prévalence d'*Es. coli* résistantes aux antibiotiques

Tableau I

Espèces d'entérobactéries résistantes aux C3G identifiées chez les rats de Mayotte et de la Réunion et résistance des entérobactéries identifiées aux antibiotiques testés en 2013-2014

Espèce identifiée	N	CFM	CTX	CAZ	FEP	TIC	ERM	AMC	TE	CFR	SXT	OFX
Réunion												
*Citrobacter freundii**	5[a]	4R/1S	4R/1S	4R/1S	5S	5R	5S	5R	5S	5R	5S	4S/1R
*Enterobacter cloacae**	5	4R/1S	5R	4R/1I	4S/1I	5R	5S	5R	5S	5R	5S	5S
Hafnia alvei	1	1R	1R	1R	1S	1S	1S	1R	1S	1R	1S	1S
Mayotte												
Citrobacter freundii	7	7R	7R	7R	6S/1R	7R	6S/1R	7R	6S/1R	6R/1S	7S	6S/1R
Enterobacter cloacae	3	3R	3R	3R	1S/2I	3R	1S/2I	3R	3S	3R	3S	3S
Escherichia coli	1[a]	1R	1R	1R	1S	1R	1S	1S	1R	1R	1R	1S
Enterobacter aerogenes	1	1R	1R	1R	1S	1R	1S	1R	1S	1R	1S	1S
Total	23	21R/2S	22R/1S	21R/1I/1S	19S/3I/1R	22R/1S	20S/2I/1R	22R/1S	21S/2R	22R/1S	22S/1R	21S/2R

CFM : céfixime ; CTX : céfotaxime ; CAZ : ceftazidime ; FEP : céfépime ; TIC : ticarcilline ; ERM : ertapénème ; AMC : amoxicilline + acide clavulanique ; TE : tétracycline ; CFR : céfadroxil ; SXT : bactrim ; OFX : ofloxacine. R : résistant ; I : intermédiaire ; S : sensible

* Un rat était porteur de deux espèces différentes d'entérobactéries résistantes aux C3G.

[a] Une entérobactérie productrice de bêta-lactamases à spectre étendu identifiée

Toutes les espèces d'entérobactéries testées étaient résistantes à l'amoxicilline, et sensibles aux antibiotiques gentamicine, amikacine et acide nalidixique.

Tableau II

Déterminants du portage d'ERC3G chez *Rattus* sp. à la Réunion et à Mayotte, 2013-2014

Territoire	Variable	Moyenne rats positifs	Moyenne rats négatifs	P
Réunion	Longueur du corps (cm)	196,1	185,5	0,06
	Longueur de la queue (cm)	386,9	207,9	0,04
	Masse (%)	155,2	132,2	0,05
	Proportion de terrains agricoles (rayon 200 m) (%)	25,4	10,5	0,05
Mayotte	Site de capture « forêt de convalescence »	–	–	0,05
	Masse (%)	126,8	148,4	0,02
Tout territoire	Longueur de la queue (cm)	295,2	214,4	0,06

était significativement plus élevée chez les rats capturés à proximité d'élevages au Vietnam (Nhung et al., 2015). L'hypothèse d'une contamination environnementale par l'épandage de lisier devrait-être testée à la Réunion.

A Mayotte aucun déterminant du portage d'ERC3G lié à l'occupation du sol n'a été identifié (figure 2 ; tableau II). Les rats capturés provenaient principalement de milieux peu perturbés, l'ensemble des types d'habitats dans ce territoire n'a pas été échantillonné. En effet, seuls cinq sites de capture ont été utilisés avec l'absence de représentation des milieux urbains et agricoles. Un effet site significatif a été observé sur le portage d'ERC3G dans la « forêt de convalescence ». Par conséquent, une (des) caractéristique(s) du site de la forêt, non identifiable(s) par la cartographie de l'occupation du sol, pourrai(en)t influencer le portage d'ERC3G par les rats. Une étude complémentaire devrait être réalisée afin de confirmer une exposition réelle au ERC3G dans la zone et identifier une potentielle source de contamination. Un plan d'échantillonnage assurant une meilleure représentation des différents types d'occupation du sol devrait être envisagé à Mayotte pour affiner l'étude des zones d'exposition au ERC3G.

Des tendances particulières relatives aux traits d'histoires de vie des rats propres à chaque territoire ont été observées. A la Réunion, les

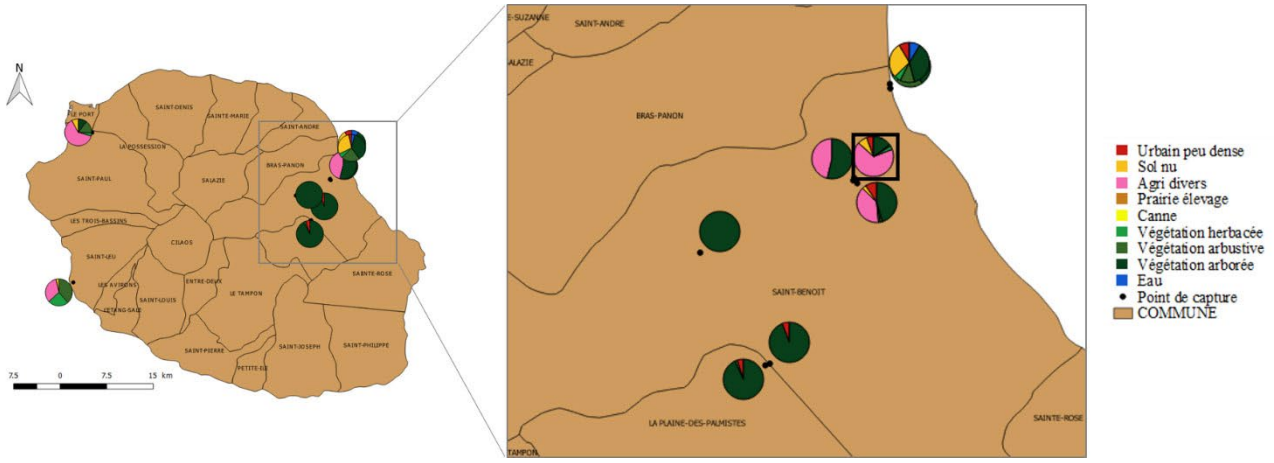

Figure 1 : *distribution spatiale et caractérisation du milieu de vie autour du point de capture des rats porteurs d'entérobactéries résistantes aux céphalosporines de troisième génération à la Réunion en 2013-2014. L'encadré correspond au rat porteur d'une entérobactérie productrices de β-lactamases à spectre étendu.*

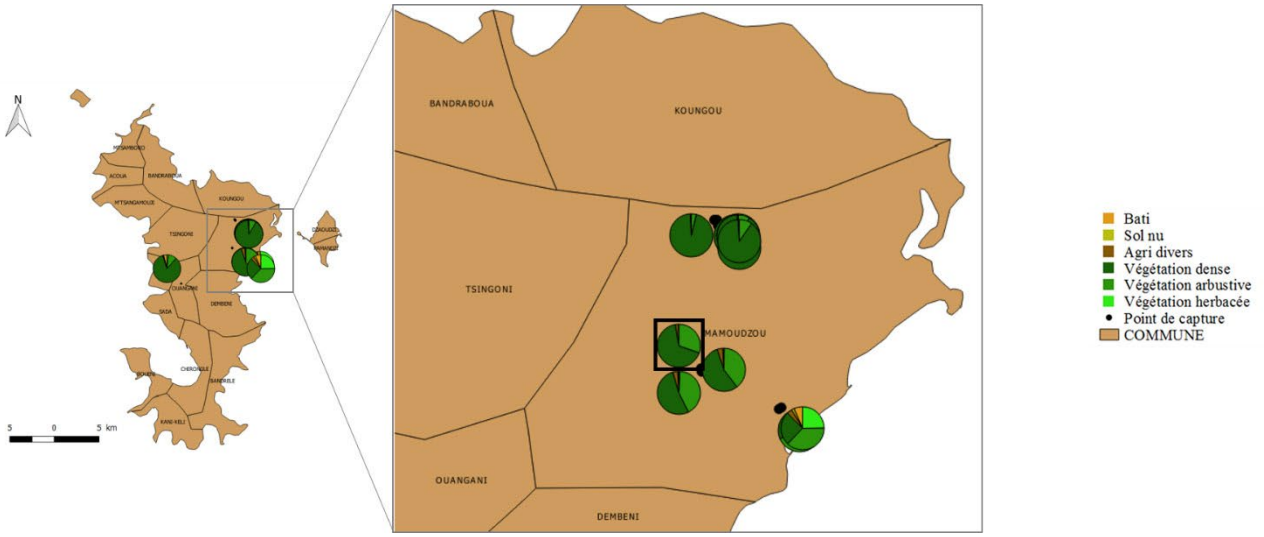

Figure 2 : *répartition spatiale et caractérisation du milieu de vie autour du point de capture des rats porteurs d'entérobactéries résistantes aux céphalosporines de troisième génération à Mayotte en 2014. L'encadré correspond au rat porteur d'une entérobactérie productrices de β-lactamases à spectre étendu.*

rats présentant des masses élevées et une queue plus allongée étaient plus porteurs d'ERC3G. La masse des rats est connue pour être un bon estimateur de l'âge et de la maturité sexuelle des individus (Morris, 1972). Cette observation est en accord avec une plus grande capacité de dispersion des individus matures qui leur confère une probabilité de rencontre accrue avec l'agent pathogène. Cela a été observé pour le portage de leptospirose chez les rats plus âgés (Himsworth et al., 2013).

A l'inverse, à Mayotte, les animaux de faible masse présentaient un portage supérieur. Il est connu chez les rats que les réponses morphologiques peuvent varier en fonction d'un ensemble de déterminants intrinsèques (par exemple régime alimentaire, prédation, compétitions, génétique) (Russell et al., 2011) ou extrinsèques (latitude, température, précipitations) (Yom-Tov and Geffen 2006). Ces différences entre les deux territoires pourraient donc être expliquées par d'autres paramètres non mesurés par notre plan d'étude (coupe transversale) ; leur étude nécessiterait un suivi longitudinal des animaux. Aucune différence morphologique entre rats de sites différents n'a été observée à Mayotte.

Cette étude est la première tentative pour évaluer la distribution des BMR dans l'environnement à l'échelle des territoires de Mayotte et de la Réunion. L'utilisation des rats comme indicateurs biologiques de la contamination environnementale par les ERC3G a permis de soulever l'hypothèse d'une source de contamination par l'épandage de lisier de porc à la Réunion. Cette hypothèse devrait être confirmée par des études environnementales. En effet, identifier puis contrôler les sources d'exposition aux BMR des populations animales et humaines est un pilier des actions de lutte contre le fléau sanitaire de l'antibiorésistance.

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L'étude a été réalisée sans aucun conflit d'intérêts.

Déclaration des contributions des auteurs

NG, PT, EC, PM ont participé à la conception et la planification de l'étude ; AC, AL, OB, MAE, GLM, EL, PT ont collecté les données ; SB, AC, NG ont analysé et interprété les données : AC, NG ont rédigé la première version du manuscrit ; AC, NG ont révisé de manière critique le manuscrit.

REFERENCES

Almakki A.Q.M., 2017. Résistance aux antibiotiques dans des eaux urbaines péri-hospitalières considérées dans un continuum hydrologique. Thèse Doct., Université Montpellier, Montpellier, France, 239 p.

Antenne SEAS-OI, 2014. Spot5. SEAS-OI, IRD, ESPACE-DEV, Université de La Réunion, http://www.seas-oi.org/web/guest/ressources (consulté le 20/01/2018)

EUCAST. 2015. Comité de l'antibiogramme de la Société Française de Microbiologie, Société Française de Microbiologie, Paris, France

Gay N., Belmonte O., Collard J.M., Halifa M., Issack M.I., Mindjae S., Palmyre P., et al. 2017. Review of Antibiotic Resistance in the Indian Ocean Commission: A Human and Animal Health Issue, *Front Public Health*, **5**: 162, doi:10.3389/fpubh.2017.00162

Guenther S., Wuttke J., Bethe A., Vojtech J., Schaufler K., Semmler T., Ulrich R.G., et al., 2013. Is fecal carriage of extended-spectrum-β-lactamase-producing Escherichia coli in urban rats a risk for public health?, *Antimicrob. Agents Chemother.*, **57**: 2424-2425, doi: 10.1128/AAC.02321-12

Guernier V., Lagadec E., Cordonin C., Le Minter G., Gomard Y., Pages F., Jaffar-Bandjee M. C., et al., 2016. Human Leptospirosis on Reunion Island, Indian Ocean: Are Rodents the (Only) Ones to Blame?, *PLoS Negl. Trop. Dis.*, doi: 10.1371/journal.pntd.0004733

Hawkey P.M. 2008. Prevalence and clonality of extended-spectrum beta-lactamases in Asia, *Clin. Microbiol. Infect.*, **14** (Suppl 1): 159-165, doi: 10.1111/j.1469-0691.2007.01855.x

Himsworth C.G., Kirbee L.P., Jardine C., Patrick D.M., 2013. Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers, *Vector-Borne Zoonot.*, **13**: 349-359, doi: 10.1089/vbz.2012.1195

Himsworth C.G., Parsons K.L., Feng A.Y.T., Kerr T., Jardine C., Patrick D.M., 2014. A mixed methods approach to exploring the relationship between Norway rat (*Rattus norvegicus*) abundance and features of the urban environment in an inner-city neighborhood of Vancouver, Canada. *PLoS One*, **9**: e97776, doi: 10.1371/journal.pone

Himsworth C.G., Zabek E., Desruisseau A., Parmley E.J., Reid-Smith R., Jardine C.M., Tang P., et al.. 2015. Prevalence and Characteristics of Escherichia Coli and Salmonella Spp. In the Feces of Wild Urban Norway and Black Rats (Rattus Norvegicus and Rattus Rattus) from an Inner-City Neighborhood of Vancouver, Canada, *J. Wildl. Dis.*, **51**: 589-600, doi: 10.7589/2014-09-242

Jazmati N., Hein R., Hamprecht A., 2016. Use of an Enrichment Broth Improves Detection of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae in Clinical Stool Samples, *J. Clin. Microbiol.*, **54**: 467-470, doi: 10.1128/JCM.02926-15

Kozak G.K., Boerlin P., Janecko N., Reid-Smith R.J., Jardine C.. 2009. Antimicrobial resistance in Escherichia coli isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada, *Appl. Environ. Microbiol.*, **75**: 559-566, doi: 10.1128/AEM.01821-08

Lagadec E., Gomard Y., Le Minter G., Cordonin C., Cardinale E., Ramasindrazana B., Dietrich M., et al., 2016. Identification of Tenrec ecaudatus, a Wild Mammal Introduced to Mayotte Island, as a Reservoir of the Newly Identified Human Pathogenic Leptospira mayottensis, *PLoS Negl. Trop. Dis.*, **10** (8), doi: 10.1371/journal.pntd.0004933

Magiorakos A.P., Srinivasan A., Carey R.B., Carmeli Y., Falagas M.E., Giske C.G., Harbarth S., et al. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, *Clin. Microbiol. Infect.*, **18**: 268-281, doi: 10.1111/j.1469-0691.2011.03570.x

Morris P., 1972. A review of mammalian age determination methods. *Mammal review*, 2: 69-104

Nhung N.T., Cuong N.V., Campbell J., Hoa N.T., Bryant J.E., Truc V.N., Kiet B.T., et al., 2015. High levels of antimicrobial resistance among escherichia coli isolates from livestock farms and synanthropic rats and shrews in the Mekong Delta of Vietnam, *Appl. Environ. Microbiol.*, **81**: 812-820, doi: 10.1128/AEM.03366-14

Rahelinirina S., Duplantier J.M., Ratovonjato J., Ramilijaona O., Ratsimba M., Rahalison L., 2010. Study on the movement of Rattus rattus and evaluation of the plague dispersion in Madagascar, *Vector Borne Zoonotic Dis.*, **10**: 77-84, doi: 10.1089/vbz.2009.0019

Russell J.C., Ringler D., Trombini A., Le Corre M., 2011. The island syndrome and population dynamics of introduced rats, *Oecologia*, **167**: 667-676., doi: 10.1007/s00442-011-2031-z

Schaufler K., Nowak K., Dux A., Semmler T., Villa L., Kourouma L., Bangoura K., 2018. Clinically Relevant ESBL-Producing K. pneumoniae ST307 and E. coli ST38 in an Urban West African Rat Population, *Front. Microbiol.*, **9**: 150, doi: 10.3389/fmicb.2018.0015

Yom-Tov Y., Geffen E., 2006. 'Geographic variation in body size: the effects of ambient temperature and precipitation', *Oecologia*, **148**: 213-218, doi: 10.1007/s00442-006-0364-9

Zahar J.R., Bille E., Schnell D., Lanternier F., Mechai F., Masse V., Nassif X., et al., 2009. Extension of beta-lactamases producing bacteria is a worldwide concern, *Med. Sci. (Paris)*, **25** (11): 939-944, doi: 10.1051/medsci/20092511939

Summary

Cohard A., Leclaire A., Belmonte O., Benkimoun S., Etheves M.-A., Le Minter G., Lagadec E., Mavingui P., Tortosa P., Cardinale E., Gay N. Prevalence and determinants of carrying third-generation cephalosporin-resistant enterobacteria in *Rattus* sp. in Reunion and Mayotte

Enterobacteriaceae resistant to third generation cephalosporins (ER3GC) constitute a major health issue for humans and animals in the Indian Ocean. Rats are synanthropic reservoirs. We have used rats as environmental bioindicators of the occurrence of ER3GC. The main objective of this exploratory study was to generate hypotheses on environmental contamination by ER3GC in the two French territories of the Indian Ocean. This objective was addressed by i) estimating the prevalence of ER3GC and of extended-spectrum β-lactamase producing enterobacteria (ESBL-E) in rats from the two territories in 2013-2014, and ii) identifying the determinants of this carriage in rats (life history traits and land use). In 2013-2014, rats were sampled according to an altitudinal gradient in Reunion and Mayotte on several sites with low anthropization. From a convenience sample of 198 and 138 rats in Reunion and Mayotte, respectively, the prevalence of ER3GC was 5.1% and 8.7%, and the prevalence of ESBL-E was 0.5% and 0.8%. The mass, tail length, and proportion of agricultural land in the home range of the rat were determinants of ER3GC carriage in Reunion. In Mayotte, the determinants of this carriage were a low mass and the capture site of the rat with a cluster of positive cases at a specific location. Finally, the results obtained seem to indicate a low environmental contamination by ER3GC in Reunion and Mayotte in 2013-2014. In Reunion, the hypothesis of environmental contamination by slurry spraying was raised, requiring further investigations.

Keywords: rats, resistance to antibiotics, Enterobacteriaceae, epidemiology, Réunion, Mayotte

Resumen

Cohard A., Leclaire A., Belmonte O., Benkimoun S., Etheves M.-A., Le Minter G., Lagadec E., Mavingui P., Tortosa P., Cardinale E., Gay N. Prevalencia y determinantes del carga de enterobacterias resistentes a cefalosporinas de tercera generación en *Rattus* sp. en la Reunión y Mayotte

Las Enterobacteriaceae resistentes a cefalosporinas de tercera generación (ERC3G) representan un peso importante para la salud de humanos y animales en el Océano Índico. Las ratas, con una forma de vida sinantrópica, son reservorios comprobados. Usamos ratas como bioindicadores ambientales de la aparición de ERC3G. El objetivo principal de este estudio exploratorio fue generar hipótesis sobre la contaminación ambiental por ERC3G en los dos territorios franceses del Océano Índico. Se aspiró a alcanzar este objetivo mediante a) la estimación de la prevalencia de ERC3G y enterobacterias productoras de betalactamasas de espectro extendido (EBLSE) en ratas en los dos territorios, en 2013-2014, y b) la identificación de los determinantes de esta carga en ratas (caracteres de la historia de vida y uso de la tierra). En 2013-2014, se muestrearon ratas según un gradiente altitudinal en la Reunión y Mayotte en varios sitios poco antropizados. En una muestra de concordancia de 198 y 138 ratas, en la Reunión y Mayotte, respectivamente, la prevalencia de ERC3G fue de 5,1% y 8,7%, y la de EBLSE de 0,5% y 0,8%. La masa, la longitud de la cola y la proporción de los terrenos agrícolas en el área vital de la rata fueron determinantes de la carga de ERC3G en la Reunión. En Mayotte, los determinantes de la carga fueron una masa baja y el sitio de captura de la rata con una agrupación de casos positivos en una localidad específica. Finalmente, los resultados obtenidos parecen indicar una baja contaminación ambiental por ERC3G en la Reunión y Mayotte en 2013-2014. En la Reunión, se planteó la hipótesis de contaminación ambiental por esparcimiento de abono que requirieron investigaciones adicionales.

Palabras clave: rata, resistencia a los antibióticos, Enterobacteriaceae, epidemiología, Reunión, Mayotte

Antibiotics use and gentamicin residues in commercial poultry and chicken eggs from Oyo and Lagos States, Nigeria

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Keywords

Poultry, layer chickens, eggs, antibiotic residues, food safety, Nigeria

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Summary

Nigeria poultry industry is fast growing to meet the demand of the increasing population with overdependence on antibiotics for production leading to consumer safety and public health concerns. The antibiotic use in poultry farms and presence of gentamicin residues in chicken eggs from city markets of Southwest Nigeria were investigated. A semi-structured questionnaire was administered to 45 poultry farmers to determine the patterns of antibiotic use, knowledge of withdrawal periods and food safety implications. Furthermore, 270 egg samples from six retail markets in Oyo and Lagos states were analyzed with ELISA for gentamicin residues. Residue concentrations were compared with Student's t test and ANOVA ($p < 0.05$). About 90% of the respondents reported they frequently administered antibiotics, 85% engaged in non-prescribed medication, and about 80% did not observe a withdrawal period before selling the eggs from treated chickens. In addition, 60% and 80% of pooled eggs from Oyo and Lagos states, respectively, contained gentamicin residues with means of 1461 ± 74 and 1350 ± 92 µg/kg, respectively. The mean residues obtained from the two states were higher than the maximum recommended residue limits. High levels of gentamicin residues, from unbridled use of antibiotics in poultry production, detected in retail eggs from markets rendered the eggs unsafe for human consumption. Therefore, regulatory control and veterinary supervision of antibiotic use are advocated to ensure Nigerian consumer protection.

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■ INTRODUCTION

The Nigerian poultry industry constitutes an important agricultural enterprise, contributing significantly to the nation's gross domestic product (GDP) and about 9–10% of the agricultural GDP (Heise et al., 2015). Poultry and poultry products are an important and cheap source of animal protein in Nigeria. Broiler and cockerel chickens are commonly reared for meat and as layers for egg production under intensive and free-range management systems. The Nigerian poultry industry provides employment for a fair proportion of the population. Poultry production is a fast means to bridge the protein deficiency gap common in many developing countries (Heise et al., 2015). It is a good means to supply the fast growing human population with high quality protein (Gueye, 2009).

In developing countries, frequent use of antibiotics, such as tetracycline, gentamicin, neomycin, fluoroquinolones and/or quinolone compounds, tylosine, erythromycin, virginiamycin, ceftiofur, and bacitracin, is a common practice in food poultry. Several authors report that these antibiotics are most commonly used as preventive and chemotherapeutic agents against poultry diseases such as respiratory diseases, necrotic enteritis, fowl typhoid, coryza, pullorum disease, and coccidiosis (Alhendi et al., 2000; Zakeri and Wright, 2008; Soni, 2012; Filazi et al., 2005). Indiscriminate use of antibiotics in livestock production is a common practice in Nigeria that is characterized by their frequently non-prescribed administration by farmers (Adesokan, et al., 2015). They are also used in low and therapeutic levels as part of the contents of commercial poultry and livestock feeds, and extra-label use is very common since the drugs are easily purchased without prescriptions (Lawal et al., 2015). Tetracycline, chloramphenicol, penicillin and gentamicin are the most commonly used antibiotics in poultry production in Nigeria (Adebowale et al., 2016). As a result of their administration, trace quantities of parent drugs and their metabolites may be present as residues in eggs. Moreno-Bondi et al., 2009 wrote: "The residues of these substances, their metabolites in eggs and other foods of animal origin may cause adverse effects on consumers' health."

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Gentamicin is a broad-spectrum aminoglycoside antibiotic commonly used in veterinary medicine against a wide range of bacterial infections (Goetting et al., 2011). It is usually administered by intramuscular injection with poor oral absorption. Its administration usually results in persistent tissue residues thereby requiring prolonged withdrawal times. (Filazi et al., 2005). When gentamicin is administered to laying hens via the intramuscular or subcutaneous route, it is deposited in egg yolk and albumen with the residues persisting longer in the yolk (Filazi et al., 2005). Residues of gentamicin can cause mutagenic, nephropathic, and hepatotoxic effects, as well as they may lead to antibiotic resistance, reproductive abnormalities or bone marrow toxicity in humans (Nisha, 2008). There is no Codex Alimentarius Commission on maximum residue limits (MRL) for gentamicin residues in poultry and poultry products, but the use of gentamicin in laying birds is prohibited by the European Union (Goetting et al., 2011). Consequently, the presence of any gentamicin residues in eggs is not permitted and such eggs are unsafe for human consumption.

In Nigeria because of little regulatory control and lack of routine monitoring of veterinary drug use and drug residues in foods of animal origin (Olatoye et al., 2012), several cocktails of antibiotics including gentamicin are being administered to poultry and other livestock indiscriminately. Several studies have reported residues of different antibiotics in these animal products (Ezenduka et al., 2011; Olatoye et al., 2012; Olatoye and Kayode, 2012), but none has reported the presence of gentamicin in commercial chicken eggs. This study therefore assessed the use of antibiotics in poultry and analyzed the levels of gentamicin residues in retail chicken eggs from commercial poultry from city markets of Oyo and Lagos states, Nigeria.

■ MATERIALS AND METHODS

Materials

Study location and sample collection

The study was carried out in selected poultry farms across Oyo and Lagos states. These states are in Southwestern Nigeria at 8° N 4° E. Oyo State is the hub of poultry production in Nigeria; Lagos State is also a major poultry producing state receiving supplies of chickens and eggs from other states of Nigeria to meet the increasing daily demand.

Questionnaire on antibiotic use

A cross-sectional survey was carried out using a pretested semi-structured questionnaire administered to commercial chicken egg producers (poultry farmers) on antibiotic use patterns.

Egg sample collection

A total of 270 chicken egg samples supplied to retail markets from commercial layer farms in Oyo and Lagos states were randomly collected three times at two weeks' intervals. Egg samples were obtained from three major markets in Oyo State (Ibadan, Oyo, and Ogbomosho cities) and in Lagos State (Ikorodu, Agege, Lagos Island). From each market, 45 eggs from 15 retailers were obtained and transported to the Food and Meat Hygiene Laboratory in the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan, Oyo State.

Equipment and ELISA kit

The equipment used included ELISA plate reader 450/630 nm, a centrifuge, weighing balance, graduated pipette, water bath, homogenizer, vortex mixer, extraction cartridges, micropipettes and glassware. The reagents included deionized water, distilled water, monobasic and dibasic sodium phosphate (NaHPO4.2H2O, NaH2PO4.2H2O).

The gentamicin ELISA test kit comprised microwell strips, six different concentrations of gentamicin standard solutions (1 ml each of 0 ppb, 0.05 ppb, 0.15 ppb, 0.45 ppb, 1.35 ppb or 4.05 ppb), an enzyme conjugate, an antibody working solution, a substrate A and B solution, a stop solution, a concentrated washing buffer, a concentrated redissolving solution, and Green Spring ELISA software (Green Spring, Shenzhen Lvshiyuan Biotechnology, China).

Methods

Questionnaire administration

The pretested questionnaire was administered to poultry farmers randomly selected from the register of the Poultry Association of Nigeria. Data on reasons for antibiotic administration to poultry and on types of antibiotic used were recorded. In addition, the farmers' knowledge of antibiotic residues and practice of withdrawal periods were assessed.

Sample pretreatment

Sample preparation was carried out according to ELISA kit instructions. Each sample comprised three eggs pooled and homogenized, followed by the addition of 8 ml of 0.2 M phosphate buffer to two grams of each pooled-egg sample. The mixture was thoroughly shaken for 5 min and incubated in a water bath at 56°C for 30 min. The solution was centrifuged at 4000 r/min at room temperature (20–25ºC) for 10 min, then 450 µl of the diluted redissolving solution was added to 50 µl of the supernatant (upper layer) and thoroughly mixed by vortex, after which 50 µl of the solution was used for ELISA test.

ELISA procedure

This test kit is based on the competitive enzyme immunoassay for the detection of gentamicin in the sample. Briefly, according to the manufacturer, washing buffer, samples and standard solutions were prepared, whereas all the microtiter wells were numbered according to samples and standard solutions. Fifty microliters each of sample and standard solutions was dispensed in duplicate into the wells of a microtiter plate with each position recorded. This was followed by dispensing 50 µl of enzyme conjugate into each of standard and sample, and thereafter 50 µl of antibody solution to each well. The plate was gently shaken manually, sealed with a membrane cover and incubated at 25°C for 60 min. Then, the liquid inside the wells was poured out of the wells and 250 µl of washing solution was added to wash each well for 30 s with five repetitions. The plate was flapped and dried with absorbent paper and bubbles were cut with clean tips. Thereafter, 50 µl of substrate A and substrate B solutions were successively added into each well, mixed gently, and incubated for 15 min in the dark. Then, 50 µl of stop solution was added to each well and mixed gently. The plates were measured for the optical density (OD) using ELISA plate reader at 450 nm wavelength. Green Spring ELISA software was used for analysis of gentamicin concentrations in the standard and sample solutions. The test has been validated with ISO Certificate: ISO9001&ISO14001&OHSMS18001; detection limits = 2.5 - 3 ppb; sensitivity = 0.05 ppb; and recovery rate 80 ± 20% - 90 ± 20%.

Calibration curve

The mean OD of the duplicate gentamicin standard solution (standard 1–6) was plotted against the logarithms of the mean gentamicin standard concentrations to obtain the calibration curve (Figure 1). The software was integrated with a formula for interpretation of gentamicin concentrations in the samples.

Gentamicin concentrations

OD data of duplicate gentamicin concentrations obtained from ELISA readings were recorded in the software. The OD value of the sample had a negative correlation with the gentamicin in it. Mean sample OD were converted to obtain the logarithms of mean gentamicin

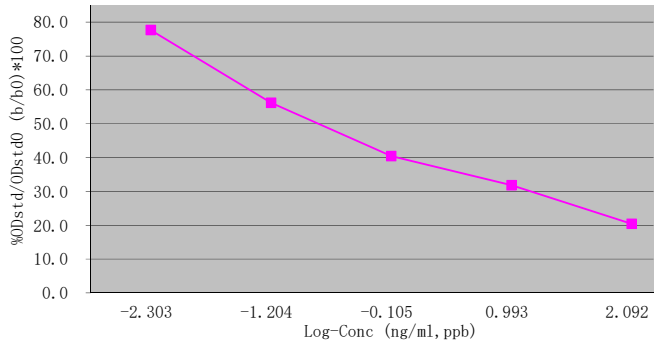

Figure 1: *Standard curve of gentamicin constructed from c-ELISA. b is the optical density (OD) value obtained in the presence of gentamicin standard concentrations, and b0 is the OD value obtained in the absence of gentamicin concentration.*

concentrations through the standard curve in the software. Residue concentrations in the samples were obtained and interpreted by the kit software. The values were compared to the standard curve and gentamicin concentrations were subsequently obtained. According to the software program, residue concentrations above the MRL in eggs (i.e. greater than or equal to 100 ppb) were judged positive for gentamicin residues (Figure 1).

Data analysis

Data collation and management were performed with Microsoft Excel. Responses to the questionnaire were presented in simple frequency tables. The Statistical Package for Social Sciences (SPSS) version 10.0 was used to analyze the mean residue concentrations from different locations. ANOVA Duncan's multiple-range test and the paired-t test were performed to determine the significant differences of gentamicin residues in the chicken eggs from the two states.

■ RESULTS

Antibiotic use pattern in poultry farms in Oyo and Lagos states

Table I shows the antibiotics commonly administered by the interviewed farmers. The most frequently used were tetracycline (98% of the farmers), enrofloxacin (92%) and gentamicin (88%). The farmers routinely used drugs for prevention and treatment of poultry diseases, whereas 86% routinely administered antibiotics as feed additives. Only 15% of the farmers reported treating their poultry on veterinary prescription following specified doses, whereas 85% engaged in non-prescribed medication based on experience and according to the drug instructions on the label. In addition, 20% of respondents claimed to give the antibiotic treatment according to specified periods and had knowledge of withdrawal periods, but they did not discard the eggs because they could not afford to lose the eggs produced during treatment and withdrawal periods. The majority (80%) of the poultry farmers claimed they did not practice a withdrawal period and were not aware of negative health consequences of antibiotic residues.

Gentamicin residues in eggs

Gentamicin residues were detected in 70.0% of all the pooled eggs sampled in this study (Table II). The overall mean gentamicin residue concentrations of 1350 ± 9.2 and 1460 ± 7.4 µg/kg were obtained in egg samples from Lagos and Oyo states, respectively (Table III). There was no significant difference ($p < 0.05$) in the mean gentamicin residue concentrations in eggs from the different market locations and across the two states.

Table I

Antibiotic use pattern in poultry farms (n = 45) in Oyo and Lagos states, Nigeria

Item	Response	Num. of farms	%
Antibiotic use	Yes	40	90.0
	No*	5	10.0
Antibiotics frequently used	Tetracycline	44	98.0
	Gentamicin	40	88.0
	Enrofloxacin	41	92.0
Prescription source	Veterinarian	7	15.0
	Farmer	38	85.0
Reason for administration	Feed additives	39	86.0
	Treatment	6	14.0
Withdrawal period practice	No	36	80.0
	Yes	9	20.0

* Farmers did not use antibiotics directly in drinking water or by injection to birds, but they used antibiotics in feeds.

Table II

Presence of gentamicin residues in chicken eggs from Oyo and Lagos states, Nigeria

Location	Total num. of samples	Num. of positive samples
Oyo State	45	27 (60%)
Lagos State	45	36 (80%)
Total	90	63 (70%)

Table III

Gentamicin concentrations in chicken eggs from markets in Oyo and Lagos states, Nigeria

Location	Total pooled eggs	Total positive samples	Mean gentamicin concentration ± SD (µg/kg)
Oyo State			
Ibadan	15	9	964 ± 43.0
Oyo	15	10	1807 ± 69.0
Ogbomosho	15	8	1587 ± 78.0
Total	45	27	1460 ± 74.0
Lagos State			
Lagos Island	15	12	1147 ± 9.2
Agege	15	12	1148 ± 6.4
Ikorodu	15	12	1783 ± 10.1
Total	45	36	1350 ± 9.2

SD: standard deviation

■ DISCUSSION

The global challenge of efficacy of antibiotics is a major threat to public health, and their use in livestock has been under critical scrutiny for the control of antimicrobial resistance. "Drug residues in chicken eggs are of concern because relatively few drugs are labeled for laying hens, although several medications are approved for other production classes of poultry" (Hofacre, 2006; Castanon, 2007). The dependence

on antibiotics for poultry production without stringent regulations on use and residue monitoring portend food safety risks. In the present study, residues of such antibiotic use may present a risk as none of the farmers discarded the eggs from treated chickens during treatment but rather offered them for sale and human consumption due to economic consideration. This supports the report of Donoghue (2003) that antimicrobials are used by poultry farms to enhance growth, feed efficiency, and to reduce bacterial diseases.

The majority of the egg sampled in this study had detectable gentamicin residues. The mean residues detected in eggs from within each state and across the two states were not significantly different ($p < 0.05$) but were above the permissible level. The detection of gentamicin in these samples correlated with the results of antibiotic use by the poultry farmers surveyed who mostly engaged in misuse and failed to observe withdrawal periods without consideration for consumers' protection. These results agree with the observation that non-adherence to antibiotic withdrawal periods is the major cause of antimicrobial residues in food animals (Donoghue, 2003; Young et al., 2010). The detection of gentamicin in eggs is of further concern as it persists in both the yoke and albumen. However, residues are rapidly accumulated in the yolk and persist longer, i.e. 10 or more days following treatment (Etches, 1996). Most of the laying chickens could have been treated parenterally with injectable gentamicin against common bacterial diseases of poultry such as salmonellosis, pasteurellosis and colibacillosis that are enzootic in the study area. Although Brown and Riviere (1991) and Bennett et al. (2001) reported that orally administered gentamicin in birds were excreted in the feces and thus that it was rare to find aminoglycoside residues in eggs following oral administration, Filazi et al. (2005) observed persistent residues of gentamicin in eggs from laying hens following administration of gentamicin for chemotherapy. Gentamicin accumulates for a long period at a high concentration in egg yolk as a result of accumulation of the yolk material during the intense laying period of hens with a direct correlation between dosage increase and drug residue concentration in eggs (Filazi et al., 2005).

Thus, the indiscriminate use of gentamicin by egg producers without consideration for consumer safety and adherence to a withdrawal period causes the residues to enter the human food chain with public health consequences. The majority of the eggs sampled in this study contained concentrations of gentamicin residues higher than those recommended by European Union MRL, i.e. levels unsafe for human consumption.

■ CONCLUSION

These findings established an unregulated antibiotic use, and the detection of gentamicin in eggs with a high proportion containing residue levels in breach with international food standards. The majority of poultry farmers in the study areas and by implication across Nigeria are not adhering to the prudent use of antibiotics and are not applying withdrawal periods thereby producing and selling eggs with antibiotic residues. The laws regulating the sale and administration of antibiotics should be strengthened and enforced to prevent indiscriminate administration of antibiotics to livestock. Veterinary supervision of drug administration to poultry and routine residue testing are also critical to ensure food safety and consumer protection in Nigeria.

Author contributions statement

OIO and YJA engaged in the conception and design of the study; YJA collected samples; OIO and TOO participated in sample preparation; OIO and YJA performed the laboratory analysis and drafted the manuscript first version; OIO performed data analysis and interpretation; OIO critically reviewed the manuscript.

REFERENCES

Adebowale O.O., Adeyemo O.K., Awoyomi O., Dada R., 2016. Antibiotic use and practices in commercial poultry laying hens in Ogun State Nigeria. *Rev. Elev. Med. Vet. Pays Trop.*, **69** (1): 41-45, doi: 10.19182/remvt.31170

Adesokan H.K., Akanbi I.O., Akanbi I.M., Obaweda R.A., 2015. Pattern of antimicrobial usage in livestock animals in south-western Nigeria: The need for alternative plans. *Onderstepoort J. Vet. Res.*, **82** (1): 1-6, doi: 10.4102/ojvr.v82i1.816

Alhendi A.B., Homeida A.A.M., Galli E.S., 2000. Drug residues in broiler chicken fed with antibiotics in rations. *Vet. Arhiv*. (70): 199-205

Bennet M., Sommervillae L.M., Taylor M.A., 2001. Drug used in the treatment of bacteria, fungal, viral, and protozoal infections. In: The veterinary formulary (Ed. Bishop Y.). Pharmaceutical Press, Cambridge, UK, 135-218

Brown S.A., Riviere J.E., 1991. Comparative pharmacokinetics of amyloglucosidase antibiotics. *J. Vet. Pharmacol. Ther.*, **14**: 1-35, doi: 10.1111/j.1365-2885.1991.tb00801.x

Castanon J.I.R., 2007. History of the use of antibiotics as growth promoters in European poultry feed. *Poult. Sci.*, **86** (11): 2466-2471, doi: 10.3382/ps.2007-00249

Donoghue D.J., 2003. Antibiotic residues in poultry tissue and eggs: human health concerns? *Poult. Sci.*, **82** (4): 618-621, doi: 10.1093/ps/82.4.618

Etches R.J., 1996. Reproduction in poultry. CAB International, Wallingford, UK

Ezenduka E.V., Oboegbulem S.I., Nwanta J.A., Onunkwo J.I., 2011. Prevalence of antimicrobial residues in raw table eggs from farms and retail outlets in Enugu State, Nigeria. *Trop. Anim. Health Prod.*, **43**: 557-559, doi: 10.1007/s11250-010-9730-z

Filazi A., Sireli U.T., Cadirci O., 2005. Residues of gentamicin in eggs following medication of laying eggs. *Br. Poult. Sci.*, **46** (5): 580-583, doi: 10.1080/00071660500273243

Goetting V., Lee K.A., Tell L.A., 2011. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of literature. *J. Vet. Pharmacol. Ther.*, **34** (6): 521-56, doi: 10.1111/j.1365-2885.2011.01287.x

Gueye E.F., 2009. The role of networks in information dissemination to family poultry farmers. *World Poult. Sci. J.*, **65** (1): 115-124, doi: 10.1017/S0043933909000009

Heise H., Crisan A., Theuvsen L., 2015. The poultry market in Nigeria: Market structures and potential for investment in the market. *Int. Food Agribusiness Manage. Rev.,* **18** (A): 197-222

Hofacre C.L., 2006. Antimicrobial drug use in poultry in antimicrobial therapy. In: Veterinary Medicine (Eds. Prescott S., Baggot J.F., Walker J.D., Dowling P.M.). Blackwell, Ames, IA, USA, 545-554

Lawal J.R., Saleh M.J., Yaqub A.G., Amina M.B., Yakaka W., Muhammad M., 2015. Antibiotic residues in edible poultry tissues and products in Nigeria: A potential public health hazard. *Int. J. Anim. Vet. Adv.*, **7** (3): 55-61, doi: 10.19026/ijava.7.5241

Moreno-Bondi M.C., Marazuela M.D., Herranz S., Rodriguez E., 2009. An overview of sample preparation procedures for LC-MS multiclass antibiotic determination in environmental and food samples. *Anal. Bioanal. Chem.*, **395** (4): 921-946, doi: 10.1007/s00216-009-2920-8

Nisha, A.R., 2008. Antibiotic residues. A global health hazard. *Vet. World*, **1** (12): 375-377, doi: 10.5455/vetworld.2008.375-377

Olatoye O., Kayode S.T., 2012. Oxytetracycline residues in retail chicken eggs in Ibadan, Nigeria. *Food Addit. Contam. Part B Surveill.*, **5**: 255-259, doi: 10.1080/19393210.2012.702791

Olatoye I.O., Oyelakin E.F., Adeyemi I.G., Call D.R., 2012. Chloramphenicol use and prevalence of its residues in broiler chickens and eggs in Ibadan, Nigeria. *Nig. Vet. J.*, **4** (33): 643-650

Young I., Rajic A., Letellie A., Cox B., Leslie M., Sanei B., McEwen S.A., 2010. Knowledge and attitudes toward food safety and use of good production practices among Canadian broiler chicken producers. *J. Food Prot.*, **73** (7): 1278-1287, doi: 10.4315/0362-028X-73.7.1278

Zakeri B., Wright G.D., 2008. Chemical biology of tetracycline antibiotics. *Biochem. Cell Biol.*, **86**: 124-136, doi: 10.1139/O08-002

Résumé

Olatoye O.I., Ojomo T.O., Adeseko Y.J. Utilisation d'antibiotiques et résidus de gentamicine dans la volaille commercialisée et les œufs de poule des états d'Oyo et de Lagos, Nigeria

L'industrie avicole nigériane se développe rapidement pour répondre à la demande d'une population croissante et dépendante des antibiotiques pour la production, ce qui entraîne des problèmes de sécurité des consommateurs et de santé publique. L'utilisation d'antibiotiques dans les élevages avicoles et la présence de résidus de gentamicine dans les œufs de poulets de marchés urbains du sud-ouest du Nigeria ont été étudiées. Un questionnaire semi-structuré a été proposé à 45 aviculteurs afin de déterminer leurs habitudes relatives à l'utilisation d'antibiotiques, leur connaissance des délais de retrait de la vente, et les conséquences pour la sécurité sanitaire des aliments. En outre, 270 échantillons d'œufs provenant de six marchés de détail des états d'Oyo et de Lagos ont été analysés avec Elisa pour détecter la présence de résidus de gentamicine. Les concentrations de résidus ont été comparées avec le test t de Student et Anova ($p < 0,05$). Environ 90 % des répondants ont déclaré administrer fréquemment des antibiotiques, 85 % le faisaient sans prescription et environ 80 % n'observaient pas de période de retrait des œufs des poulets traités. En outre, respectivement 60 % et 80 % des œufs mis en commun dans les états d'Oyo et de Lagos contenaient des résidus de gentamicine avec des moyennes de 1461 ± 74 et 1350 ± 92 µg/kg. Les résidus moyens obtenus dans les deux états étaient supérieurs aux limites maximales de résidus recommandées. Les niveaux élevés de résidus de gentamicine, liés à l'utilisation incontrôlée d'antibiotiques dans la production avicole et détectés dans les œufs vendus au détail sur les marchés, rendaient les œufs impropres à la consommation humaine. Par conséquent, le contrôle réglementaire et la supervision vétérinaire dans l'utilisation d'antibiotiques sont indispensables pour assurer la protection des consommateurs nigérians.

Mots-clés : volaille, poule pondeuse, œuf, résidu d'antibiotique, sécurité sanitaire des aliments, Nigeria

Resumen

Olatoye O.I., Ojomo T.O., Adeseko Y.J. Uso de antibióticos y residuos de gentamicina en aves comerciales y huevos de gallina en los estados de Oyo y Lagos, Nigeria

La industria avícola de Nigeria esta creciendo rápidamente, para satisfacer la demanda de una población creciente, con una dependencia excesiva en los antibióticos para la producción, generando problemas de seguridad y salud pública para el consumidor. Se investigó el uso de antibióticos en granjas avícolas y la presencia de residuos de gentamicina en huevos de gallina en los mercados citadinos del suroeste de Nigeria. Se administró un cuestionario semi estructurado a 45 avicultores, con el fin de determinar patrones de uso de antibióticos, conocimiento sobre periodos de abstinencia e implicaciones para la seguridad alimenticia. Se analizaron además mediante ELISA 270 muestras de huevos provenientes de seis mercados públicos en los estados de Oyo y Lagos, con el fin de detectar residuos de gentamicina. Las concentraciones de residuos se compararon mediante la prueba t de Student y ANOVA ($p < 0,05$). Alrededor de 90% de los encuestados informaron que frecuentemente administraban antibióticos, con 85% utilizando antibióticos sin prescripción medica y 80% sin respetar períodos de abstinencia antes de vender los huevos de las aves medicadas. Además, 60% y 80% de los huevos examinados en Oyo y Lagos, respectivamente, contenían residuos de gentamicina, con medias de 1461 ± 74 y 1350 ± 92 µg/kg, respectivamente. Los residuos medios obtenidos en ambos estados fueron superiores a los límites máximos de residuos recomendados. Los altos niveles de residuos de gentamicina, provenientes de un uso excesivo de antibióticos en la producción avícola, detectados en los huevos vendidos en mercados, los hicieron inseguros para el consumo humano. Por lo tanto, se recomienda reglamentación de control y supervisión veterinaria del uso de antibióticos para garantizar la protección del consumidor nigeriano.

Palabras clave: aves de corral, gallina ponedora, huevos, residuos de antibióticos, inocuidad alimentaria, Nigeria

Antibiorésistance des souches de *Salmonella gallinarum* isolées en aviculture moderne en zones périurbaines au Mali

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Mots-clés

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Résumé

L'objectif de l'étude, menée d'août 2014 à décembre 2015, était de tester la résistance aux antibiotiques de 52 isolats de *Salmonella gallinarum* obtenus à partir d'élevages avicoles modernes situés en zones périurbaines du district de Bamako (n = 27), et des villes de Ségou (n = 16) et Sikasso (n = 9). Les taux de résistance moyens obtenus ont été de 98,08 % à l'érythromycine, 94,23 % à la colistine, 90,38 % à la streptomycine, 67,31 % à la kanamycine, 65,38 % à la fluméquine, 63,46 % à la doxycycline, 59,61 % à la tétracycline et 21,15 % à la gentamicine. Tous les isolats de salmonelles issus des élevages du district de Bamako se sont avérés résistants à la tétracycline, à la doxycycline et à l'érythromycine. De même, une résistance à l'érythromycine, à la tétracycline, à la colistine et à la streptomycine a été mise en évidence pour tous les isolats issus des élevages du site de Sikasso. Les résultats ont montré un développement de la résistance de la plupart des souches de salmonelles isolées à la majorité des antibiotiques usuels et dans une moindre mesure à la gentamicine.

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■ INTRODUCTION

Le cheptel aviaire au Mali compte près de 38 590 000 têtes, toutes espèces confondues (DNPIA, 2016). Le secteur moderne représente plus de 10 % de cet effectif et est constitué d'exploitations comportant jusqu'à 30 000 têtes (DNPIA, 2016). L'aviculture moderne est la principale source d'approvisionnement des populations en œufs et poulets de chair. A la différence des fermes avicoles traditionnelles villageoises, ces exploitations hébergent des effectifs plus importants par bandes, composés principalement de races exotiques de poules pondeuses dont la Leghorn, la Lhomann Rouge, la Rhode Island, ou de poulets de chair de races Cobb 500 ou Ross élevés sur des espaces assez réduits (7–8 volailles/m^2) ; elles bénéficient de meilleures conditions d'habitat, d'hygiène, d'alimentation et de suivi sanitaire.

Les poussins sont en général importés, même si des embryons d'unités industrielles de production locale de poussins d'un jour commencent à émerger. Bien que les fournisseurs de poussins mettent à la disposition des aviculteurs des plans de prophylaxie, les volailles continuent d'être lourdement impactées par les pathologies, notamment les salmonelloses (Arbelot., 1997 ; Arbelot et al., 1997) qui constituent une préoccupation dans les élevages avicoles africains (Allaoui et al., 2017 ; Elared et al., 2001 ; Arbelot, 1997 ; Arbelot et al., 1997 ; Kounta, 1992 ; Kounta, 1993). Ainsi, des salmonelles ont été isolées en culture dans plusieurs pays d'Afrique subsaharienne aussi bien à partir d'élevage que sur des abats ou sur les carcasses de volailles (Combari, 2014 ; Toko, 2010 ; Coulibaly et al., 2005).

En Afrique de l'Ouest, les salmonelloses causent d'importantes pertes économiques lors de mortalités pouvant atteindre 90 %. En outre, les humains peuvent également être contaminés à travers la consommation de produits issus de volailles (Kimura et al., 2004 ; Sannat et al., 2017 ; Cardinale et al., 2005). Au Mali, les salmonelloses à

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S. pullorum/gallinarum font partie des pathologies dominantes en aviculture moderne avec des taux de prévalences respectivement de 20,09 % et de 19,53 % pour les infections d'organes et d'œufs (Sidibé et al., 2013). La lutte contre les salmonelloses aviaires en Afrique est surtout basée sur l'utilisation abusive de produits antimicrobiens, en l'occurrence les antibiotiques. Pour une production alimentaire sans salmonelles, Awad et Ghareeb (2014) recommandent, entre autres, l'application de la vaccination et de mesures de biosécurité. Au Mali, notamment dans les petites exploitations avicoles, les mesures d'hygiène et de biosécurité pour minimiser le risque d'introduction et de diffusion d'agents pathogènes ne sont pas correctement appliquées. De même, la vaccination contre les salmonelloses n'est pas systématique en raison de son coût.

Dans ce contexte, différents complexes médicamenteux (Neotreat, Panteryl, Tetracolivit, Alfacéryl et Gentadox) renfermant un ou plusieurs antibiotiques, comme l'érythromycine, la colistine, la streptomycine, la doxycycline, la tétracycline, la gentamicine, sont fréquemment utilisés pour prévenir ou traiter les salmonelloses en aviculture moderne. Ils sont également utilisés chez la volaille comme promoteurs de croissance. Suite à la persistance de cas de salmonelloses dans les exploitations avicoles modernes, plusieurs cas d'échecs thérapeutiques ont été observés après l'usage de ces médicaments. Par ailleurs, le profil de résistance aux antibiotiques des souches de salmonelles isolées demeure mal connu.

L'usage incontrôlé d'antibiotiques peut conduire à une sélection de bactéries pathogènes résistantes (Sanders et al., 2012). De même, l'augmentation de l'incidence des souches de *Salmonella* résistantes aux antibiotiques dans les élevages semi-intensifs a été évoquée par Casin et al. (1996). L'antibiorésistance est devenue un sérieux problème de santé publique. L'objectif de la présente étude était de déterminer le profil de résistance à huit antibiotiques usuels (gentamicine, fluméquine, tétracycline, doxycycline, colistine, streptomycine, érythromycine et kanamycine) de souches de *S. gallinarum* isolées dans des élevages avicoles modernes.

■ MATERIEL ET METHODES

L'étude a été réalisée d'août 2014 à décembre 2015 en deux phases : le suivi clinique épidémiologique et la collecte de prélèvements, puis les examens de laboratoire.

Choix des sites et des élevages

Cette étude prospective a été réalisée dans les zones périurbaines du district de Bamako et des villes de Ségou et Sikasso (figure 1). Le développement et l'importance de l'aviculture moderne dans ces sites ont justifié ces choix. En zone périurbaine de Bamako, 96 élevages avicoles ont été visités sur six axes : Bamako-Koulikoro, Bamako-Siby, Bamako-Kangaba, Bamako-Ségou, Bamako-Kati et Bamako-Bougouni. A Ségou, 43 élevages ont été choisis sur trois axes : Ségou-Markala, Ségou-Cinzana, Ségou-Bamako. A Sikasso, 43 fermes avicoles ont été ciblées sur quatre axes : Sikasso-Koutiala, Sikasso-Nièna, Sikasso - Bobo Dioulasso, Sikasso-Missiricoro. Les élevages ont été choisis en fonction de l'importance des effectifs de volailles qui variaient entre 1 000 et 30 000 têtes, du type de spéculation (poules pondeuses), de l'adhésion des aviculteurs à l'esprit de l'étude, de l'accessibilité des élevages et de la fréquence de cas suspects de salmonelloses.

Collecte des échantillons

Au cours de visites bimensuelles, des écouvillonnages cloacaux ont été réalisés sur les poules pondeuses présentant des signes cliniques suspects de salmonelloses (dépression, anorexie, diarrhées blanchâtres, déshydratation, anémie et cyanose sur la crête et les barbillons). Des prélèvements d'organes (foie, rate, intestins, grappes ovariennes, poumons) ont été collectés en cas de présence de signes lésionnels spécifiques de la maladie (décoloration du foie, ovarite, salpingite, splénomégalie, entérite, myopéricardite) à l'autopsie de sujets malades ou récemment morts (moins de quatre heures). Les prélèvements ont été identifiés en notant les informations relatives à l'élevage, aux signes cliniques et lésionnels, et à la nature du prélèvement. Ils ont ensuite été conservés sous glace et acheminés au Laboratoire central vétérinaire pour les examens. Au total, 526 prélèvements, dont 515 écouvillons cloacaux et 11 prélèvements d'organes, ont été collectés dans 182 élevages (tableau I).

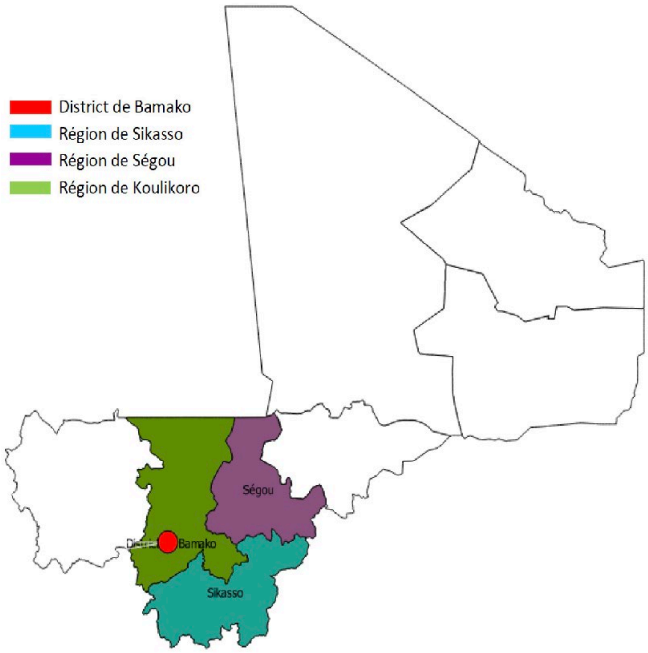

Figure 1 : *localisation des trois sites de l'étude au Mali.*

Méthodes d'analyse de laboratoire

Recherche des salmonelles

L'analyse des échantillons soumis au laboratoire a été effectuée en trois étapes. D'abord, chaque écouvillon a été trempé dans de l'eau peptonée, puis incubé à 37 °C pendant 24 h. Les organes ont été au préalable broyés, dilués à 10 % (P/V) dans de l'eau peptonée (1 ml/g de broyat pesé) puis incubés pendant 24 h. Ensuite, le bouillon au tétrathionate a été ensemencé avec un millilitre de culture de pré-enrichissement sur eau peptonée, puis incubé à 37 °C pendant 24 h. Enfin, à partir des cultures obtenues sur les milieux d'enrichissement, les milieux sélectifs, comme les géloses Mac Conkey et Salmonella-Shigella, ont été ensemencés, puis incubés à 37 °C pendant 24 h. Les

Tableau I

Nombre d'échantillons collectés chez des poules pondeuses en zones périurbaines au Mali

Echantillon	Bamako	Ségou	Sikasso	Total
Ecouvillon cloacal	273	98	144	515
Prélèvement d'organes	04	01	06	11
Total	277	99	150	526

colonies caractéristiques de *Salmonella*, paraissant incolores avec un centre noir sur géloses Salmonella-Shigella et Mac Conkey, ont été observées. Les cultures pures obtenues sur gélose tryptone soja ont été utilisées pour la réalisation de tests biochimiques classiques ou de systèmes API (galeries biochimiques miniaturisées).

Technique de l'antibiogramme

Le test de sensibilité aux antibiotiques a été effectué selon la méthode de diffusion en milieu gélosé de Kirby-Bauer et les recommandations du Comité de l'antibiogramme de la société française de microbiologie (CA-SFM). Le profil de résistance à huit antibiotiques présents dans des complexes médicamenteux couramment utilisés en aviculture moderne a été déterminé : la gentamicine (10 µg), la fluméquine (30 µg), la kanamycine (30 µg), la tétracycline (30 µg), la doxycycline (30 µg), la colistine (10 µg), la streptomycine (10 µg) et l'érythromycine (15 µg). Pour ce faire, chaque souche a été repiquée sur gélose tryptone soja puis incubée à 37 °C pendant 24 h afin d'obtenir des cultures pures. A partir de colonies de la culture de 24 h, une suspension turbide a été réalisée dans deux millilitres d'eau physiologique (NaCl 85 %) à l'échelle 0,5 de Mac Farland. L'inoculum a été obtenu en émulsionnant 100 µl de cette suspension dans 10 ml d'eau physiologique. La gélose Mueller Hinton en boîte de Petri a été ensemencée par strie à l'aide d'écouvillons imbibés de suspension bactérienne. Les disques des huit antibiotiques ont été placés à l'aide d'un distributeur de disques. Les boîtes contenant les disques ont été laissées à la température ambiante sous hotte pendant 30 min puis incubées pendant 24 h à 37 °C. Les résultats ont été interprétés comme sensibles, intermédiaires ou résistants en fonction du diamètre d'inhibition sur gélose Mueller Hinton selon les recommandations du CA-SFM et la méthode de Quinn et al. (1994) (tableau II). Cependant, seuls les résultats résistants et sensibles ont été considérés lors de la synthèse des tests d'antibiogramme. La doxycycline et la fluméquine ne figurent pas sur la fiche de lecture proposée par Quinn et al. (1994), expliquant leur absence du tableau II.

Tableau II

Interprétation des résultats de tests de résistance de souches de *Salmonella gallinarum* à six antibiotiques selon Quinn et al. (1994)

Antibiotique	Resistant ≤	Intermédiaire	Sensible ≥
Gentamicine (10 µg)	12	13–14	15
Kanamycine (30 µg)	13	14–17	18
Tétracycline (30 µg)	14	15–18	19
Colistine (10 µg)	8	9–10	11
Streptomycine (10 µg)	11	12–14	15
Erythromycine (15 µg)	13	14–22	23

■ RESULTATS

La mise en culture des 526 prélèvements a permis d'établir un taux de prévalence bactériologique global de 9,9 % (52 cas positifs) pour *S. gallinarum*. Par ailleurs, 27 échantillons provenant de 96 élevages de la zone périurbaine du district de Bamako, 15 échantillons issus de 43 élevages de la zone de Ségou et 12 échantillons issus de 43 élevages de la zone de Sikasso ont été positifs à la culture des salmonelles. Les résultats relatifs au profil de résistance de *S. gallinarum* aux huit antibiotiques sont présentés dans le tableau III. En moyenne, pour l'ensemble des trois sites, les taux de résistance ont été de 98,1 %

Tableau III

Profil de résistance des souches de *Salmonella gallinarum* isolées chez des poules pondeuses en zones périurbaines au Mali

Antibiotique	Bamako n = 27 (%)	Ségou n = 16 (%)	Sikasso n = 9 (%)	Total n = 52 (%)
Gentamicine (10 µg)	6 (22,22)	3 (18,70)	2 (22,22)	11 (21,15)
Fluméquine (30 µg)	16 (59,25)	11 (68,70)	7 (77,77)	34 (65,38)
Kanamycine (30 µg)	19 (70,37)	9 (56,25)	7 (77,77)	35 (67,31)
Tétracycline (30 µg)	22 (81,48)	0	9 (100)	31 (59,61)
Doxycycline (30 µg)	25 (92,59)	0	8 (88,88)	33 (63,46)
Colistine (10 µg)	25 (92,59)	15 (93,75)	9 (100)	49 (94,23)
Streptomycine (10 µg)	25 (92,59)	13 (81,27)	9 (100)	47 (90,38)
Erythromycine (15 µg)	26 (96,29)	0	9 (100)	51 (98,08)

à l'érythromycine, 94,2 % à la colistine, 90,4 % à la streptomycine, 67,3 % à la kanamycine, 65,4 % à la fluméquine, 63,5 % à la doxycycline, 59,6 % à la tétracycline et 21,2 % à la gentamicine. Pour les 27 isolats de Bamako, le taux de résistance à l'érythromycine a été le plus élevé (96,3 %), suivi de ceux à la doxycycline, à la colistine et à la streptomycine (92,6 % chacun). Sur 16 isolats obtenus à Ségou, le taux de résistance à la gentamicine a été de 18,7 % et celui à la colistine de 93,7 % a été le plus élevé. Tous les isolats de ce site se sont avérés sensibles à la tétracycline, à la doxycycline et à l'érythromycine. Enfin à Sikasso, sur neuf isolats de *S. gallinarum* mis en évidence, le taux de résistance à la doxycycline (88,9 %) a été le plus élevé.

■ DISCUSSION

L'analyse du profil de résistance aux antibiotiques usuels sur des isolats de *S. gallinarum* a montré que les taux de résistance les plus élevés étaient avec l'érythromycine (98,1 %), puis la colistine (94,2 %) et la streptomycine (90,4 %). Celui pour l'érythromycine était cependant inférieur à celui observé par Combari au Sénégal (2014) dans les fermes avicoles de la zone périurbaine de Dakar, qui rapporte que 100 % des souches de salmonelles étaient résistantes à cet antibiotique. Cette similarité pourrait s'expliquer par l'utilisation abusive des promoteurs de croissance contenant en majorité de la colistine et de la streptomycine, et à la résistance naturelle des bactéries à Gram négatif à l'érythromycine. Le pourcentage de résistance le plus faible a été observé pour la gentamicine (21,2 %). Il était comparable à celui de 8,8 % obtenu par Elared et al. (2001) au Maroc. Cet antibiotique est très peu utilisé en aviculture à cause de son coût élevé. Le taux de résistance aux tétracyclines (59,6 %) était comparable à celui obtenu au Sénégal par Combari (2014) et inférieur à celui de Toko (2010) en Côte d'Ivoire (100 %). Cette différence pourrait s'expliquer par le niveau d'utilisation des antibiotiques qui varie d'un pays à l'autre.

En France, il a été établi que les résistances aux tétracyclines sont les plus élevées (ANSES, 2010). Ces dernières sont d'usage courant

en thérapeutique vétérinaire et appartiennent à la famille d'antibiotiques la plus utilisée chez les humains. La mise en évidence d'isolats de *S. gallinarum* résistants à la fluméquine (62,6 %) (classe des fluoroquinolones) est très inquiétante car cette famille d'antibiotiques est utilisée en derniers recours lors du traitement des salmonelloses humaines graves (Weill, 2008). Ce taux était supérieur à celui de 25,5 % obtenu par Combari (2014) au Sénégal.

Le développement de la résistance antimicrobienne à l'échelle mondiale a conduit l'Organisation mondiale de la santé (OMS, 2016), l'Organisation mondiale de la santé animale (OIE), et l'Organisation des Nations unies pour l'alimentation et l'agriculture (ONU-FAO) à adopter une approche concertée en vue d'élaborer un plan mondial pour combattre la résistance aux antimicrobiens. Au Mali, l'élaboration d'un « Plan d'action national de lutte contre la résistance aux antimicrobiens » est en cours de finalisation. Ce plan prévoit la mise en place d'un cadre législatif pour une meilleure lutte contre la résistance antimicrobienne dans le pays. Ainsi, la mise en œuvre des activités clés du plan (information, éducation, sensibilisation des acteurs sur l'ampleur et les mesures de contrôle du phénomène, réglementation rigoureuse de la vente et de l'usage des antibiotiques) contribuerait à réduire le développement de la résistance antimicrobienne. Des campagnes de sensibilisation et de formation des utilisateurs doivent transmettre des messages visant à éviter l'usage d'antibiotiques comme facteurs de croissance, de ne les utiliser qu'en cas de salmonellose confirmée suivie d'un antibiogramme, et de procéder à l'usage rationnel et responsable des antibiotiques.

■ CONCLUSION

La majorité des souches de *S. gallinarum* isolées dans les trois sites de l'étude se sont révélées résistantes à la plupart des antibiotiques usuels. Les techniciens avicoles doivent privilégier la réalisation d'antibiogrammes avant d'entreprendre des traitements. Dans le cadre de la thérapeutique des salmonelloses, l'utilisation de produits à base de gentamicine doit être privilégiée. Des études complémentaires doivent être réalisées en élargissant le test d'antibiogramme à un nombre plus important de sites, en vue d'obtenir des résultats plus complets sur le profil de résistance des salmonelles en aviculture moderne au Mali.

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Déclaration des contributions des auteurs

SS a coordonné les travaux de conception, de planification de l'étude, et la mise en œuvre des analyses de laboratoire ; YSK et AF ont participé à la conception, planification et réalisation de l'étude ; ABT et ABD ont participé à la collecte des prélèvements sur le terrain, et à la rédaction de l'article ; ABT, ABD et KWC ont participé à la mise en œuvre des examens de laboratoire ; AB et OT ont participé aux analyses de laboratoire.

REFERENCES

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, 2013. Résapath - Réseau d'épidémiosurveillance de l'antibiorésistance des bactéries pathogènes animales. Bilan 2012. ANSES, Maisons-Alfort, France, 164 p.

Allaoui A. El, Filali F.R., Ameur N., Bouchrif B., 2017. Contamination des élevages de dinde de chair par *Salmonella* spp. au Maroc : prévalence, antibiorésistance et facteurs de risque associés. *Rev. Sci. Tech. Off. Epizoot.*, **36** (3) : 935-946, doi : 10.20506/rst.36.3.2726

Arbelot B., 1997. Rapport de fin de contrat. Laboratoire de pathologie aviaire ISRA LNERV, Prodec 5B. Cirad-EMVT, Montpellier, France

Arbelot B., Dayon J.F., Mamis D., Gueye J.C., Tall F., Samb H., 1997. Seroprevalence survey of dominant avian diseases in Senegal: mycoplasmoses, fowl typhoid and pullorum disease, Newcastle, infectious bursal and infectious bronchitis diseases. *Rev. Elev. Med. Vet. Pays Trop.*, **50** (3): 197-203, doi: 10.19182/remvt.9569

Awad W.A., Ghareeb K., 2014. Some aspects of control of *Salmonella* infection in poultry for minimizing contamination in food chain. *Salmonella* control in poultry industry. *World Poult. Sci. J.,* **70** (3): 519-530, doi: 10.1017/S0043933914000579

Cardinale E., Perrier Gros-Claude J.D., Rivoal K., Rose V., Tall F., Mead G.C., Salvat G., 2005. Epidemiological analysis of *Salmonella enterica* ssp. *enterica* serovars Hadar, Brancaster and enteritidis from humans and broiler chickens in Senegal using pulsed-field gel electrophoresis and antibiotic susceptibility. *J. Appl. Microbiol.*, **99**: 968-977, doi: 10.1111/j.1365-2672.2005.02618.x

Casin I., Brisabois A., Berger N., Breuil J., Collatz E., 1996. Phénotypes et génotypes de résistance de 182 souches de *Salmonella* sérotype Typhimurium résistantes à l'ampicilline d'origine humaine et animale. *Med. Mal. Infect.*, 26 : 426-430, doi : 10.1016/S0399-077X(96)80187-9

Combari A., 2014. Niveau de contamination par les salmonelles antibiorésistantes des élevages de poulet de chair en zone péri-urbaine de Dakar. Thèse Doct. Méd. Vét., Ecole inter-Etat des sciences et médecines vétérinaires, Dakar, Sénégal, 64 p.

Coulibaly E.K., Bakayogo S., Karou T.G., Coulibaly K.J., Goualie G.B., Dosso M., Diopoh K.J., 2005. Sérotypage et antibiorésistance des souches de *Salmonella* isolées dans les foies de poulet vendus sur les marchés de Yopougon (Abidjan, Côte d'Ivoire) en 2005. *Rev. Afr. Santé Prod. Anim.*, **8** (s)

Direction nationale des productions et industries animales, 2016. Rapport annuel d'activités. DNPIA, Bamako, Mali, 137 p.

Elared O., Amara A., Faid M., Alaoui M.A., Tahri El H., 2001. Antibiorésistance des souches de *Salmonella enteritidis* isolées dans la zone Rabat-Casablanca à partir de l'œuf de consommation, de l'aliment et des fientes de poules pondeuses. *Actes Inst. Agron. Vet (Maroc).*, **21** (3) : 147-150

Kimura A.C., Reddy V., Marcus R., Cieslak P.R., Moehle-Boetani J.C., Kassenborg H.D., Segler S.D., et al., 2004. Chicken consumption is a newly identified risk factor for sporadic *Salmonella enterica* serotype Enteridis infections in the United States: a case-control study in Foodnet sites. *Clin. Infect. Dis.*, **38** (Suppl. 3): 244-252, doi: 10.1086/381576

Kounta A.O.S., 1992. Note technique sur le développement de l'aviculture au Mali. *Tropicultura*, **10** : 103-105

Kounta A.O.S., 1993. Vers un développement de l'aviculture villageoise au Mali. *Afric. Agric.* (223) : 56

OMS, 2016. Plan d'action mondial pour combattre la résistance aux antimicrobiens. OMS, Genève, Suisse, 32 p.

Quinn P.J., Carter M.E., Markey B., Carter G.R., 1994. Clinical veterinary microbiology. Wolfe, London, UK

Sanders P., Granier S.A., Blanc-Gonnet A., Santolini J., 2012. Les plans de surveillance de l'antibiorésistance en santé animale : le contexte européen et les évolutions récentes. Bull. Epidémiol. Santé Anim. Aliment. (53 Spécial Antibiot. Antibiorésistances) : 25-29

Sannat C., Patyal A., Rawat N., Ghosh R.C., Jolhe D.K., Shende R.K., Hirpurkar S.D., et al., 2017. Characterization of *Salmonella* Gallinarum from an outbreak in Raigarh, Chhattisgarh. *Vet. World*, **10** (2): 144-148, doi: 10.14202/vetworld.2017.144-148

Sidibé S.S., Coulibaly M.K.., Sylla M., Traoré B., Kéita S., Doumbia M.R., Diallo M., et al., 2013. Pathologies dominantes identifiées en aviculture semi-industrielle au Mali (zones périurbaines du District de Bamako et des villes de Sikasso et Ségou). *Bull Anim. Health Prod. Afr.*, **61** (3) : 427-433

Toko A., 2010. Evaluation du niveau de résistance de salmonelles d'origine aviaire vis-à-vis de la tétracycline et du sulfaméthoxazole. Thèse Doct. Méd. Vét., Ecole inter-Etat des sciences et médecines vétérinaires, Dakar, Sénégal, 84 p.

Weill F.X., 2008. Salmonelles non-typhiques d'origine animale et résistance aux antibiotiques. Bull. Acad. Vét. France, **61** (3), doi : 10.4267/2042/47946

Summary

Sidibé S., Traoré A.B., Koné Y.S., Fané A., Coulibaly K.W., Doumbia A.B., Bamba A., Traoré O. Antibiotic resistance of isolated *Salmonella gallinarum* strains in modern poultry farming in suburban areas in Mali

The objective of the study, conducted from August 2014 to December 2015, was to test the antibiotic resistance of 52 *Salmonella gallinarum* isolates obtained from modern poultry farms located in suburban areas of Bamako District (n = 27), and of Segou (n = 16) and Sikasso (n = 9) cities. The average resistance rates obtained were 98.08% to erythromycin, 94.23% to colistin, 90.38% to streptomycin, 67.31% to kanamycin, 65.38% to flumequine, 63.46% to doxycycline, 59.61% to tetracycline and 21.15% to gentamicin. All *Salmonella* isolates from Bamako District farms were resistant to tetracycline, doxycycline and erythromycin. Similarly, all isolates from Sikasso area farms showed resistance to erythromycin, tetracycline, colistine and streptomycin. The results showed a development of resistance of most isolated salmonella strains to the majority of common antibiotics, and to a lesser extent to gentamicin.

Keywords: poultry, *Salmonella gallinarum*, resistance to antibiotics, Mali

Resumen

Sidibé S., Traoré A.B., Koné Y.S., Fané A., Coulibaly K.W., Doumbia A.B., Bamba A., Traoré O. Resistencia a los antibióticos de cepas de *Salmonella gallinarum* aisladas en avicultura moderna en zonas periurbanas en Mali

El objetivo de este estudio, llevado a cabo entre agosto 2014 y diciembre 2015, fue el de examinar la resistencia a antibióticos en 52 aislamientos de *Salmonella gallinarum*, obtenidos a partir de granjas avícolas modernas, situadas en zonas periurbanas del distrito de Bamako (n = 27), y de las ciudades de Segou (n = 16) y Sikasso (n = 9). Las tasas de resistencia promedio obtenidas fueron de 98,08% a la eritromicina, 94,23% a la colistina, 90,38 a la estreptomicina, 67,31 a la kanamicina, 65,38% a la flumequina, 63,46% a la doxiciclina, 59,61% a la tetraciclina y 21,15% a la gentamicina. Todos los aislamientos de salmonelas provenientes de granjas del distrito de Bamako fueron resistentes a la tetraciclina, a la doxiciclina y a la eritromicina. También se demostró una resistencia a la eritromicina, a la tetraciclina, a la colistina y a la estreptomicina en todos los aislamientos provenientes de Sikasso. Los resultados mostraron un desarrollo de la resistencia en la mayoría de las cepas de salmonelas aisladas a la mayoría de los antibióticos usuales y en menor medida a la gentamicina.

Palabras clave: aves de corral, *Salmonella gallinarum*, resistencia a los antibióticos, Malí

Antimicrobial resistance profiles of *Salmonella* serovars isolated from dressed chicken meat at slaughter in Kaduna, Nigeria

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Keywords

Salmonella, resistance to antibiotic, chicken meat, slaughtering, Nigeria

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Summary

Invasive non-typhoidal salmonellosis characterized by gastroenteritis and bacteremia is endemic in sub-Sahara Africa. Most infections are foodborne with animals serving as asymptomatic carriers. We investigated *Salmonella* serovars and associated resistance genes in chicken meat using culture, minimum inhibitory concentrations and PCR amplification of resistance genes. Of 100 samples examined, 28 (28%) were *Salmonella* positive and spread across six serovars: Haifa (71.4%), Chomedey (7.1%), Saintpaul (7.1%), Kainji (7.1%), Derby (3.6%), and Blockley (3.6%). Antimicrobial resistance was observed to ciprofloxacin (85.7%), nalidixic acid (75.0%), sulfamethoxazole (67.8%), tetracycline (89.3%), trimethoprim (42.9%), gentamicin (35.7%), streptomycin (32.1%), ampicillin (10.7%), chloramphenicol (10.7%), kanamycin (7.1%) and florfenicol (3.6%). All isolates were susceptible to cefotaxime, ceftazidime and colistin, whereas 19 (67.9%) showed multidrug resistance to at least three antimicrobials. The predominant resistance type was Cip-Gen-Nal-Smx-Tet-Tmp detected in six (21%) isolates. Multidrug resistance of *Salmonella* serovars was high in the sampled chicken meat with resistance most observed against ciprofloxacin. This suggests possible horizontal transfer of plasmid-mediated quinolone resistance genes, which may compromise the clinical use of fluoroquinolones. Thus, improved hygiene and provision of adequate facilities at meat processing centers could help limit meat contamination and foodborne transmission of multiresistant non-typhoidal *Salmonella* serovars from chickens to humans.

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■ INTRODUCTION

Salmonella is an enteric bacterial pathogen of the family Enterobacteriaceae and has the potential to invade tissue and cause bacteremia (de Jong et al., 2012). Globally, *Salmonella* serovars are among the leading cause of foodborne gastroenteritis and constitute health and socioeconomic burdens in both developed and developing countries. Non-typhoidal *Salmonella enterica* causes an annual estimate of 93.8 million cases of gastroenteritis and 155,000 deaths worldwide, with over 85% of *Salmonella* gastroenteritis attributed to foodborne transmission (Majowicz et al., 2010). Food-producing animals have been implicated as major reservoirs and sources of zoonotic transmission of non-typhoidal salmonellosis (Kagambèga et al., 2013). In particular, poultry and poultry products play significant roles in the transmission of salmonellosis to humans. Apparently, healthy chickens may harbor non-typhoidal zoonotic *S. enterica* serovars in their gastrointestinal tracts, which are transmitted to humans through contact with carrier birds, feces-contaminated environment and consumption of contaminated food and water (Kagambèga et al., 2011; 2013).

In developing countries, cases of salmonellosis are often misdiagnosed because of inadequate laboratory investigations (Feasey et al., 2012). Moreover, unhygienic practices in food processing and in distribution chains, low-level awareness of non-typhoidal salmonellosis, malnutrition and concurrent infections may contribute to spread and severity of

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foodborne infections. Self-medication, delayed or lack of access to good health care, inappropriate prescriptions and prevailing cultural practices are additional factors that may contribute to complications, widespread dissemination, increased morbidity and case fatality of *Salmonella* gastroenteritis (Morpeth et al., 2009). Generally, the incidence of foodborne infection in developing countries is grossly underestimated because of poor record keeping, inadequate disease reporting and lack of coordinated monitoring and surveillance of important foodborne pathogens.

The increasing incidence of antimicrobial resistance by pathogenic and commensal bacteria is of public health concern. In sub-Saharan Africa, antimicrobial chemotherapy is an integral part of the management of invasive non-typhoidal *Salmonella* infections (Feasey et al., 2012). However, widespread acquisition and dissemination of antimicrobial resistance traits are continuously narrowing the antimicrobial therapeutic options available for the treatment of bacterial infections. Antimicrobial-resistant *Salmonella* spp. have been isolated from different foods of animal origin around the world (Garedew et al., 2015; Löfström et al., 2015; Saravanan et al., 2015). Extensive use of antimicrobial agents in animal production is common and selects for multidrug resistant (MDR) pathogens (Marshall and Levy, 2011; Oluwasile et al., 2014). Fluoroquinolones are broad-spectrum antimicrobials effective in the treatment of a wide variety of clinical and veterinary infections, particularly for life-threatening salmonellosis treatment.

Quinolone resistance in *Salmonella* is mainly attributed to point mutations in chromosomal genes encoding gyrase subunits A (*gyrA*) and B (*gyrB*), and topoisomerase subunits C (*parC*) and E (*parE*) (Hopkins et al., 2005). The fluoroquinolone resistance phenotype might be further modulated with the appearance of several plasmid-mediated quinolone resistance mechanisms (PMQR) (Luo et al., 2011; Wasyl et al., 2014) encoded by *qnrA*, *qnrB*, *qnrS*, *qepA* genes.

Factors responsible for the endemicity of non-typhoidal *Salmonella* infections in Africa are poorly understood (Morpeth et al., 2009). There is a dearth of information on the dynamics of *Salmonella* serovars and their antimicrobial resistance properties in livestock and food of animal origin. To prevent the continuous spread of antimicrobial resistance and zoonotic transmission of foodborne pathogens from animals to humans, there is a need for regular monitoring and reporting of resistant bacteria in food of animal origin. The present study investigated the occurrence of *Salmonella* serovars as well as their phenotypic and genotypic antimicrobial resistance properties in freshly dressed chicken meat in Kaduna in Northern Nigeria.

■ MATERIALS AND METHODS

Sample collection

One hundred neck skin samples were collected from freshly dressed chicken meat sold to consumers at two chicken processing facilities in Kaduna, capital of Kaduna State, Nigeria. Neck skin sampling was selected in this study since it provides higher *Salmonella* yields than whole carcass rinse and other skin parts (Diezhang et al., 2014). Fifty samples were collected from each facility, and sampling was carried out three times daily (morning, afternoon and evening) for two weeks in March 2012. In each center, one and sometimes two samples per visit were collected from different processors selling dressed chickens. Samples were collected in pre-labeled sample bags and preserved with ice pack in sterile containers before transport to the laboratory for microbiological analysis.

Isolation and presumptive identification of Salmonella

Salmonella isolation was performed according to ISO 6579 standard (2002) with minor modifications. In particular, each neck sample (10 g) was pre-enriched after thorough homogenization in 90 ml of sterile buffered peptone water (BPW, Oxoid CM0509, UK), then incubated at 37°C for 18–24 hours. From the BPW culture, 0.1 ml was transferred into 9.9 ml of modified semi-solid Rapport-Vassiliadis (MSRV) selective enrichment broth (Oxoid CM0910) supplemented with novobiocin (Oxoid SR0161), then incubated at 42°C for 24–48 hours. Afterwards, MSRV was observed for the typical migration pattern of *Salmonella enterica* (≥ 2.0 mm migration) every 24 hours until 72 hours. A loopful of observable bacterial spread was taken from the periphery of MSRV medium and streaked simultaneously onto plates of brilliant green agar (BGA, Oxoid CM0263) supplemented with sulphamandelate (Oxoid SR0087) and onto xylose lysine desoxycholate agar (XLD, Oxoid). The inoculated plates were incubated at 37°C for 18–24 hours, then examined for bacterial colonies. Typical *Salmonella* colonies were picked and subjected to biochemical and serological tests.

Serotyping of Salmonella *isolates*

Presumptive *Salmonella* isolates were shipped to the Office International des Epizooties (OIE) Reference Laboratory for *Salmonella*, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe, Italy). All strains were serotyped by agglutination tests with specific O and H antisera and classified according to Kauffman-White's scheme as previously described (Grimont and Weill, 2007).

Phenotypic antimicrobial susceptibility testing

Susceptibility to 14 antimicrobials was carried out to determine the minimum inhibitory concentrations by means of broth microdilution following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The CMV2AGNF (sensititre, Trek Diagnostic Systems, USA) susceptibility plates of the National Antimicrobial Monitoring System containing 14 antimicrobials was used. The antimicrobials tested were ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, colistin, florfenicol, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim. The breakpoints used for sulfamethoxazole and kanamycin were CLSI (2009) breakpoints, whereas for other antimicrobials, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off was used (Aarestrup and McDermott, 2007; EUCAST, 2012). *Escherichia coli* (ATCC 25922) was used as the quality control strain.

Determination of antimicrobial resistance genes

DNA templates used for PCR were prepared by boiling as described by Barco et al. (2011). Molecular characterization of genetic antimicrobial resistance determinants in all isolates was undertaken by PCR amplification according to Beutlich et al. (2011). The isolates were analyzed for the presence of resistance genes corresponding to their resistance phenotypes. The resistance-associated genes investigated included those encoding resistance to beta-lactam antibiotics (bla_{PSE1}, $bla_{TEM1\text{-}like}$, $bla_{OXA1\text{-}like}$), chloramphenicol and florfenicol (*catA1*, *cmlA*, *floR*), fluoroquinolones (*gyrA*, *qnrA*, *qnrB*, *qnrS*, *qepA*), gentamicin [*aacC2*, *aacC4*, *aac*(3)-Ie (*aacC5*), *aadB*, *armA*], kanamycin [*aphA1*, *aphA2*, *aac*(6)-1b], streptomycin (*aadA1*-like, *strA*, *strB*), sulfamethoxazole (*sul1*, *sul2*, *sul3*), tetracycline [*tet*(A), *tet*(B), *tet*(G)], and trimethoprim (*dfrA1*-like, *dfrA5*-14, *dfrA7*-17, *dfrA12*). In particular, PCR amplification of *gyrA* gene and sequencing of purified amplicons were performed on both nucleotide strands to detect substitutions using ABI 100 DNA sequencer (Applied Biosystems, USA) as previously reported (Capoor et al., 2009). In each case, the *gyrA* sequence obtained was compared with wild-type sequences (GenBank accession number AE006468.1).

■ RESULTS

Prevalence of Salmonella *serovars in chicken meat*

Culture and serology confirmed almost one third (28%, 28/100) of the samples as *Salmonella* positive; they were spread across six serovars:

S. Haifa, *S*. Chomedey, *S*. Saintpaul, *S*. Derby, *S*. Blockley and *S*. Kainji (Table I).

Phenotypic antimicrobial resistance of S. *isolates*

The highest antimicrobial resistance was to ciprofloxacin (85.7%, 24/28), followed by nalidixic acid (75%), sulfamethoxazole (67.8%) and tetracycline (89.3%) (Table I). About half of *Salmonella* isolates showed resistance to trimethoprim, gentamicin and streptomycin, whereas small numbers of isolates were resistant to ampicillin, chloramphenicol, kanamycin and florfenicol. All isolates were susceptible to cefotaxime, ceftazidime and colistin, whereas the two representatives of *S*. Kainji were susceptible to all tested antimicrobial agents (Table I). Of the 26 resistant isolates, nineteen (67.9%) showed multidrug resistance to at least three different classes of antimicrobials. The most prevalent resistance type (R-type) was Cip-Gen-Nal-Smx-Tet-Tmp observed in six (21%) isolates, followed by Cip-Nal-Tet in four (14%) isolates (Table II).

Antimicrobial resistance genes in Salmonella *isolates*

The bla_{TEM} gene coding for beta-lactam resistance was detected in all three ampicillin-resistant isolates (Table I). The *floR* gene encoded resistance to chloramphenicol and florfenicol in one isolate. Two chloramphenicol-resistant isolates carried *cmlA* gene. Streptomycin was mainly encoded by *strB* (6 isolates) and/or *strA* (1 isolate) genes except for two isolates that showed only *aad1*-like gene. Gentamicin resistance was mediated by *aacC2* (7/10 resistant isolates) and *acc(3)-le* (3/10 resistant strains) genes. Sulfamethoxazole resistance was mostly associated with *sul1* (15/19), however, *sul2* (2/19) and *sul3* (2/19) were also detected. Tetracycline resistance was uniquely encoded by *tet*(A) in 25 isolates. Trimethoprim resistance was uniquely encoded by *dfrA5*-14 (12 isolates). Substitutions in the codons of *gyrA* were responsible for fluoroquinolone resistance in 21 isolates. Sequence analysis revealed mutation of amino acid at position 83 (TCC→TAC, Ser83→Tyr) in 17 isolates and (TCC→TTC, Ser83→Phe) in four isolates. Three fluoroquinolone-resistant isolates with similar R-type (Cip-Gen-Nal-Smx-Str-Tet-Tmp) had double point mutations in their *gyrA* gene. The first mutation occurred at position 83 (TCC→TTC, Ser83→Phe), the second at position 87 (GAC→GGC, Asp87→Gly). Three (10.7%) of the *Salmonella* isolates carried a *qnr* gene. These isolates were characterized by a reduced level of fluoroquinolone resistance (0.5–1 mg/l) and no simultaneous chromosomal mutations occurred in gyrase or topoisomerase. Two isolates (*S*. Chomedey) carried *qnrB* gene and one isolate (*S*. Derby) *qnrS* gene.

■ DISCUSSION

The results showed high contamination of poultry meat during processing with potentially zoonotic non-typhoidal *Salmonella* serovars. The serovars detected have been associated throughout the world with foodborne infections in humans. For instance, Kaibu et al. (2005) report fatal cases of food intoxication by *Salmonella* Haifa in an older person and in a child. *S*. Derby was reported in an outbreak of foodborne

Table I

Distribution of resistant isolates and resistance genes among *Salmonella* serovars isolated from fresh chicken meat in Kaduna, Nigeria

Antimicrobial agent	Resistance gene	*Salmonella* serovars*					Total (n = 28)	% (95% CI)
		S. Haifa (n = 20)	*S*. Derby (n = 1)	*S*. Chomedey (n = 2)	*S*. Blockley (n = 1)	*S*. Saintpaul (n = 2)		
Ampicillin	bla_{TEM}	1	–	–	–	2	3	10.7 (2.9–28.0)
Cefotaxime	–	–	–	–	–	–	–	–
Ceftazidime	–	–	–	–	–	–	–	–
Chloramphenicol	*cmlA1*	–	–	–	–	2	2	7.1 (0.9–23.7)
Chloramphenicol/ florfenicol	*floR*	1	–	–	–		1	3.6 (0.0–19.2)
Colistin	–	–	–	–	–	–	–	–
Fluoroquinolone (ciprofloxacin)	*gyrA* mutation	20	–	–	1	–	21	75 (56.4–87.6)
Fluoroquinolone (ciprofloxacin)	*qnrB*	–	–	2	–	–	2	7.1 (0.9–23.7)
Fluoroquinolone (ciprofloxacin)	*qnrS*	–	1	–	–	–	1	3.6 (0.0–19.2)
Gentamicin	*aacC2*	7	–	–	–	–	7	25 (12.4–43.6)
Gentamicin	*aac(3)-Ie*	3	–	–	–	–	3	10.7 (2.9–28.0)
Kanamycin	*aphA1*	–	–	–	1	1	2	7.1 (0.9–23.7)
Nalidixic acid	–	20	–	–	1	–	21	75 (56.4-87.6)
Streptomycin	*strA*	1	–	–	–	–	1	3.6 (0.0–19.2)
Streptomycin	*strB*	3	–	2	1	–	6	21.4 (9.9–39.9)
Streptomycin	*aad-1like*	–	–	–	–	2	2	7.1 (0.9–23.7)
Sulfamethoxazole	*sul1*	15	–	–	–	–	15	53.6 (35.8–70.5)
Sulfamethoxazole	*sul2*	–	–	2	–	–	2	7.1 (0.9–23.7)
Sulfamethoxazole	*sul3*	–	–	–	–	2	2	7.1 (0.9–23.7)
Tetracycline	*tet*(A)	19	1	2	1	2	25	89.3 (72.0–97.1)
Trimethoprim	*dfrA5-14*	12	–	–	–	–	12	42.9 (26.5–61.0)

* No resistant isolates were found for *S*. Kainji (n = 2)

Table II

Phenotypic and genotypic resistance patterns of *Salmonella* serovars isolated from fresh chicken meat in Kaduna, Nigeria

Serial Num.	Serovar	Phenotypic resistance pattern	Resistance genes (mutations)
1	*S*. Haifa	CIP-NAL	*gyrA* (Ser83→Tyr)
2	*S*. Derby	CIP-TET	*qnrS, tet*(A)
3	*S*. Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
4	*S*. Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
5	*S*. Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
6	*S*. Haifa	CIP-NAL-TET	*gyrA* (Ser83→Tyr), *tet*(A)
7	*S*. Chomedey	CIP-SMX-STR-TET	*qnrB, sul2, strB, tet*(A)
8	*S*. Chomedey	CIP-SMX-STR-TET	*qnrB, sul2, strB, tet*(A)
9	*S*. Blockley	CIP-KAN-NAL-STR-TET	*gyrA* (Ser83→phe), *aphA1, strA, strB, tet*(A)
10	*S*. Haifa	CIP-NAL-SMX-TET	*gyrA* (Ser83→Tyr), *sul1, tet*(A)
11	*S*. Haifa	CIP-NAL-SMX-TET	*gyrA* (Ser83→Tyr), *sul1, tet*(A)
12	*S*. Haifa	CIP-NAL-SMX-TET	*gyrA* (Ser83→Tyr), *sul1, tet*(A)
13	*S*. Haifa	CIP-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *sul1, tet*(A), *dfrA5-14*
14	*S*. Saintpaul	AMP-CHL-FFN-SMX-STR-TET	bla_{TEM}, *floR, sul3, aad1-like, tet*(A)
15	*S*. Saintpaul	AMP-CHL-KAN- SMX-STR-TET	bla_{TEM}, *cmlA, aphA1, sul3, aad1-like, tet*(A)
16	*S*. Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
17	*S*. Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
18	*S*. Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
19	*S*. Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
20	*S*. Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
21	*S*. Haifa	CIP-GEN-NAL-SMX-TET-TMP	*gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
22	*S*. Haifa	AMP-CIP-GEN-NAL-SMX-TET-TMP	bla_{TEM}, *gyrA* (Ser83→Tyr), *aacC2, sul1, tet*(A), *dfrA5-14*
23	*S*. Haifa	CIP-CHL-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→Tyr), *cmlA, aad1-like, sul1, tet*(A), *dfrA5-14*
24	*S*. Haifa	CIP-GEN-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→phe, Asp87→Gly), *aac(3)Ie, sul1, strB, tet*(A), *dfrA5-14*
25	*S*. Haifa	CIP-GEN-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→phe, Asp87→Gly), *aac(3)Ie, sul1, strB, tet*(A), *dfrA5-14*
26	*S*. Haifa	CIP-GEN-NAL-SMX-STR-TET-TMP	*gyrA* (Ser83→phe, Asp87→Gly), *aac(3)Ie, sul1, strA, strB, tet*(A), *dfrA5-14*

AMP: ampicillin; CHL: chloramphenicol; CIP: ciprofloxacin; FFN: florfenicol; GEN: gentamicin; KAN: kanamycin; NAL: nalidixic acid; STR: streptomycin; SMX: sulfamethoxazole; TET: tetracycline; TMP: trimethoprim

Florfenicol: *floR*; fluoroquinolones: *gyrA*, *qnrB*, *qnrS*; gentamicin: *aacC2*, *aac*(3)-Ie; kanamycin: *aphA1*; streptomycin: *aadA1*-like, *strA*, *strB*; sulfamethoxazole: *sul1*, *sul2*, *sul3*; tetracycline: *tet*(A); trimethoprim: *dfrA5-14*

salmonellosis following the consumption of grilled meat in Fukuka City in Japan (Ebuchi et al., 2006). *S*. Saintpaul was responsible for a large foodborne outbreak of salmonellosis involving 1500 cases with 21% hospitalizations and two deaths in the United States (Barton et al., 2011). Gonose et al. (2012) reported *S*. Blockley as the etiology of foodborne illness characterized by diarrhea, stomach cramp and headaches in three adult males in South Africa.

The 28% overall prevalence rate of *Salmonella* observed in dressed chicken in our study was higher than those of 11% reported in the feces of on-farm chickens in Ibadan (Fashae et al., 2010), and of 2.3–5.2% in feces and intestinal samples of chickens in Maiduguri, Nigeria (Raufu et al., 2009). Unhygienic processing including lack of proper separation of dirty and clean areas during evisceration in the slaughterhouse, unhygienic processors and equipment contributed to the contamination of carcasses during processing. In addition, the extent of colonization of the crop due to feed withdrawal during transportation and resting exposes carcasses to possible contamination (Ramirez et al., 1997). *Salmonella* contamination of the neck skin has been linked to colonization of the crop since this organ is more likely to be ruptured during processing than other parts of the gastrointestinal tract such as the ceca (Hargis et al., 1995). Finally, successful contamination of carcasses and slaughter lines may also depend on the toughness of the contaminating *Salmonella* strain, considering that such strains survive environmental stress usually associated with slaughterhouses (Olsen et al., 2003; Rasschaert et al., 2007).

S. Haifa was the predominant (71.4%) serovar detected in the present study. Five other serovars were also detected suggesting that chicken meat may act as a vehicle for the transmission of diverse ranges of *Salmonella* serovars. Fashae et al. (2010) detected eight *S*. serovars (Virchow, Bredeney, Derby, Haifa, Havana, Muenster, Mbandaka and Onireke) in the feces of on-farm chickens in Ibadan, and *S*. Virchow accounted for 71% of all isolates. In Maiduguri, *S*. Hiduddify was the main (95%) serovar isolated from chicken feces and tissues (Raufu et al., 2009). Differences in geographical location, sampling site and type may account for the observed disparities betwen these studies. Although the *S*. serovars detected in this study could originate from the intestinal content of chickens, they may have originated from extraneous sources including the persons involved in processing chicken meat.

Fluoroquinolones, especially ciprofloxacin, are the drugs of choice in the treatment of human salmonellosis (Akinyemi et al., 2007) and they are also used frequently in livestock medicine. Resistance to ciprofloxacin was found in over 85% of all isolates. Most fluoroquinolone resistance (87.5%) was mediated by chromosomal mutations on *gyrA* gene. Resistance mutations occurring on *gyrA* gene, corresponding to amino acid changes at Ser-83 (to Phe or Tyr) or at Asp-87 (to Gly), are the most frequently observed in fluoroquinolone resistant strains (Piddock,

2002; Hopkins et al., 2005). In addition to these site mutations, there were occurrence and dissemination of *qnr* genes in *Salmonella* isolates in this study, even though they were quite low (10.7%). This suggests horizontal spread of resistance plasmid determinants in Kaduna State as reported by Fortini et al. (2011). However, it is notable that wide dissemination of PMQR genes mainly in commensal or pathogenic Gram-negative bacteria is mostly associated with defined successful plasmids which could be positively selected by other drugs because of the co-location of multiple resistance determinants (Fortini et al., 2011). In general, the acquisition of PMQR genes decreases susceptibility to fluoroquinolones and may accelerate selection of fluoroquinolone-resistant mutants (Martínez-Martínez et al., 2003; Rodríguez-Martínez et al., 2007). Given their transferability and the possibility that they may cause increases in resistance thereby affecting the clinical response to treatment, data on the occurrence of PMQR genes are important for the surveillance of quinolone resistance in humans and animals. In developing countries where poor facilities hamper accurate and timely diagnosis, antimicrobial agents are administered empirically for the treatment of suspected cases of salmonellosis without recourse to laboratory investigation. Resistance may complicate *Salmonella* infections as resistant strains are more likely to be associated with tissue invasion, severe illness and death (Helms et al., 2004).

In this study there was a high resistance to (fluoro)quinolones, sulfonamide and tetracycline, a moderate resistance to trimethoprim and aminoglycosides, and a low resistance to ampicillin and phenicol antimicrobials. This could be a reflection of the pattern of antimicrobial use in the poultry industry. Usage of any particular antimicrobial agent could exert selective pressure leading to the emergence, proliferation and spread of bacterium strains showing resistance to the antimicrobial agent. Previous studies have shown that fluoroquinolones, sulfonamides and tetracyclines are the most widely used antimicrobial agents in the Nigerian poultry industry (Ogunleye et al., 2008; Oluwasile et al., 2014). In our study resistance to tetracycline was found in five out of six serovars and was uniquely associated with *tet*(A) gene. Tetracycline is used as an additive in water and feed to improve performance, whereas ciprofloxacin is the ready choice for the treatment of bacterial infections and sometimes for prophylaxis (Oluwasile et al., 2014).

Although three serovar types (Haifa, Saintpaul and Chomedey) showed resistance to sulfamethoxazole, the resistance was mediated by different genes. The *sul1* gene was responsible for resistance in all isolates belonging to serovar Haifa. In *S*. Chomedey, sulfamethoxazole resistance was due to *sul2*, whereas in *S*. Saintpaul it was due to *sul3*. Two isolates of *S*. Saintpaul possessed *cmlA*, which accounted for resistance to chloramphenicol, whereas in *S*. Haifa resistance to chloramphenicol and its derivative (florfenicol) was mediated by *floR*. All gentamicin-resistant *Salmonella* isolates belonged to serovar Haifa and resistance was attributable mainly to *aacC2* (seven isolates) but also to *aac(3)-Ie* (three isolates). Similarly, trimethoprim resistance was observed only in *S*. Haifa and was encoded solely by *dfrA5-14* gene. Two *S*. Saintpaul isolates and one *S*. Haifa showed resistance to ampicillin and possessed bla_{TEM} gene. Resistance to streptomycin was detected in nine isolates belonging to four different serovars. Three streptomycin-associated genes were detected: *strA* and *strB* in *S*. Haifa, *strB* in *S*. Chomedey and Blockley, and *aadA-like* in *S*. Saintpaul. The *Salmonella* isolates were completely susceptible to cefotaxime, ceftazidime and colistin. These drugs are not known to be used in poultry production. Susceptibility to cefotaxime and ceftazidime indicated that cephalosporins including highly important third and fourth generation antimicrobials will be very effective in the treatment of life-threatening and complicated salmonelloses in humans.

These findings are subject to at least two limitations. Firstly, these data were mainly collected from chickens originating from Kaduna State and other parts of Northern Nigeria which produce live chickens, and secondly, the relatively small sample size, which depended on budgetary constraints since whole chickens had to be purchased to allow for sampling. Notwithstanding these limitations, this study offers some insight into the circulating non-typhoidal *Salmonella* serovars and their antibiogram.

■ CONCLUSION

The study showed that chicken meat may contribute to the transmission of non-typhoidal *Salmonella* serovars in the area. In addition, the *Salmonella* observed might have been attributed to unhygienic handling during processing. This included observed poor personal hygiene among processors during processing as well as poor slaughterhouse sanitation due to inadequate infrastructure and unclean running water. Improved hygiene, provision of slaughter facilities, as well as adequate inspection at meat processing centers may limit meat contamination and reduce the risk of zoonotic transmission of non-typhoidal salmonellosis to humans. Furthermore, the study revealed circulation of *S*. serovars that are predominantly multidrug resistant. Preventive measures against misuse of antibiotics, including strict monitoring of antibiotics use both for prophylaxis and growth stimulation in poultry farms will mitigate antibiotic resistance development.

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Author contributions statement

MA, AAL, OEO, MAD designed and planned the study; MA, AL, EM, KA, PZ, BBO, EOO performed experiments and collected data; MA, AAL, OEO analyzed and interpreted data; MA, AAL, OEO, BBO, AL, EM, KA, PZ, EOO drafted the first version of the manuscript; MA, AL, OEO, MAD critically reviewed the manuscript.

REFERENCES

Akinyemi K.O., Bamiro B.S., Coker A.O., 2007. Salmonellosis in Lagos, Nigeria: incidence of *Plasmodium falciparum*-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. *J. Health Popul. Nutr.*, **25** (3): 351-358

Arestrup F., McDermott P., Kahlmeter G., 2007. Antimicrobial susceptibility testing - clinical break points and epidemiological cut-off values. Community Reference Laboratory for Antimicrobial Resistance, European Union, Newsl. 2, 3-5

Barco L., Lettini A.A., Ramon E., Longo A., Saccardin C., Pozza M.C.D., Ricci A., 2011. A rapid and sensitive method to identify and differentiate *Salmonella enterica* serotype Typhimurium and *S. enterica* serotype 4,[5],12:i:- by combining traditional serotyping and multiplex polymerase chain reaction. *Foodborne Pathog. Dis.*, **8** (6): 741-743, doi:10.1089/fpd.2010.0776

Barton B.C., Mody K.R., Jungk J., Gaul L., Redd T.J., Chen S., Cosgrove S., et al., 2011. 2008 outbreak of *Salmonella* Saintpaul infections associated with raw produce. *N. Engl. J. Med.*, **364** (10): 918-927, doi: 10.1056/NEJMoa1005741

Beutlich J., Jahn S., Malorny B., Hauser E., Huhn S., Schroeter A., Rodicioet R.M., et al., 2011. Antimicrobial resistance and virulence determinants in European *Salmonella* genomic island 1-positive *Salmonella enterica* isolates from different origins. *Appl. Environ. Microbiol.*, **77** (16): 5655-5664, doi: 10.1128/AEM.00425-11

Capoor R.M., Nair D., Walia S.N., Routela S.R., Grover S.S., Deb M., Aggarwal P., et al., 2009. Molecular analysis of high-level ciprofloxacin resistance in *Salmonella enterica* serovar Typhi and *S.* Paratyphi A: need to expand the QRDR region? *Epidemiol. Infect.*, **137** (6): 871-878, doi: 10.1017/S0950268808001076

Clinical and Laboratory Standards Institute, 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th edn. CLSI, Wayne, PA, USA

De Jong H.K., Parry C.M., Van der Poll T., Wiersinga W.J., 2012. Host-pathogen interaction in invasive salmonellosis. *PLoS Pathog.*, **8** (10): e1002933, doi: 10.1371/journal.ppat.1002933

Diezhang Wu., Alali Q.W., Harrison A.M., Hofacre L.C., 2014. Prevalence of *Salmonella* in neck skin and bone of chickens. *J. Food Prot.*, **77** (7): 1193-1197, doi: 10.4315/0362-028X.JFP-14-006

Ebuchi S., Baba A., Uryu K., Hiwaki H., 2006. Two outbreaks caused by *Salmonella* Derby and *S*. Anatum at grilled-meat restaurants in Fukuoka City. *Jpn. J. Infect Dis.*, **59** (6): 405-406

European Committee on Antimicrobial Susceptibility Testing, 2012. Definitions of clinical breakpoints and epidemiological cut-off values. EUCAST Development Laboratory, Sweden

Fashae K., Ogunsola F., Aarestrup F.M., Hendriksen R.S., Hendriksen R.S., 2010. Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. *J. Infect. Dev. Ctries.*, **4** (8): 484-494, doi: 10.3855/jidc.909

Feasey N.A., Dougan G., Kingsley R.A., Heyderman R.S., Gordon M.A., 2012. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. *Lancet*, **379**: 2489-2499, doi: 10.1016/S0140-6736(11)61752-2

Fortini D., Fashae K., Garcia-Fernandez A., Villa L., Carattoli A., 2011. Plasmid-mediated quinolone resistance and β-lactamases in *Escherichia coli* from healthy animals from Nigeria. *J. Antimicrob. Chemother.*, **66** (6): 1269-1272, doi: 10.1093/jac/dkr085

Garedew L., Hagos Z., Addis Z., Tesfaye R., Zegeye B., 2015. Prevalence and antimicrobial susceptibility patterns of *Salmonella* isolates in association with hygienic status from butcher shops in Gondar town, Ethiopia. *Antimicrob. Resist. Infect. Control.*, **4**: 21, doi: 10.1186/s13756-015-0062-7

Gonose T., Smith M.A., Keddy H.K., Sooka A., Howell V., Jacobs A.C., Haffejee S., et al., 2012. Human infections due to *Salmonella* Blockley, a rare serotype in South Africa: a case report. *BMC Res. Notes*. **5** (1): 562, doi: 10.1186/1756-0500-5-562

Grimont P.A., Weill F.X., 2007. Antigenic formulae of the *Salmonella* serovars, 9th Edn. WHO Collaborating Centre for Reference and Research on *Salmonella*, Paris, France

Hargis B.M., Caldwell J.D., Brewer L.R., Corrier E.D., Deloach R.J., 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. *Poult. Sci.*, **74**: 1548-1552, doi: 10.3382/ps.0741548

Helms M., Simonsen J., Mølbak K., 2004. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J. Infect. Dis.*, **190** (9): 1652-1654, doi: 10.1086/424570

Hopkins K.L., Davies R.H., Threlfall E.J., 2005. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int. J. Antimicrob. Agents*, **25** (5): 358-373, doi: 10.1016/j.ijantimicag.2005.02.006

Kagambèga A., Haukka K., Siitonen A., Traore A.S., 2011. Prevalence of *Salmonella enterica* and the hygienic indicator *Escherichia coli* in raw meat at markets in Ouagadougou, Burkina Faso. *J. Food Prot.*, **74** (9): 1547-1551, doi: 10.4315/0362-028X.JFP-11-124

Kagambèga A., Lienemann T., Aulu L., Traoré S.A., Barro N., Siitonen A., Haukka K., 2013. Prevalence and characterization of *Salmonella enterica* from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates. *BMC Microbiol.*, **13** (1): 253, doi: 10.1186/1471-2180-13-253

Kaibu H., Higashine H., Iida K., Ueki S., Ehara H., 2005. A fatal food intoxication case due to *Salmonella* Haifa. *Jpn. J. Infect. Dis.*,**58** (3): 192-193

Löfström C., Hintzmann A.S., Sørensen G., Baggesen D.L., 2015. Outbreak of *Salmonella enterica* serovar Typhimurium phage type DT41 in Danish poultry production. *Vet. Microbiol.*, **178** (1): 167-172, doi: 10.1016/j.vetmic.2015.04.017

Luo Y., Li J., Meng Y., Ma Y., Hu C., Jin S., Zhang Q., et al., 2011. Joint effects of topoisomerase alterations and plasmid-mediated quinolone-resistant determinants in *Salmonella enterica* Typhimurium. *Microb. Drug. Resist.*, **17** (1): 1-5, doi: 10.1089/mdr.2010.0074

Majowicz E.S., Musto J., Scallan E., Angulo J.F., Kirk M., O'Brien J.S., Jones F.T., et al., 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.*, **50** (6): 882-889, doi: 10.1086/650733

Marshall B.M., Levy, S.B., 2011. Food animals and antimicrobials: Impacts on human health. *Clin. Microbiol. Rev.*, **24** (4): 718-733, doi: 10.1128/CMR.00002-11

Martínez-Martínez L., Pascual A., García I., Tran J., Jacoby G.A., 2003. Interaction of plasmid and host quinolone resistance. *J. Antimicrob. Chemother.*, **51** (4): 1037-1039, doi: 10.1093/jac/dkg157

Morpeth S.C., Ramadhani H.O., Crump J.A., 2009. Invasive non-Typhi *Salmonella* disease in Africa. *Clin. Infect. Dis.,* **49** (4): 606-611, doi: 10.1086/603553

Ogunleye A.O., Oyekunle M.A., Sonibare A.O., 2008. Multidrug resistant *Escherichia coli* isolates of poultry origin. *Vetrinarski Arhiv*., **78** (6): 501-509

Olsen J.E., Brown J.D., Madsen M., Bisgaard M., 2003. Cross contamination on a broiler slaughterhouse line demonstrated by use of epidemiological markers. *J. Appl. Microbiol.,* **94**: 826-835, doi: 10.1046/j.1365-2672.2003.01911.x

Oluwasile B.B., Agbaje M., Ojo O.E., Dipeolu M.A., 2014. Antibiotic usage pattern in selected poultry farms in Ogun State. *Sokoto J. Vet. Sci.*, **12** (1): 45-50, doi: 10.4314/sokjvs.v12i1.7

Piddock L.J.V., 2002. Fluoroquinolone resistance in *Salmonella* serovars isolated from humans and food animals. *FEMS Microbiol. Rev*., **26** (1): 3-16, doi: 10.1111/j.1574-6976.2002.tb00596.x

Ramirez G.A., Sarlin L.L., Caldwell J.D., Yezak Jr R.C., Hume E.M., Corrier E.D., Deloach R.J., et al., 1997. Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. *Poult. Sci.,* **76**: 654-656, doi: 10.1093/ps/76.4.654

Rasschaert G., Houf K., De Zutter L., 2007. Impact of the slaughter line contamination on the presence of *Salmonella* on broiler carcasses. *J. Appl. Microbiol.*, **103**: 333-341, doi: 10.1111/j.1365-2672.2006.03248.x

Raufu I., Hendriksen R.S., Ameh J.A., Aarestrup F.M., 2009. Occurrence and characterization of *Salmonella* Hiduddify from chickens and poultry meat in Nigeria. *Foodborne Pathog. Dis*., **6** (4): 425-430, doi: 10.1089/fpd.2008.0150

Rodríguez-Martínez J.M., Velasco C., García I., Cano M.E., Martínez-Martínez L., Pascual A., 2007. Mutant prevention concentrations of fluoroquinolones for Enterobacteriaceae expressing the plasmid-carried quinolone resistance determinant *qnr*A1. *Antimicrob. Agents Chemother.*, **51** (6): 2236-2239, doi: 10.1128/AAC.01444-06

Saravanan S., Purushothaman V., Murthy K.G.R.T., Sukumar K., Srinivasan P., Gowthaman V., Balusamy M., et al., 2015. Molecular epidemiology of nontyphoidal *Salmonella* in poultry and poultry products in India: Implications for human health. *Indian J. Med. Microbiol*., **55** (3): 319-326, doi: 10.1007/s12088-015-0530-z

Wasyl D., Hoszowski A., Zając M., 2014. Prevalence and characterisation of quinolone resistance mechanisms in *Salmonella* spp. *Vet. Microbiol*., **171** (3-4): 307-314, doi: 10.1016/j.vetmic.2014.01.040

Résumé

Agbaje M., Lettini A.A., Ojo O.E., Longo A., Marafin E., Antonello K., Zavagnin P., Oluwasile B.B., Omoshaba E.O., Dipeolu M.A. Profils de résistance antimicrobienne de sérotypes de salmonelles isolés à partir de viande de poulet apprêtée à l'abattoir à Kaduna, Nigeria

La salmonellose invasive non typhique, caractérisée par une gastro-entérite et une bactériémie, est endémique en Afrique subsaharienne. La plupart des infections sont d'origine alimentaire, les animaux servant de porteurs asymptomatiques. Nous

Resumen

Agbaje M., Lettini A.A., Ojo O.E., Longo A., Marafin E., Antonello K., Zavagnin P., Oluwasile B.B., Omoshaba E.O., Dipeolu M.A. Perfiles de resistencia antimicrobiana a serovares de *Salmonella* aislados de carne de pollo en mataderos de Kaduna, Nigeria

La salmonelosis invasiva no tifoidea, caracterizada por gastroenteritis y bacteriemia es endémica en África subsahariana. La mayoría de las infecciones son de origen alimenticio, con animales actuando como portadores asintomáticos. Investigamos

avons étudié les sérotypes de *Salmonella* et les gènes de résistance associés dans la viande de poulet à l'aide de cultures, de concentrations minimales inhibitrices et de l'amplification par PCR des gènes de résistance. Sur 100 échantillons examinés, 28 (28 %) étaient positifs pour *Salmonella* et répartis dans six sérotypes : Haïfa (71,4 %), Chomedey (7,1 %), Saintpaul (7,1 %), Kainji (7,1 %), Derby (3,6 %) et Blockley (3,6 %). Une résistance antimicrobienne à la ciprofloxacine (85,7 %), à l'acide nalidixique (75,0 %), au sulfaméthoxazole (67,8 %), à la tétracycline (89,3 %), au triméthoprime (42,9 %), à la gentamicine (35,7 %), à la streptomycine (32,1 %), à l'ampicilline (10,7 %), au chloramphenicol (10,7 %), à la kanamycine (7,1 %) et au florfenicol (3,6 %) a été observée. Tous les isolats ont été sensibles à la céfotaxime, à la ceftazidime et à la colistine ; 19 (67,9 %) ont présenté une multirésistance à au moins trois antimicrobiens. Le type de résistance prédominant était Cip-Gen-Nal-Smx-Tet-Tmp, détecté dans six isolats (21%). La multirésistance des sérovars de *Salmonella* était élevée dans la viande de poulet échantillonnée, et la résistance la plus souvent observée était contre la ciprofloxacine. Cela suggère la possibilité d'un transfert horizontal des gènes de résistance à la quinolone à médiation plasmidique, ce qui pourrait compromettre l'utilisation clinique des fluoroquinolones. Ainsi, l'amélioration de l'hygiène et la mise en place d'installations adaptées dans les centres de transformation de la viande pourraient contribuer à limiter la contamination de la viande et la transmission aux humains des sérotypes multirésistants non typhiques de *Salmonella* par la viande de poulets.

Mots-clés : *Salmonella*, résistance aux antibiotiques, viande de poulet, abattage d'animaux, Nigeria

serovares de *Salmonella* y los genes de resistencia asociados en carne de pollo, mediante cultivo, concentraciones mínimas inhibitorias y amplificación de PCR de los genes de resistencia. De las 100 muestras examinadas, 28 (28%) fueron positivas a *Salmonella* y se distribuyeron en seis serovares: Haifa (71,4%), Chomedey (7,1%), Saintpaul (7,1%), Kainji (7,1%), Derby (3,6%), y Blockley (3,6%). La resistencia antimicrobiana se observó a ciprofloxacina (85,7%), ácido nalidíxico (75,0%), sulfametoxazol (67,8%), tetraciclina (89,3%), trimetoprima (42,9%), gentamicina (35,7%), estreptomicina (32,1%), ampicilina (10,7%), cloranfenicol (10,7%), kanamicina (7,1%) y florfenicol (3,6%). Todos los aislamientos fueron susceptibles a cefotaxima, ceftazidima y colistina, mientras que 19 (67,9%) mostraron resistencia múltiple a por lo menos tres antimicrobianos. El tipo de resistencia predominante fue Cip-Gen-Nal-Smx-Tet-Tmp, detectado en seis (21%) aislamientos. La resistencia múltiple a fármacos de serovares de *Salmonella* fue elevada en las muestras de carne de pollo, con una mayor resistencia observada a la ciprofloxacina. Esto sugiere una posible transmisión horizontal de genes resistentes a quinolonas mediados por plásmidos, lo que podría comprometer el uso clínico de fluoroquinolonas. Por lo tanto, la mejora de la higiene y la provisión de instalaciones adecuadas en los centros de procesamiento de carne podrían ayudar a limitar la contaminación de la carne y la transmisión a través de los alimentos de los serotipos multi resistentes de *Salmonella* no tifoidea de pollos a humanos.

Palabras clave: *Salmonella*, resistencia a los antibióticos, carne de pollo, sacrificio, Nigeria

Evaluation des résidus de tétracyclines et de bêta-lactamines dans le lait de vache produit au Centre Bénin

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Mots-clés

Lait de vache, résidu d'antibiotique, tétracycline, bêta-lactamines, Bénin

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Résumé

L'objectif de cette étude a été d'évaluer la présence des résidus de tétracyclines et de bêta-lactamines avec le kit Twinsensor (KIT 020) dans le lait cru des vaches élevées dans les exploitations bovines sédentaires installées dans huit communes du département du Zou et une commune du département des Collines au Centre Bénin. Au total, 261 échantillons de lait cru de tank ont été collectés à raison de trois prélèvements par élevage réalisés à deux jours d'intervalle. Les proportions d'échantillons de lait positifs aux résidus de tétracyclines et de bêta-lactamines par commune ont varié respectivement de 1,4 % à 100 % et de 22,2 % à 100 %. Tous les échantillons de Zakpota étaient positifs aux tétracyclines, alors que dans les huit autres communes moins de 50 % des échantillons étaient positifs à cette famille d'antibiotiques. Les plus fortes proportions de lait (100 %) positif aux résidus de bêta-lactamines ont été observées à Abomey, Agbangnizoun, Dassa-Zoumè, Djidja et Zakpota. Etant donné que les limites de détection des tétracyclines et des bêta-lactamines par la méthode qualitative utilisée étaient inférieures aux limites maximales de résidus recommandées par l'Union européenne, les échantillons positifs devraient être confirmés par un test physicochimique. Néanmoins, le mode de suivi sanitaire et de traitement des animaux dans la zone d'étude pourrait être amélioré pour préserver la santé des consommateurs de lait.

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■ INTRODUCTION

Le lait est la sécrétion mammaire normale d'animaux de traite obtenu à partir d'une ou de plusieurs traites, sans rien y ajouter ou en soustraire, destiné à la consommation comme lait liquide ou à un traitement ultérieur (FAO/OMS, 1999). Une consommation de l'équivalent de deux à trois verres par jour de produits laitiers est d'ailleurs recommandée à toute personne (USDA, 2015). Cependant, bien que le lait soit riche en nutriments comme les glucides, les protéines, les lipides, les vitamines et les sels minéraux, sa consommation peut aussi nuire à la santé du consommateur. En effet, le lait peut contenir des agents responsables de zoonoses ou des résidus de médicaments vétérinaires notamment les antibiotiques qui constituent un risque pour le consommateur (Jones, 1999 ; Blowey et Edmondson, 2000). Les antibiotiques sont généralement utilisés en médecine humaine et vétérinaire pour détruire ou inhiber la multiplication des bactéries responsables des infections. Les familles d'antibiotiques les plus utilisés en élevage bovin pour la production de lait sont les tétracyclines et les bêta-lactamines (Goulette, 2007 ; Mensah et al., 2014b). Les tétracyclines sont des antibiotiques à large spectre efficaces contre

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les bactéries Gram-positif et Gram-négatif, mais aussi contre les rickettsies, les mycoplasmes, les spirochètes et les actinomycètes. Les bêta-lactamines sont constituées de deux groupes d'antibiotiques que sont les pénicillines et les céphalosporines. Elles sont actives sur les bactéries Gram-positif et Gram-négatif.

Au Bénin, les tétracyclines et les bêta-lactamines sont aussi fréquemment utilisées en élevage bovin (Mensah et al., 2014b). Le lait de vache produit localement est commercialisé ou consommé sans contrôle sanitaire, ce qui constitue un risque potentiel pour le consommateur. Ces antibiotiques et leurs métabolites peuvent être transférés du sang dans le lait trait après leur fixation aux protéines du sérum sanguin (Goulette, 2007). Ingérées sous forme de résidus dans le lait, les tétracyclines sont dangereuses par leur caractère tératogène et leur toxicité surtout quand elles sont administrées après la date de péremption (Goulette, 2007). Les bêta-lactamines représentent 95 % des résidus d'antibiotiques retrouvés dans le lait, ce qui pose de sérieux problèmes de santé publique (Raemy, 1977 ; Ghidini et al., 2002). Ainsi l'Union européenne à travers les règlements 470/2009 et 37/2010 a fixé la limite maximale de résidus (LMR) dans le lait à 100 µg/l pour les tétracyclines, 4 à 30 µg/l pour les pénicillines et 20 à 125 µg/l pour les céphalosporines (UE, 2009 ; 2010).

Une étude précédente réalisée dans les élevages du Centre Bénin à l'aide du kit Delvotest T (DSM Food Specialities) a révélé la présence de résidus d'antibiotiques dans le lait de vache cru produit localement sans préciser les familles d'antibiotiques et les taux de résidu (Mensah et al., 2014b). La présente étude a eu pour objectif de rechercher la présence des résidus de tétracyclines et de bêta-lactamines dans le lait de vache cru produit au Centre Bénin.

■ MATERIEL ET METHODES

Milieu d'étude

L'étude a été réalisée dans les huit communes du département du Zou et une commune du département des Collines au Centre Bénin (figure 1). La zone d'étude s'étend entre 1° 38' et 2° 32' E, et 6° 56' et 7° 55' N. Le climat y est de type subéquatorial à soudano-guinéen. La pluviométrie annuelle varie de 1100 à 1200 millimètres et l'humidité relative de 51,49 % à 93,70 %. La température moyenne est de 20,04 °C (Akoegninou et al., 2006 ; Gnanglè et al., 2011). Les deux départements avaient un effectif total de 136 822 têtes de bovin soit plus de 7 % de l'effectif bovin du pays. Ces bovins sont de races Borgou, Fulani, Goudali, Lagunaire et Somba. Ils sont spécialisés dans la production de lait et de viande et élevés dans des systèmes transhumants et sédentaires (DE, 2008). L'étude a été limitée aux élevages de type sédentaire afin de faciliter les prélèvements répétés de lait cru prévus dans le protocole.

Collecte d'échantillons de lait cru de vache

Cette étude fait suite à une enquête réalisée sur l'usage d'antibiotiques en élevage bovin (Mensah et al., 2014b) et une première étude sur la présence de résidus d'antibiotiques sans distinction de famille dans le lait cru produit dans les élevages sédentaires de bovins de la même zone (Mensah et al., 2014a). Pour la présente étude, des échantillons de lait cru de vache ont été collectés de décembre 2011 à mai 2012 dans 87 élevages sédentaires de bovins sur les 90 élevages ayant été pris en compte dans les deux précédentes études. Trois élevages de la zone ont été exclus parce que des informations sur ces élevages n'étaient plus disponibles. Dans chaque élevage sélectionné, 500 ml de lait cru ont été prélevés dans le tank contenant toute la traite du matin, avec un flacon stérile, à trois reprises et à deux jours d'intervalle pour évaluer l'évolution de la présence de résidus d'antibiotiques dans le temps. Un total de 261 échantillons de lait cru a ainsi été collecté dans les élevages des différentes communes soit 27 à Abomey, 27 à Agbangnizoun, 15 à Bohicon, 9 à Covè, 72 à Dassa-Zoumè, 66 à Djidja, 9 à Zagnanado, 15 à Zakpota et 21 à Zogbodomey. Ces échantillons ont été transportés dans un délai de trois heures maximum entre 2–4 °C vers le Laboratoire de diagnostic vétérinaire de Bohicon pour y être conservés à -20 °C. Ils ont ensuite été acheminés au Laboratoire de recherche zootechnique, vétérinaire et halieutique de l'Institut national des recherches agricoles du Bénin pour la détection de résidus de tétracyclines et de bêta-lactamines deux mois après le début des collectes.

Analyses de laboratoire

La détection des résidus de tétracyclines (chlortétracycline, doxycycline, oxytétracycline, tétracycline) et de bêta-lactamines (pénicillines, céphalosporines) dans les échantillons de lait cru de tank collectés a été faite avec le Kit 020 Twinsensor (Unisensor, Belgique). C'est

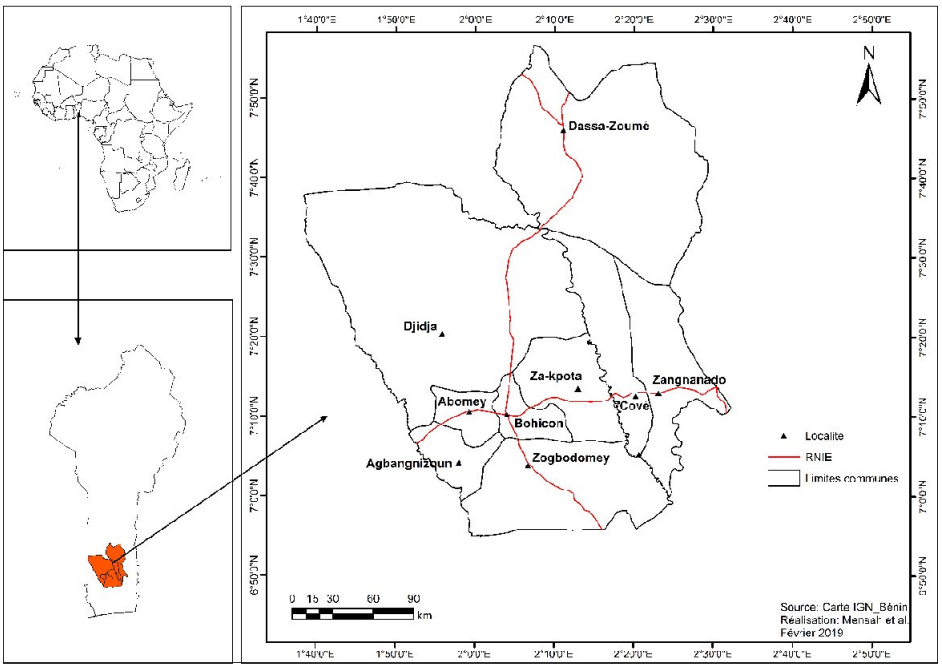

Figure 1 : *zone d'étude au Bénin.*

un test de compétition qui se base sur la reconnaissance de deux récepteurs spécifiques, l'un pour les bêta-lactamines et l'autre pour les tétracyclines. Les limites de détection des résidus de tétracyclines et de bêta-lactamines dans le lait par ce test, comparées aux LMR recommandées par l'UE, sont présentées dans le tableau I. Le test utilisé a une spécificité estimée à 100 % pour la détection des résidus de tétracyclines dans le lait de tank de vache (Perme et al., 2010) et de brebis (Beltrán et al., 2014).

Analyses statistiques

Les données ont été analysées avec le logiciel R (version 2.15.3). La distribution des données n'étant pas normale, les proportions médianes de présence des résidus de tétracyclines et de bêta-lactamines par la méthode utilisée suivant les sites et les périodes de prélèvements ont été calculées puis comparées avec un test non paramétrique des proportions de Kruskal Wallis (McDonald, 2014).

■ RESULTATS

Lait positif aux résidus de tétracyclines et de bêta-lactamines

Les proportions d'échantillons de lait positifs aux résidus de tétracyclines et de bêta-lactamines ont été respectivement de 16,5 % et 83,9 %. Ces proportions ont varié selon la commune respectivement de 1,4 % à 100 % et de 22,2 % à 100 % (tableau II). Tous les échantillons collectés à Zakpota ont été positifs aux tétracyclines, alors que dans les huit autres communes, moins de 50 % des échantillons étaient positifs aux résidus de cette famille d'antibiotique. Quant aux bêta-lactamines, plus de 92 % des échantillons collectés à Abomey, Agbangnizoun, Dassa-Zoumè, Djidja et Zakpota ont été positifs (tableau II). Par ailleurs, 22 % à 93 % des échantillons de lait étaient positifs à chacune des deux familles d'antibiotiques à Bohicon, Covè, Zagnanado, Zakpota et Zogbodomey.

Les proportions d'échantillons de lait positifs aux résidus de tétracyclines et de bêta-lactamines n'ont pas varié ($p > 0,05$) du premier prélèvement au troisième (tableau III).

Tableau I

Limites de détection des antibiotiques dans le lait par le Kit 020 Twinsensor et limites maximales recommandées (LMR) par l'Union européenne

		Limites de détection	
		KIT 020 (µg/L)	LMR UE (µg/L)
ß-lactamine	Penicilline-G	2–3	4
	Ampicilline	3–4	4
	Amoxicilline	3–4	4
	Oxacilline	12–18	30
	Cloxacilline	6–8	30
	Dicloxacilline	6–8	30
	Nafcilline	30–50	30
	Ceftiofur	10–15	125
	Cefquinome	20–30	20
	Céfazoline	18–22	60
	Céfapirine	6–8	20
	Céfacétrile	30–40	50
	Céfoperazone	3–4	50
	Céfalexine	> 750	20
	Céfalonium	3–5	100
Tétracycline	Chlortétracycline	30–40	100
	Doxycycline	10–15	100
	Oxytetracycline	50–60	100
	Tetracycline	80–100	100

■ DISCUSSION

L'évaluation des résidus d'antibiotiques dans les denrées alimentaires d'origine animale en général et dans le lait de vache en particulier présente un enjeu pour la sécurité sanitaire des aliments et la santé des consommateurs. La présence de résidus de tétracyclines et de bêta-lactamines dans les échantillons de lait de tank collectés dans les élevages sédentaires de bovins au Centre Bénin a montré que ces antibiotiques étaient utilisés dans ces élevages. Cela confirme les résultats de l'enquête précédente chez ces mêmes éleveurs par Mensah et al. (2014b) où respectivement 89 % et 34 % d'entre eux ont déclaré utiliser les tétracyclines et les bêta-lactamines. Ces deux familles d'antibiotiques sont d'ailleurs les plus utilisées en élevage bovin (Goulette, 2007 ; Reybroeck, 2010 ; Dognon et al., 2018).

La méthode de dépistage utilisée dans cette étude a détecté des résidus de tétracyclines et de bêta-lactamines dans les échantillons de lait

Tableau II

Proportions médianes des échantillons de lait positifs aux résidus d'antibiotiques dans les communes (Centre Bénin)

Commune	Nb. d'échantillons	Tétracycline	Bêta-lactamine
		% positifs (ES)	% positifs (ES)
Abomey	27	3,7 (3,7)	92,6 (3,7)
Agbangnizoun	27	7,4 (3,7)	100 (0,0)
Bohicon	15	33,3 (24,0)	40,0 (30,6)
Covè	9	33,3 (33,3)	55,6 (22,2)
Dassa-Zoumè	72	1,4 (1,4)	94,4 (3,7)
Djidja	66	9,1 (6,9)	95,5 (2,6)
Zagnanado	9	22,2 (22,2)	22,2 (22,2)
Zakpota	15	100 (0,0)	93,3 (6,7)
Zogbodomey	21	38,1 (4,8)	42,9 (8,3)
Total	261	16,5 (2,3)	83,9 (2,3)

ES : erreur standard

Tableau III

Proportions médianes d'échantillons de lait positifs aux résidus d'antibiotiques en fonction du rang de prélèvement (Centre Bénin)

Rang	Tétracycline	Bêta-lactamine
	% (ES)	% (ES)
1	17,1[a] (11,4)	77,8[a] (11,8)
2	37,2[a] (13,6)	71,8[a] (13,2)
3	28,5[a] (12,7)	62,5[a] (12,2)

ES : erreur standard ; [a] les fréquences interclasses sur une même colonne suivies d'une même lettre ne sont pas significativement différentes au seuil de 5 %.

à des limites inférieures pour la plupart aux LMR recommandées (UE, 2010). Pour une bonne appréciation du risque lié à la présence de résidus d'antibiotiques dans le lait collecté, il aurait fallu confirmer les échantillons positifs avec une méthode physicochimique (Gaudin, 2016).

Présence de résidus de bêta-lactamines dans le lait

Les proportions d'échantillons de lait cru positifs aux bêta-lactamines observées dans notre étude étaient globalement supérieures aux 32,9 % obtenus par Mokhtari et al. (2013) en Iran. L'utilisation d'antibiotiques par les éleveurs est importante pour lutter contre certaines des maladies des bovins très fréquentes dans ces régions : 51 % contre les affections du pied, 29 % contre les mammites, 19 % contre les infections ombilicales et 13 % contre les infections respiratoires (Mensah et al., 2014b). Mais au-delà de leur utilisation normale dans la pratique vétérinaire, le problème demeure le non-respect des délais d'attente pour la vente du lait. Cela semble justifier le fait que trois (Dassa-Zoumè, Djidja et Zakpota) des cinq communes où les bêta-lactamines étaient supposées présentes dans plus de 92 % des échantillons soient classées comme à risque moyen à élevé concernant l'usage des antibiotiques (Mensah et al., 2014b). Paradoxalement, les proportions d'élevages positifs aux bêta-lactamines dans les quatre autres communes classées à risque moyen à élevé (Bohicon, Covè, Zagnanado et Zogbodomey) par la même étude varient de 22,2 % à 56,6 %. Cette classification des communes de la zone suivant le niveau de risque lié à l'usage des antibiotiques en élevage sédentaire bovin par Mensah et al. (2014b) a été basée sur les déclarations des éleveurs. Elle a utilisé des paramètres comme le lieu d'achat des antibiotiques, le recours à la prescription ou au conseil du vétérinaire pour leur utilisation et le respect de la dose, de la fréquence et de la durée d'administration. Les déclarations des éleveurs concernant leur usage d'antibiotiques ne semblent donc pas refléter leur pratique réelle, du moins durant la période de la présente étude. Une absence de maladies nécessitant l'administration d'antibiotiques aux bovins ou un respect des délais d'attente après l'utilisation d'antibiotiques dans les élevages de ces communes à risque autour de la période de la présente étude pourrait aussi être envisagés.

Le mode d'administration des antibiotiques peut aussi expliquer ces fortes proportions d'échantillons positifs au test. En effet, une injection intramammaire est faite en cas de mammite sévère et une injection intramusculaire pour les cas moins graves. Or, 26–49 % de la pénicilline G est excrétée si elle est injectée par voie intramammaire (Hargrove et al., 1950) et sa présence sous forme de résidu est particulièrement détectée dans le lait trait immédiatement après le traitement (Hovmand et al., 1954 ; Raemy, 1977). Reybroeck (2010) a ainsi estimé que de grandes quantités de lait sont exposées à un risque de contamination aux concentrations supérieures à la LMR suite à des injections par voie intramammaire.

Présence de résidus de tétracyclines dans le lait

Les proportions d'échantillons de lait positifs aux tétracyclines observées dans la plupart des communes (Abomey, Agbangnizoun, Bohicon, Covè, Dassa-Zoumè, Djidja, Zogbodomey et Zagnanado) étaient comparables aux 18,7 % d'échantillons de lait cru positifs aux tétracyclines rapportés par Al-Zuhair (2012) en Palestine et aux 28,6 % rapportés par Mesgari Abbasi et al. (2011) en Iran. Aussi, les proportions d'échantillons positifs aux tétracyclines par commune reflétaient-elles bien le classement des communes par Mensah et al. (2014b) suivant le risque lié à l'utilisation d'antibiotiques. En effet, moins de 8 % des échantillons des communes classées à risque faible étaient positifs. De plus, excepté Dassa-Zoumè, près d'un tiers des échantillons étaient positifs dans les communes classées à risque moyen. Enfin, jusqu'à 100 % des échantillons de certaines communes classées à risque élevé étaient positifs.

Présence simultanée de résidus de tétracyclines et de bêta-lactamines dans le lait

La présence simultanée de résidus des deux familles d'antibiotiques, les tétracyclines et les bêta-lactamines, pose le problème de l'association d'antibiotiques dans les traitements effectués sur les bovins de la zone d'étude. Cela confirme l'existence de mauvaises pratiques d'utilisation des antibiotiques dans la zone d'étude et au Nord Bénin comme le montrent Mensah et al. (2014b) puis Dognon et al. (2018). Ainsi, selon Mensah et al. (2014b), dans sept des neuf communes de la zone, plus de 80 % des éleveurs n'ont pas recours au vétérinaire pour l'utilisation des antibiotiques et plus de 64 % d'entre eux se procurent les antibiotiques sur le marché local au lieu d'une pharmacie vétérinaire. De plus, 92,9 % des éleveurs de bovins du nord-est du Bénin ne respectent pas le délai d'attente après l'administration d'antibiotiques au bétail (Dognon et al., 2018). Cette substitution des éleveurs aux professionnels de santé animale constitue un risque sérieux pour la santé des consommateurs. Par ailleurs, les associations d'antibiotiques non indiquées et mal administrées augmentent le risque de présence de résidus dans les denrées alimentaires d'origine animale avec ses conséquences sur la santé du consommateur.

■ CONCLUSION

L'étude a mis en évidence la présence de résidus de tétracyclines et de bêta-lactamines dans le lait cru de tank échantillonné dans les élevages bovins du Centre Bénin. D'autre part, l'absence de résidus d'antibiotiques dans les élevages d'une commune ne semble pas toujours liée à la présumée bonne utilisation des antibiotiques dans cette commune. De façon globale, le rang de prélèvements des échantillons de lait n'a eu aucune influence sur la présence des résidus d'antibiotiques.

Une étude similaire pourrait être envisagée dans la région du Nord Bénin qui abrite les plus grands effectifs bovins du pays, afin de mieux cerner le problème de présence de résidus d'antibiotiques dans le lait à l'échelle du pays. Par ailleurs les méthodes immunologiques de dépistage comme le Twinsensor utilisé dans cette étude devraient être confirmées par une méthode physicochimique.

Conflits d'intérêts

L'étude a été réalisée sans aucun conflit d'intérêts.

Déclaration des contributions des auteurs

SEPM, ODK, PS et FAA ont participé à la conception et à la planification de l'étude ; SEPM, et BAA ont recueilli les données ; SEPM et KYKBA ont effectué les analyses statistiques et interprété les données ; SEPM, BAA et KYKBA ont rédigé la première version du manuscrit ; ODK, SS, GAM et FAA ont révisé le manuscrit.

REFERENCES

Akoegninou A. Van Der Burg W.J., Maesen L.I.G., 2006. Flore analytique du Bénin. Backhuys Publishers, Université d'Abomey-Calavi, Cotonou, Bénin. 1034 p.

Al-Zuhair I.M., 2012. Detection of β–Lactams and Tetracyclines antimicrobial residues in raw dairy milk for human consumption in Palestine. *Walailak J. Sci. Tech.*, **9** (3): 277-279

Beltrán M.C., Althaus R.L., Berruga M.I., Molina A., Molina M.P., 2014. Detection of antibiotics in sheep milk by receptor-binding assays. *Int. Dairy J.*, **34** (2): 184-189, doi: 10.1016/j.idairyj.2013.09.006

Blowey R., Edmondson P., 2000. Mastitis control in dairy herds: an illustrated practical guide, Farming Press, Tombridge, 138 p.

Direction de l'Elevage (DE), 2008. Rapport Annuel, DE, Cotonou, Bénin, 93 p.

Dognon S.R., Antoine-Moussiaux N., Douny C., Gustin P., Moula N., Scippo M.L., Youssao A.K.I., 2018. The use of antibiotics in cattle in North-East Benin: pharmaceutical inventory and risk practices of cattle breeders. *Trop. Anim. Health Prod.*. **50** (7): 1683-1699, doi : 10.1007/s11250-018-1612-9

FAO/OMS, 1999. Norme générale pour l'utilisation de termes de laiterie (site consulté 12 nov. 2020)

Gaudin V., 2016. Caractérisation de la performance et validation des méthodes de dépistage des résidus d'antibiotiques dans les denrées alimentaires. Thèse Doct., Université Rennes 1, France, 245 p.

Ghidini S.M., Zanardi E., Varisco G., Chizzolini R., 2002. Prevalence of Molecules of β-lactam Antibiotics in Bovine Milk in Lombardia and Emili Romagna (Italy). *Ann. Fac. Medi. Vet. Di Parma*, **22:** 245-252

Gnanglè P.C., Glèlè Kakaï R., Assogbadjo A.E., Vodounnon S., Yabi J.A., Sokpon N., 2011. Tendances climatiques passées, modélisation, perceptions et adaptations locales au Bénin. *Climatol.*, **8:** 27-40., doi: 10.4267/climatologie.259

Goulette R.R., 2007. Investigation of Safe-Level Testing for Beta-lactam Sulfonamide, and Tetracycline Residues in Commingled Bovine Milk. https://core.ac.uk/download/pdf/56509354.pdf (Consulté 4 nov. 2020)

Hargrove R.E., Walter H.E., Malkames J.P., Maskell K.T., 1950. The effects of penicillin and streptomycin of Swiss cheese starters (Abs.). *J. Dairy Sci.*, **33** (6): 401

Hovmand H.C., Jespen A., Overby A.J., 1954. Two years of collective mastitis campaign in cooperative dairies with practical control of penicillin-induced starter failures by use of penicillinase or penicillin resistant starter. *Nord. Vet. Med.*, **6**: 591

Jones G.M., 1999. On farm test for drug residues in milk, Virginia cooperative extension, knowledge for the common wealth, Virginia Polytechnic and State University, U.S.A.

McDonald J.H., 2014. Handbook of Biological Statistics (3rd ed.). Sparky House Publishing, Baltimore, Maryland, USA

Mensah S.E.P., Aboh A.B., Salifou S., Mensah G.A., Sanders P., Abiola F.A., Koudandé O.D., 2014a. Risques dus aux résidus d'antibiotiques détectés dans le lait de vache produit dans le Centre Bénin. *J. Appl. Biosci.*, **80**: 7102-7112. doi: 10.4314/jab.v80i1.9

Mensah S.E.P., Laurentie M., Salifou S., Sanders P., Mensah G.A., Abiola F.A., Koudandé O.D., 2014b. Usage des antibiotiques par les éleveurs au Centre du Bénin, quels risques pour la santé publique ? *Bul. Rech. Agr. Bénin*, **75** (1)**:** 1-16

Mesgari Abbasi M., Babaei H., Ansarin M., 2011. Simultaneous determination of tetracyclines residues in bovine milk samples by solid phase extraction and HPLC-FL method. *Adv. Pharm. Bull.*, **1** (1): 34-39

Mokhtari A., Hosseini, B., Panahi P., 2013. β-Lactams and tetracyclines antibiotic residue detection in bulk tank milk in Iran. *Iranian J. Publ. Health*, **42** (4): 447-448

Perme T., Bizjak M., Gačnik K.Š., Kirbiš A., 2010. Validation of twinsensorbt, screening test for the Detection of ß-lactams and tetracyclines in milk, and comparison to DELVOTEST SP NT. *Slov. Vet. Zb.*, **47** (3): 97-106

Raemy, O., 1977. Residues of antibiotics in milk after intramammary application on high grade cows in Kenya (Zebu x Frisian and Zebu x Ayrshire). *Rev. Elev. Med. Vet. Pays Trop.*, **30** (1): 61-65, doi: 10.19182/remvt.8106

Reybroeck W., 2010. Screening for residues of antibiotics and chemotherapeutics in milk and honey. Thèse Doct. Faculteit Diergeneeskunde, Universiteit Gent, Belgium. 295 p.

Union européenne (UE), 2009. Règlement (CE) No 470/2009 du Parlement Européen et du Conseil du 6 mai 2009 (site consulté 15 avril 2020)

Union européenne (UE), 2010. Règlement (UE) No 37/2010 de la Commission du 22 décembre 2009 (site consulté 15 avril 2020)

United States Department of Agriculture (USDA), 2005. "Food and Nutrition" (site consulté 28 Nov. 2015)

Summary

Mensah S.E.P. Koudande O.D., Aboh B.A., Adjahoutonon K.Y.K.B., Salifou S., Mensah G.A., Sanders P., Abiola F.A. Evaluation of tetracycline and beta-lactam residues in cow milk produced in Central Benin

The objective of this study was to evaluate the presence of tetracycline and beta-lactam residues with the Twinsensor kit (KIT 020) in the raw milk of cows raised in sedentary cattle farms located in eight communes of Zou Department and one commune of Collines Department in Central Benin. A total of 261 samples of raw bulk milk were collected at the rate of three samples per farm taken two days apart. The proportions of tetracycline- and beta-lactam-residue positive milk samples per commune ranged from 1.4% to 100% and 22.2% to 100%, respectively. All samples in Zakpota were positive for tetracyclines, whereas in the other eight communes less than 50% of the samples were positive for this family of antibiotics. The highest proportions of milk (100%) positive for beta-lactam residues were observed in Abomey, Agbangnizoun, Dassa-Zoumè, Djidja and Zakpota. Since the detection limits for tetracyclines and beta-lactam antibiotics by the qualitative method used were below the maximum residue limits recommended by the European Union, positive samples should be confirmed by a physicochemical test. Nevertheless, health monitoring and treatment of animals in the study area could be improved to preserve the health of milk consumers.

Keywords: cow milk, antibiotic residues, tetracyclines, beta-lactam antibiotics, Benin

Resumen

Mensah S.E.P. Koudande O.D., Aboh B.A., Adjahoutonon K.Y.K.B., Salifou S., Mensah G.A., Sanders P., Abiola F.A. Evaluación de residuos de tetraciclinas y betalactámicos en la leche de vaca en el centro de Benin

El estudio se realizó con el objetivo de evaluar la presencia de residuos de tetraciclina y beta-lactámicos en la leche cruda de vacas criadas en explotaciones bovinas sedentarias de ocho municipios del departamento de Zou y una comuna en el departamento de Colinas en el centro de Benin. Se recolectaron un total de 261 muestras de leche cruda de tanque, tres muestras por finca con dos días de intervalo. Las proporciones de muestras de leche positivas para residuos de tetraciclina y betalactámicos por comuna variaron respectivamente de 1,39 a 100% y de 22,22 a 100%. Todas las muestras de Zakpota fueron positivas para tetraciclinas, mientras que en las otras ocho comunas menos del 50% de las muestras fueron positivas para esta familia de antibióticos. Las proporciones más altas de leche (100%) positiva para residuos de betalactámicos se observaron en Abomey, Agbangnizoun, Dassa-Zoumè, Djidja y Zakpota. Dado que los límites de detección de tetraciclinas y betalactámicos por el método cualitativo utilizado eran inferiores a los límites máximos de residuos recomendados por la Unión Europea, las muestras positivas deberían confirmarse mediante una prueba fisicoquímica. No obstante, el modo de seguimiento sanitario y el tratamiento de los animales en el área de estudio, podría mejorarse para preservar la salud de los consumidores de leche.

Palabras clave: leche de vaca, residuos de antibióticos, tetraciclina, betalactámicos, Benin

Field assessment of antibiotic use in fish farms in Southwestern Nigeria

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Keywords

Aquaculture, good agricultural practices, antibiotics, resistance to antibiotics, tetracycline, Nigeria

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Summary

Antibiotic resistance is a global public health issue linked to antimicrobial use in food animals. However, little is known of antibiotic use in aquaculture in developing countries and its link to antibiotic resistance. This study investigated antibiotic use in 50 aquaculture farms in Southwestern Nigeria using a structured questionnaire. Twenty-seven (54%) farms used antibiotics (n = 24), antiparasitics (n = 2) or both (n = 1) as additive to feed or water. The most frequently used antibiotics were tetracycline (11 farms), beta-lactams and furazolidone (4 farms each), aminoglycosides and chloramphenicol (3 farms each), enrofloxacin (2 farms) and metronidazole (1 farm). Only 16 farmers knew the antibiotics used in their ponds and no farm had data on the quantity used. The drugs were sourced from veterinary (59%) and human medicine stores (15%), veterinary doctors (4%), or other fish farmers (4%); 19% did not answer this question. Many farmers (n = 15), among whom 11 held a technician diploma or a university degree, did not know of any risk entailed by the use of antibiotics in their ponds. Direct addition of antibiotics to pond water practiced in these farms can create local reservoirs of antibiotic resistance and is a risk to public health.

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■ INTRODUCTION

Resistance to antimicrobial drugs is presently a key challenge in the control of infectious diseases worldwide (UN-IACG, 2019). Human and agricultural uses of antibiotics have been implicated as important contributors to the development and spread of antibiotic resistance in pathogenic and commensal bacteria strains (Tang et al., 2017). Currently, more antibiotics are used in food animal production to promote growth and prevent disease than in the entire human population. Global antibiotic consumption in livestock was conservatively estimated at 63,200 tons in 2010 (van Boeckel et al., 2015), accounting for about two-thirds of the estimated worldwide annual production of 100,000 tons of antibiotics.

Among intensive types of livestock production that use antimicrobial agents, particular attention should be paid to fish farms because of their direct impact on the aquatic environment (Cabello et al., 2013). Large quantities of antimicrobials are used in aquaculture in low- and middle-income countries, often without professional supervision with consequences for development and spread of resistance and global public health. In Nigeria, policy regulating the availability and use of pharmaceutical substances are weakly enforced; hence, antimicrobial drugs are used widely in human, veterinary and agricultural applications often without professional advice or supervision. Accordingly, Nigeria was cited among the 50 countries with the largest amounts of antibiotic use in livestock in 2010 (Van Boeckel et al., 2015).

Recommended action plans to combat the spread of antibiotic resistance include the collation of national and local data on antibiotic use in human, veterinary, and other agricultural applications (O'Neill and

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the Review Committee, 2016) which should be made publicly available to inform policy decisions on the control of antibiotic resistance. Very little is known about the types and quantity of antibiotics used in livestock production in Nigeria. However, available evidence suggests that antibiotics are used widely in poultry, swine and aquaculture production (Adelowo et al., 2009; 2014; Adebowale et al., 2016) with little or no information on the types and quantity used, frequency of administration, and reasons for use. This knowledge gap limits the understanding of the link between antimicrobial use and the development and spread of antibiotic resistance in the Nigerian fish production chain, creating an urgent need for studies investigating these issues. The primary objective of this paper was to investigate antibiotic use in selected fish farms in Southwestern Nigeria. The information generated will be an important addition to the knowledge of antibiotics use in food animal production in the country.

■ MATERIALS AND METHODS

Participating farms and data collection

The procedure used for data collection was a volunteer-based convenience sampling method. Prospective participants were enlisted by peer contact using one of the farmers in the study area who had earlier been briefed about the overall objective of the study. The peer contact made a preliminary visit to the fish farmers in the study area, who generally operate in clusters, to solicit consent before questionnaire administration. During the preliminary visit (as well as during questionnaire administration), a detailed description of the study and the type of data to be collected were presented to the farmers after which questions raised were addressed as much as possible before the farmers were asked to indicate their interest in the study. The peer-contact method was adopted because a previous attempt to collect similar data among poultry farmers in the same area failed as many of the farmers expressed open reservations about the intention of the researchers.

Antimicrobial-use information was collected from each participating farmer on a second visit using a structured questionnaire administered to either farm owners or farm hands in a guided format. A brief description of what antimicrobials are and some examples that are used in aquaculture production were orally provided to each respondent when necessary. Only one questionnaire was administered per farm. The questionnaire was designed to capture basic information about the farm and the respondent, including: educational qualification of the respondent; age of the farm; fish species raised; culture method; type of feed used and their components; drugs used and categories; frequency of use; dosage and types used; reason for use; administration mode; source of the drugs and who the farmers consulted before antibiotic use; and whether the farmers were aware of any hazard associated with antibiotic use on their fish farms.

Data manipulation and statistical analysis

Data generated by the questionnaire were analyzed by descriptive statistics using Excel 2010 (Microsoft, USA).

■ RESULTS AND DISCUSSION

Participating farms

At the end of the peer contact's work, 50 farms made up of 3 farms in Lagos and 47 farms in Oyo town, Southwestern Nigeria, agreed to participate in the study. The peer-contact person was based in Oyo town, a reason that might be responsible for the large number of participants from this location. The average age of the participating farms was 7.5 years with the youngest and oldest being 2 and 24 years, respectively. All farms were involved in small- to medium-scale operation with a minimum of two and a maximum of seven ponds. Thirty-five farmers (70%) were involved in the production of *Clarias gariepinus*, 2 (4%) of *Heterotis* spp. in monoculture, in either earthen ponds (64%), artificial recirculation tanks (28%), or a combination of both (8%). The remaining 13 did not respond to the question. The educational qualification of the farmers ranged from basic elementary education (23.5%), to secondary education (17.5%), technician diploma (39%) and university degree (20%).

Antibiotics use on the study farms

Twenty-five farmers (50%) (including one who used a combination of antibiotics and antiparasitics) admitted using antibiotics in their farms, whereas two (4%) used only antiparasitics. Among these groups, nine (36%) had no idea of the type of antibiotics they were using. Surprisingly, three held higher education degrees whereas the remaining had elementary or secondary education. Two farmers in this group provided us with the packages of the products used and the information indicated the products contained a combination of "antibiotics and anti-stress". However, the specific nature of these molecules was not listed. The responses also showed that six of the antibiotic users used more than one class of antibiotics, with the most common (n = 3) combination being neomycin, tetracycline and furazolidone. The other combinations were ampicillin, tetracycline and metronidazole (n = 1), and ampicillin and tetracycline (n = 2). These findings were not unexpected, as policy regulating the availability and use of antimicrobial substances in developing countries is either non-existent or weakly enforced. In Nigeria, antimicrobials and other important drugs are available over the counter, making it easy for fish farmers to obtain them for use in their farms. The ease of availability may be a relevant factor promoting the use of these drugs in Nigerian fish farms. Dispensing antibiotics without prescription has been implicated as a link in the antibiotic resistance chain (Plachouras et al., 2010).

None of the 27 farmers using antibiotics and/or antiparasitics could supply information on their quantities used on their farms. Neither did they have any idea of the effective dose of the antibiotics administered. However, 16 farmers (59%) were able to supply the type(s) of antibiotics used in fish health management (Table I). The others either did not know the type used or did not respond to the question. Tetracyclines (oxytetracycline and doxycycline) were the most commonly administered antibiotics, used in 11 farms. Beta-lactams and furazolidone were each used in 4 farms, neomycin and chloramphenicol were each used in 3 farms, enrofloxacin (fluoroquinolone family) was used in 2 farms and metronidazole in 1 farm. None of the farmers using antiparasitics had an idea of the name of the product. It is of concern that all antibiotics mentioned as used on farms are categorized as important, highly important or critically important for human medicine (Table I) by WHO (2019). Thus, development of resistance to any of these drugs will have an effect on the ability to treat human infections.

This scarcity of data for quantitative antibiotic use from most countries engaged in aquaculture (Chuah et al., 2016) makes assessing the risk associated with their use a difficult task. This situation is compounded by the present rapid growth of the aquaculture industry in Nigeria where a large proportion of the unemployed population is embracing small-scale agro-enterprises as a means of generating much needed income. Under this condition, the pressure to maximize profit in a very short time will result in widespread use of antibiotics to prevent bacterial infections.

An important discovery worth mentioning among the study population is that 82% of the farmers used poultry waste in their pond either as feed supplement or for pond fertilization, although residues of antibiotics are often found in poultry waste (OIE/FAO/WHO, 2006). This

Table I

Common antibiotics cited as being used by fish farmers and administration mode in Southwestern Nigeria

Antibiotic class	Importance to human medicine[a]	Priority level	Use of specific class[b] (%)	Administration mode			
				Feed	Water	Feed + water	DNS
Tetracycline Doxycycline and oxytetracycline	High	NA	44	3	7	1	0
β-lactam Penicillin and ampicillin	Critical	High	16	1	2	1	0
Nitrofuran Furazolidone	Important	NA	16	1	3	0	0
Aminoglycoside Neomycin	Critical	High	12	0	3	0	0
Amphenicol Chloramphenicol	High	NA	12	0	3	0	0
Fluoroquinolone Enrofloxacin	Critical	High	8	2	0	0	0
Nitroimidazole Metronidazole	Important	NA	4	0	0	1	0
No idea[c]			36	3	0	0	6

Of the 50 farmers surveyed, 27 used antibiotics (n = 24), antiparasitics (n = 2) or both (n = 1)

[a] WHO (2019); [b] The total percentage sums up to more than 100% because some farmers were using more than one antibiotic class; [c] Have no idea of the type used or antibiotic class; NA: Not applicable (prioritization is only applied to critically important antimicrobials); DNS: Did not specify

practice is therefore an additional risk factor for the development and spread of antibiotic resistance (OIE/FAO/WHO, 2006).

The antibiotic types used in fish production in the present study were very similar to those used in poultry and swine production in Nigeria (Adelowo et al., 2014; Amaechi, 2014; Oluwasile et al., 2014; Adebowale et al., 2016). Fewer varieties of antibiotics were, however, used in fish production. Whereas Adelowo et al. (2014), Oluwasile et al. (2014) and Adebowale et al. (2016) report, respectively, the use of 16, 12 and 11 antibiotics, belonging to 8 antibiotic classes, among poultry farmers in Ogun and Oyo states, results of the present study showed that a total of 9 antibiotics belonging to 7 classes were used in fish production within the same region. In sharp contrast, 23 antibiotics belonging to 10 antibiotic classes were used in 94 Vietnamese aquaculture farms (Pham et al., 2015).

Tetracyclines were also reported as the most commonly used antibiotics in two studies dealing with the same issue in various countries (Tuševljak et al., 2013; Pham et al., 2015). However, in addition to tetracycline, Tuševljak et al. (2013) report a high prevalence of fluoroquinolone use among fish farmers in the United States (70%) and Canada (67%), whereas Pham et al. (2015) report a similar high prevalence use of trimethoprim and sulfonamides in addition to tetracyclines in Vietnamese aquaculture farms. In the present study, only two farms used fluoroquinolone and no farm used trimethoprim and/or sulfonamides.

The main drug (antibiotics and antiparasitics) administration modes were addition to feed (n = 10) or water (n = 8). One farm used both modes whereas six did not specify the mode of administration (Table I). Administering antibiotics through these modes is risky from the point of view of public health as synthetic antibiotics such as fluoroquinolones are expected to be persistent in aquatic sediments where they can select for fluoroquinolone resistance in sediment bacteria (Martinez, 2009). The persistence of the residues of some of the drugs used in the pond environment or in aquatic feeds could in addition modify the composition and activity of the pond microbiota (Martinez, 2009), or expose aquatic-food consumers to risks associated with drug residues. Concerns have been expressed about the genotoxicity, embryo- and feto-toxicity, and carcinogenic potentials of chloramphenicol and its metabolites in humans as well as the carcinogenic and mutagenic potential and thyroid toxicity of sulfonamides. Similarly, beta-lactams, streptomycin (and other aminoglycosides), sulfonamides and, to a lesser extent, tetracyclines are known to cause allergic reactions in sensitive persons (OIE/FAO/WHO, 2006). Thus, most countries have banned the use of these antibiotics in food-animal production, including aquaculture.

Most farmers (n = 14) used drugs (antibiotics and antiparasitics) in the ponds when they noticed infection among the fish stock whereas nine (including two farmers using antiparasitics) used drugs in disease prevention. One farmer did not know the reason behind his use of drugs and three did not respond to the question (Table II). Sixteen of the farmers sourced the antibiotics used from local veterinary medicine stores, four from local human medicine stores, and one each from

Table II

Sources and reasons for antibiotic and antiparasitic use by fish farmers in Southwestern Nigeria

Source	Num. (%) of farmers
Veterinary medicine store	16 (59)
Patient medicine store	4 (15)
Veterinary doctor	1 (4)
Other farmer	1 (4)
Local pharmacy	0
DNS	5 (19)
Most common reason for use	
Treatment of diseased fish	14 (52)
Disease prevention	9 (33)
Did not know reason for use	1 (4)
DNS	3 (11)

Of the 50 farmers, 27 mentioned using the drugs; DNS: Did not specify

local veterinary doctors and other fish farmers. Five of the farmers did not respond to this question. Surprisingly, all respondents to this question used only one source (Table II).

None of the farmers retained the services of a veterinary or a fishery specialist, but seven farmers consulted them occasionally, and three farmers consulted with other fish farmers. However, although six farmers did not respond to the question, the remaining four indicated they did not consult with anybody before drug administration on their farms because "they consider their experience sufficient to qualify them as specialists." It is interesting to report that three of these farmers held a technician diploma or a university degree.

Among the 25 farmers who used antibiotics, a large number (n = 15; 60%) did not know of any risk associated with their indiscriminate use in ponds or did not respond to the question (n = 8). Among the two who responded in the affirmative, one did not specify the risk involved, and the other mentioned weight loss as a consequence of antibiotic use in animals. It appeared that the level of education was not positively correlated with an appreciation of the risk as 11 of those who did not know the risk involved in antibiotic use in fish production held either a technician diploma or a university degree.

■ CONCLUSION

Antimicrobial use has been identified as a risk factor for the development and spread of antibiotic resistance. However, the lack of accurate information on the quantity of antimicrobials used in animal production is an important drawback in the evaluation of the impact of veterinary antibiotic utilization on the selection of resistance and release of antibiotics into the environment. Although the present study covered a narrow geographical region, it provided important data on antibiotic use in fish production in Southwestern Nigeria. However, it is not yet clear how much selection pressure is being exerted by this practice and how such pressure is contributing to the development and spread of antibiotic resistance from Nigerian fish farms. A much wider study designed to examine antimicrobial drug use and its link to resistance on fish farms will go a long way in answering this important question.

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Conflicts of interest

The study was carried out without any conflict of interest.

Author contributions statement

OOA conceived and designed the study, analyzed and interpreted data and wrote the first draft of the manuscript. IO collected data and reviewed the manuscript.

REFERENCES

Adebowale O.O., Adeyemo O.K., Awoyomi A., Dada R., Adebowale O., 2016. Antibiotic use and practices in commercial laying hens in Ogun state Nigeria. *Rev. Elev. Med. Vet. Pays Trop.,* **69** (1): 41-45, doi: 10.19182/remvt.31170

Adelowo O.O., Ojo F.A., Fagade O.E., 2009. Prevalence of Multiple Antibiotic Resistance among Bacterial Isolates from Selected Poultry Waste Dumps in Southwestern Nigeria. *World J. Microbiol. Biotechnol.,* **25**: 713-719, doi: 10.1007/s11274-008-9940-y

Adelowo O.O., Fagade O.E., Agerso Y.A., 2014. Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. *J. Infect. Dev. Ctries.,* **8** (9): 1103-1112, doi: 10.3855/jidc.4222

Amaechi N., 2014. A survey of antibiotics usage in pigs and poultry birds in Abia State, Nigeria. *Global J. Med. Res.,* **14** (5): 11-17

Cabello F.C., Godfrey H.P., Tomova A., Ivanova L., Dölz H., Millanao, A., Buschmann A.H., 2013, Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.* **15:** 1917–1942. doi: 10.1111/1462-2920.12134

Chuah L., Effarizah M.E., Goni A.M., Rusul G., 2016, Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. *Curr. Envir. Health Rpt.,* **3**: 118–127, doi: 10.1007/s40572-016-0091-2

Martinez J.L., 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ. Pollut.*, **157**: 2893–2902. doi: 10.1016/j.envpol.2009.05.051

Oluwasile B.B., Agbaje M., Ojo O.E., Dipeolu M.A., 2014. Antibiotic usage pattern in selected poultry farms in Ogun State. *Sokoto J. Vet. Sci.,* **12**(1): 45-50, doi: 10.4314/sokjvs.v12i1.7

O'Neill J., The Review Committee, 2016. Tackling drug resistant infections globally: Final Report and Recommendations. The UK Government and Wellcome Trust, London, UK

OIE/FAO/WHO, 2006. Antimicrobial use in aquaculture and antimicrobial resistance. Report of a joint FAO/OIE/WHO expert consultation on antimicrobial use in aquaculture and antimicrobial resistance. Seoul, Republic of Korea, 13-16 June 2006

Pham D.K., Chu J., Do N.T., Brose F., Degand G., Delahaut P., Pauw E.D., et al., 2015. Monitoring Antibiotic Use and Residue in Freshwater Aquaculture for Domestic Use in Vietnam. *EcoHealth.,* **12**: 480–489, doi: 10.1007/s10393-014-1006-z

Plachouras D., Kavatha D., Antoniadou A., Giannitsioti E., Poulakou G., Kanellakopoulou K., Giamarellou H., 2010. Dispensing of antibiotics without prescription in Greece, 2008: Another link in the antibiotic resistance chain. *Eurosurveillance,* **15** (7), doi: 10.2807/ese.15.07.19488-en

Tang K.L., Caffery N. P., Nóbrega D.B., Cork S.C., Ronksley P.E., Barkema H.W., Polachek, A.J., et al., 2017. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet Planet Health,* **1**: e316-e327. doi: 10.1016/S2542-5196(17)30141-9

Tuševljak N., Dutil L., Rajić A., Uhland F.C., McClure C., St-Hilaire S., Reid-Smith R.J., et al., 2013. Antimicrobial use and resistance in aquaculture: findings of a globally administered survey of aquaculture-allied professionals. *Zoonoses Public Health,* **60** (6): 426-36. doi: 10.1111/zph.12017

Van Boeckel T.P., Brower C., Gilbert M., Grenfell B.T., Levin S.A., Robinson T.P., Teillant A., et al., 2015. Global trends in antimicrobial use in food animals. *Proc. Nation. Acad. Sci. USA,* **112** (18): 5649–5654. doi: 10.1073/pnas.1503141112

UN-IACG, 2019, No Time to Wait–Securing the Future from Drug-resistant Infections. Report to the Secretary General of the United Nations. www.who.int/antimicrobial-resistance/interagency-coordinationgroup/IACG_final_report_EN.pdf?ua Accessed [25 June, 2019].

WHO, 2019. Critically important antimicrobials for human medicine. Sixth Revision

Résumé

Adelowo O.O., Okunlola I. Evaluation de l'utilisation d'antibiotiques dans les élevages piscicoles du sud-ouest du Nigeria

La résistance aux antibiotiques est un problème de santé publique mondial lié à l'utilisation d'antimicrobiens en production animale. Cependant, on connaît peu de choses sur l'utilisation d'antibiotiques en aquaculture dans les pays en développement et sur son lien avec la résistance aux antibiotiques. Cette étude s'est intéressée à l'utilisation des antibiotiques dans 50 fermes aquacoles du sud-ouest du Nigeria en se fondant sur un questionnaire structuré. Vingt-sept (54 %) exploitations utilisaient des antibiotiques (n = 24), des antiparasitaires (n = 2) ou les deux (n = 1) comme additifs dans l'alimentation ou l'eau. Les antibiotiques les plus fréquemment utilisés étaient la tétracycline (11 fermes), les bêta-lactamines et la furazolidone (4 fermes chacune), les aminoglucosides et le chloramphénicol (3 fermes chacun), l'enrofloxacine (2 fermes), et le métronidazole (1 ferme). Seuls 16 éleveurs connaissaient les antibiotiques utilisés dans leurs mares et aucune ferme ne disposait de données sur les quantités utilisées. Les médicaments provenaient de magasins de médecine vétérinaire (59 %) ou humaine (15 %), de vétérinaires (4 %), ou d'autres pisciculteurs (4 %) ; 19 % n'ont pas répondu à cette question. De nombreux éleveurs (n = 15), parmi lesquels 11 détenaient un diplôme de technicien supérieur ou universitaire, ignoraient les risques liés à l'utilisation d'antibiotiques dans leurs mares. L'ajout direct d'antibiotiques dans l'eau des mares pratiqué dans ces fermes peut créer des réservoirs locaux de résistance aux antibiotiques et constitue un risque pour la santé publique.

Mots-clés : aquaculture, bonnes pratiques agricoles, antibiotiques, résistance aux antibiotiques, tétracyclines, Nigeria

Resumen

Adelowo O.O., Okunlola I. Evaluación de campo del uso de antibióticos en explotaciones piscícolas de Nigeria sudoccidental

La resistencia a los antibióticos es un problema de salud pública mundial, relacionado con el uso de antimicrobianos en los animales destinados a la alimentación. Sin embargo, se sabe poco sobre el uso de antibióticos en la acuicultura en los países en desarrollo y su relación con la resistencia a los antibióticos. En el presente estudio investigó el uso de antibióticos en 50 fincas de acuicultura en el sudoeste de Nigeria, mediante un cuestionario estructurado. Veintisiete (54%) explotaciones utilizaron antibióticos (n = 24), antiparasitarios (n = 2) o ambos (n = 1) como aditivo en el alimento o el agua. Los antibióticos más utilizados fueron la tetraciclina (11 explotaciones), los betalactámicos y la furazolidona (4 explotaciones cada uno), los aminoglucósidos y el cloranfenicol (3 explotaciones cada uno), la enrofloxacina (2 explotaciones) y el metronidazol (1 explotación). Sólo 16 agricultores conocían los antibióticos utilizados en sus estanques y ninguna finca poseía datos sobre la cantidad utilizada. Los medicamentos procedían de comercios de medicina veterinaria (59%) y humana (15%), de médicos veterinarios (4%) o de otros piscicultores (4%); el 19% no respondió a esta pregunta. Varios productores (n = 15), entre los cuales 11 con un diploma de técnico o un título universitario, no sabían de ningún riesgo relacionado con el uso de antibióticos en sus estanques. La adición directa de antibióticos al agua de los estanques practicada en estas explotaciones, puede crear reservorios locales de resistencia a los antibióticos y constituye un riesgo para la salud pública.

Palabras clave: acuicultura, buenas prácticas agrícolas, antibióticos, resistencia a los antibióticos, tetraciclina, Nigeria