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Antibiotic resistance patterns of *Escherichia coli* strains isolated from pig's (*Sus scrofa domesticus*) casings in Lomé, Togo

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Optimal treatment and prudent use of antimicrobials for pigs is imperative to secure animal health and mostly prevent the development of critical resistance which can be transmitted to humans. An important step in this one-health context is to monitor resistance patterns of important animal pathogens. The aim of the study was to investigate the antibiotic resistance patterns of *Escherichia coli* strains in the intestinal flora of pigs in Lome, Togo. Thus 41 *E. coli* strains were isolated from 50 non duplicate samples of pig's faeces collected in various pig's casing in Lome. Disk diffusion antimicrobial susceptibility testing against a panel of antibiotics was carried out for the isolates. Susceptibility was interpreted using clinical breakpoints. Various resistance patterns were obtained. Both imipenem and fosfomycin were 100% sensitive; ampicillin, ofloxacin and nalidixic acid were the top three least active molecules on the isolated *E. coli*. The mean resistance rates were 10.8% for cephalosporins, 28% for penicillins, and 29.6% for other antibiotics. A total of 18 multidrug-resistant strains were found (43.9%). The resistance phenotypes found were: ESBL (4.9%), low level (14.6%) and high level (9.8%) penicillinase, low level (7.3%) and high level cephalosporinase (2.4%). With the presence of these critical resistant phenotypes, continuous surveillance of resistance patterns in pig pathogenic bacteria is urgent.

Key words: *Escherichia coli*, resistance, antibiotic, pig, Lome, Togo.

INTRODUCTION

Antibiotic resistance (AMR) is actually one of the most serious threats to infectious diseases therapy with serious impact on the global health, food security and development. An increasing number of infections, such as pneumonia, tuberculosis or salmonellosis, are becoming more difficult to treat, as antibiotics used to treat them become less effective (Brogan and Mossialos, 2016). The death rate associated with infection due to AMR is

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evaluated at 700,000 annually and is projected to increase up to 10 million fatalities worldwide by 2050 (Interagency Coordination Group on Antimicrobial Resistance, 2019). Many factors as over-prescription of antibiotics, overuse of antibiotics in livestock and fish farming, poor infection control in health care settings and genetic plasticity are highly contributing to AMR appearance and development. The uncontrolled use of antibiotics is one of the factors widely implicated, hence the renewed interest in breeding where it is practiced as a rapid growth supplement and in veterinary medicine. It is associated with the development of drug resistance within the commensal and pathogenic floras of treated animals (Bedekelabou et al., 2020). The spread of zoonotic transmission between humans and animals has long been known, but the current status of resistance patterns of pathogenic found in animals as well as a possible resistance vector to humans remain less explored (Madec, 2013). Animal's gut microbiota can harbour antimicrobial-resistant bacteria, potentially transmitted to humans through food, by direct contact or through the environment. Several studies have incriminated the promiscuity between humans and animals as a possible way of spreading mobile genetic elements carrying antimicrobial resistant genes between bacteria that impact all activities in humans, veterinary and environmental health (Partridge et al., 2018; Hernando-Amado et al., 2019).

Pork is one of the most widely consumed meats in the world (Hernando-Amado et al., 2019) and has been shown through several studies to being a reservoir of pathogenic and non-pathogenic bacteria (White et al., 2019; Wu et al., 2020). Several resistance profiles have been isolated from pig farmers on the digestive flora of wild boars and deer, in piglets with diarrheal signs, or on healthy animals in poultry and pig farms, in France, Belgium and Ivory Coast (Guessennd et al., 2012; Roguet et al., 2017). The predominant human pathogen species isolated with various resistance profiles was *Escherichia coli* and can be a possible resistance vector (Castro et al., 2005).

E. coli has historically been considered as one of the most common agents associated with diarrhea in suckling and post-weaned piglets (Fairbrother and Nadeau, 2019). There is high diversity and variants of *E. coli* strains integrating the normal gut microbiota, with most of them being considered non-pathogenic (DebRoy and Maddox, 2001). The characterization of pathogenic *E. coli* strains is usually based on the presence of virulence factors (Mainil, 2013). In piglets, *E. coli* pathogenic strains can be classified into different pathotypes: enterotoxigenic (ETEC) strains releasing heat-labile (LT) and heat-stable Sta and Stb exotoxins, intimin (eae)-producing enteropathogenic (EPEC) strains and verotoxigenic (VTEC) strains producing VT1/VT2 verotoxins (Robins-Browne et al., 2016).

The systematic use of antimicrobials in livestock, especially in the growing pig industry, either for "prophylaxis" or "metaphylaxis" poses a serious danger to the breeding of multidrug resistant bacteria (MDR). Thus, faced with the constantly growing use of antibiotics in pig breeding and the place of pork in culinary cultures, it is urgent to localize the surveillance of the resistance profiles of the bactectial species that can be transmitted to humans.

In this study, we aim to determine and explore the antibiotic resistance of *E. coli* isolates in the guts of pigs *(Sus scrofa domesticus)* from pork selling points in Lomé,Togo.

MATERIALS AND METHODS

Sampling

A cross-sectional study was conducted from July to November 2020 on five (5) informal pork sales sites in the urban region (Sagbado, Adidogome, Avedji, Agoe, Bè) of Lomé in Togo. Ten (10) non replicates individual feces samples were collected from each site by rectal swabbing, for a total of 50 samples. The swabs were immediately discharged into 2 ml of physiological water (NaCl 0.9%) stored in a cooler (4-8°C) and sent to the laboratory, within 1 to 2 h after collection.

Isolation and identification of strains

Isolation and identification of *E. coli* strains from samples are done using the procedure adapted from the Manual for Medical Bacteriology Standard Operating Procedures in Togo (Ministère de la Santé et de l'Hygiène Publique, 2020).

A first culture was done on the EMB medium, and suspicious green colonies with a characteristic metallic reflection were transferred into a UTI medium. Confirmation of *E. coli* strains from pure cultures was made by the indole test, as described by Perry et al. (2003).

Antibiotic susceptibility testing (AST)

Confirmed strains of *E. coli* were tested in triplicate against 19 antibiotics by agar diffusion method, according to European Committee of Antimicrobial Susceptibility Testing recommendations (European Committee on Antimicrobial Susceptiblity Testing, 2020). Antibiotic discs (Oxoid) used included: Betta-lactams (Penicillins: ampicillin, amoxicillin+clavulanic acid, piperacillin, ticarcillin; Cephalosporins: cefalotin, cefoxitin, cefotaxime, ceftazidime, cefepime; Carbapenem: imipenem; Monobactam: aztreonam), Fluoroquinolones (nalidixic acid, ciprofloxacin, ofloxacin), Aminosides (amikacin, gentamicin), Phenicol(chloramphenicol), Sulfamid (trimethoprim) and Fosfomycin. The reference strain *E. coli* ATCC 25922 was used for internal quality control. The inhibition diameter was measured and strains are categorized as "S" for sensitive and "R" for resistant. An isolate with resistance of at least one agent in three or more antimicrobial families was considered as multidrug resistant strain (Basak et al., 2016). We systematically looked for an extended spectrum beta lactamase (ESBL) using the synergy test. A positive synergy test is materialized by a champagne cork image between the clavulanic acid amoxicillin disc and a third-generation cephalosporin. The other Beta-lactam resistance phenotypes were also determined based on susceptibility test data (Piéboji et al., 2004):

1. Wild type: Strains susceptible to all Betta-lactams used.

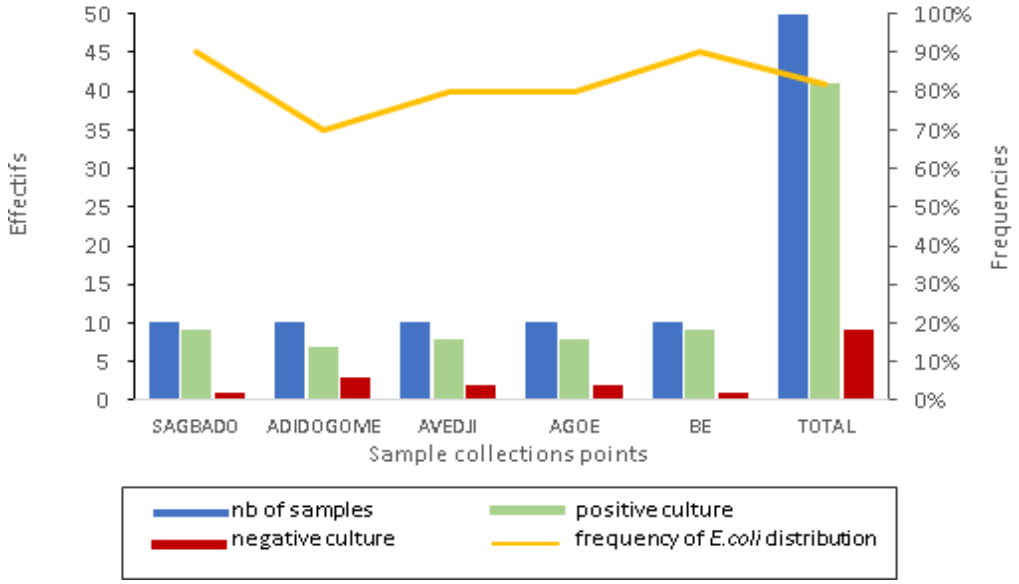

Figure 1. Frequency of isolated *E. coli* strains.

2. Low-level penicillinase: Strains resistant to ampicillin and piperacillin and moderately resistant to cefalotin.
3. High-level penicillinase: Strains resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin and cefalotin.
4. Low-level cephalosporinase: Strains resistant to ampicillin, amoxicillin/clavulanic acid, cefalotin and cefoxitin.
5. High-level cephalosporinase: Strains resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin, cefalotin, cefoxitin, moderately resistant to cefotaxime, ceftazidime and aztreonam.

Data analysis

Statistical data analysis was performed in Microsoft Excel 2019 and SphinxPlus2V5 software. The Chi-square test was used for comparison between the groups and a p-value < 0.05 was considered statistically significant. Cramer's V test was used to study the distribution between two qualitative variables.

Ethical approval and consent to participate

Pork sellers gave their consent by a signed paper for samples and information collection.

RESULTS

Frequency of *Escherichia coli* strains

Of the 80% isolated samples analyzed, a similarity between the obtained frequencies of positive *E. coli* cultures across the different sampling sites was observed with a slight increase in the frequencies of the Sagbado and Bè sites (Figure 1). A total of 41 strains (Ec-1 to Ec-41) of *E. coli* were obtained.

Antibiotic susceptibility

Tested against a panel of 19 antibiotics, various phenotypes and resistance's frequencies were obtained. High resistance rates (>30%) were obtained against Ampicillin, Nalidixic acid and Ofloxacin and low resistance frequencies against Fosfomycin and Imipenem (Table 1). The most represented phenotypes were Wild phenotypes with 61% of frequency followed by low-level penicillinase (14.6%) and high-level penicillinase (9.8%). Over the 41 strains, 2 were Extended Spectrum Beta-Lactamase (ESBL) phenotypes (Table 2).

A total of 18 multidrug resistant strains were found, with a rate of 43.9% and various resistance patterns (Table 3).

DISCUSSION

Antimicrobial resistance is a worldwide concern of the one health concept, affecting humans and animals. It is accepted that *E. coli* is a good candidate for the study of antibiotics (Sorum and Sunde, 2001) performance. Sensitivity tests were conducted on molecules representing different classes of antibiotics, with the aim of documenting the potential risks of emergence of resistant strains through food and contact with animals. Forty-one strains of *E. coli,* isolated from the guts of pigs for human consumption in Lomé, Togo, were tested against antibiotics fairly representative of classes of antibiotics used in veterinary medicine and of interest in human medicine. Our results showed that *E. coli* is highly represented (82%) in the commensal digestive flora of the 56.89% prevalence found in samples from farms pig in Togo (Bedekelabou et al., 2019), but less than the prevalence obtained in north- eastern Thailand which was 90% (Lunha et al., 2020). The difference here may be due to the direct environment and feeding (McCormack et al., 2017). There was no statistically verified link between the sampling sites and the

Table 1. *E. coli* strain sensitivity profile for each antibiotic.

Antibiotic	Profile		Frequency of Resistance (%)	p-value
	R (n)	S (n)		
Ampicillin (AMP)	15	26	36.6	0.0858
Amoxicillin+clavulanic acid (AMC)	9	32	22.0	0.0030
Piperacillin (PRL)	11	30	26.8	0.0030
Ticarcillin (TI)	11	30	26.8	0.0030
Cefalotin (KF)	3	38	7.3	0.0001
Cefoxitin (FOX)	6	35	14.6	0.0001
Cefotaxim (FEP)	5	36	12.2	0.0001
Ceftazidim (CAZ)	4	37	9.8	0.0001
Cefepim (CEF)	2	39	4.9	0.0001
Imipenem (IMP)	0	41	0.0	N/A
Aztreonam (ATM)	6	35	14.6	0.0001
Nalidixic acid (NA)	14	27	34.1	0.0423
Ciprofloxacin (CIP)	10	31	24.4	0.0010
Ofloxacin (OFX)	13	28	31.7	0.0191
Amikacin (AK)	11	30	26.8	0.0030
Gentamicin (CN)	12	29	29.3	0.0079
Chloramphenicol (C)	6	35	14.6	0.0001
Trimethoprim (W)	9	32	22.0	0.0003
Fosfomycin (FOS)	0	41	0.0	N/A

R: Resistant, S: Sensitive, n: number of isolates.

Table 2. Beta-lactam resistance phenotypes.

Phenotype	Number	Frequencies (%)
Wild	25	61.0
Low-level penicillinase	6	14.6
High-level penicillinase	4	9.8
Low-level cephalosporinase	3	7.3
High-level cephalosporinase	1	2.4
ESBL	2	4.9
Total	41	100

All of the strains studied were sensitive to Fosfomycin and Imipenem. Similar studies of Guessennd et al. (2012) and Kone et al. (2019) on piglets in Ivory Coast also reported a lack of resistance for Imipenem and 66.7% sensitivity for Fosfomycin in piglets treated with Tetracyclin (Mensah et al., 2014). The same sensitivity to Imipenem has been reported by studies on strains of the same species, isolated in hospitals in Togo by Salou et al. (2011) and by Mlaga et al. (2019). Salah et al. (2016) also found very high rates of sensitivity in 2016 (97.8% for Imipenem and 95.6% for Fosfomycin). An almost high sensitivity hospital isolates of *E. coli* to Imipenem was observed in 2012 in Morocco (Mohammad-Jafari et al., 2012) and in 2015 in Benin (Anago et al., 2020), at rates of 96.7 and 96.4% respectively. These results therefore support the assumption that Imipenem remains the molecule of last resort in therapeutic efficacy on *E. coli* strains because of the stability and high activity of carbapenem against most beta-lactamases (Mlaga et al., 2019; Anago et al., 2020).

No strain was resistant to all antibiotics. The difference between resistance (36.6%) and sensitivity (63.4%) to Ampicillin was insignificant (p-value= 0.0858<0.005).

The lowest resistance rates were observed for Ceftazidim (4.9%), Cefalotin (7.3%) and Cefotaxim (9.8%). On the other hand, in Ivory Coast, Guessennd et al. (2012) found high rates of Cefotaxime resistance (66.7%). Kone et al. (2019) reported 7 and 38% resistance to Cefotaxime compared to 23.2 and 83.3% to Ceftazidime on piglets treated and not treated with

Table 3. Antibiotic resistance patterns of multi drug resistant *E. coli* isolates detected.

Isolate	Resistance Pattern
Ec- 03	AMP, AMC, KF, FEP, ATM, NA, CIP, AK, CN
Ec- 07	AMP, AMC, KF, FEP, CAZ, NA, CIP, AK, CN
Ec- 08	AMP, AMC, PRL, KF, FOX, FEP, CAZ, NA, W
Ec- 11	AMP, AMC, PRL, TI, NA, CN
Ec- 12	AMP, PRL, TI, FOX, FEP, CAZ, ATM, NA, CIP, AK, C, W
Ec- 16	KF, FOX, FEP, NA, CIP, OFX, AK, CN, W
Ec- 17	AMP, PRL, NA, CIP, CN, OFX, W
Ec- 21	FEP, CEF, ATM, CN, C, W
Ec- 22	AMP, AMC, PRL, FOX, FEP, CAZ, NA, W
Ec- 24	AMP, AMC, PRL, TIC, CAZ, ATM, AK, CN, C, W
Ec- 25	AMP, AMC, PRL, FOX, NA, CN
Ec- 28	PRL, TI, KF, FOX, CEF, ATM, NA, OFX
Ec-29	AMP, PRL, NA, CIP, CN, OFX, W
Ec- 33	AMP, PRL, TI, KF, FOX, FEP, CAZ, ATM, NA, CN, CIP, AK, C
Ec- 35	AMP, PRL, TI, NA, CN, C
Ec- 37	PRL, TI, KF, FOX, CEF, ATM, NA, OFX
Ec- 39	AMP, AMC, PRL, TI, NA, CN
Ec- 41	AMP, AMC, PRL, CN, C

Ampicillin (AMP), Amoxicillin+clavulanic acid (AMC), Piperacillin (PRL), Piperacillin (PRL), Ticarcillin (TI), Cefalotin (KF), Cefoxitin (FOX), Cefotaxim (FEP), Ceftazidim (CAZ), Cefepim (CEF), Imipenem (IMP), Aztreonam (ATM), Nalidixic acid (NA), Ciprofloxacin (CIP), Ofloxacin (OFX), Amikacin (AK), Gentamicin (CN), Chloramphenicol (C), Trimethoprim (W), Fosfomycin (FOS).

Tetracyclin respectively. These differences could be better elucidated if our sampling was conducted under the same conditions as these previous studies. But we preferred the sites of sale of pig meat, rather than the livestock farms, for a better representation of our study population, because, sellers used to buy the pigs from diversified livestock farms. Also, Guessennd et al. (2012) selected only piglets with diarrhea, unlike our study which involved clinically healthy animals.

Average resistance rates were 10.8% for Cephalosporins, 28% for Penicillins (the least active class), 19% for beta-lactams and 29.6% for other antibiotics, with an overall resistance average of 18.9% for all molecules tested. In a similar study by Guessennd et al. (2012), 44.4% of the strains were sensitive to beta-lactams.

The highest resistance rates were 36.6, 34.1 and 31.7% respectively for Nalidixic acid and Ofloxacin. Similar resistance patterns to Nalidixic acid (42%) were also found in the study of Martin et al. (2007) in Belgium.

We found a resistance rate of 22.0% to Amoxicillin +Acid Clavulanic, very close to what was found by Guessennd et al. (2012) in Ivory Coast (20.0%).

Multi-resistance was observed on 43.9% (18/41) of our strains, compared to 26.0 and 37.8% of multi-resistance in the studies of Guessennd et al. (2012) and Bedekelabou et al. (2020) respectively. The difference between sensitivity and resistance profiles was significant for all molecules (p-value=0.001<0.05).

ESBL strains accounted for 4.9%. Penicillinases-producing strains accounted for 14.6 and 9.8% respectively for low and high levels, corroborating the high rates of penicillin resistance observed. One (01) high-level cephalosporinase-producing strain was found, while three (3) strains were identified as low-level cephalosporinase producers. In Guessennd et al. (2012), no ESBL strains were found; however, low-level penicillinase-producing strains were also the most represented (40.7%) among the isolates. The difference between the proportions of phenotypes we encountered is significant (p-value =0.0001<0.05).

Conclusion

This study is one of the first on the extent of drug resistance in the animal health sector in Togo. Imipenem and Fosfomycin were 100% active on the strains of our study. Beyond the satisfaction that this could generate, it must appeal to public health actors in general and animal health in particular, on the regular surveillance of these molecules in order to prevent any outbreak of resistance and its spread to humans, which could aggravate cases of therapeutic failures. Cephalosporins remain effective overall with only 10.8% resistance. Penicillins represented by ampicillin, Amoxicillin-Clavulanic acid, Piperacillin and Ticarcillin are found to be the least active class of isolated strains in our study. Of course, only two (2) ESBL strains have been found, but there were also penicillinase and cephalosporinase producers among the isolated strains

of our study.

However, our findings deserve further study, including genotypic studies of antimicrobial resistance, the risk assessment of the circulation of antimicrobial-resistant strains on farms, and the analysis of the circumstances and conditions of exposure related to practices, in order to propose mitigation measures.

Limitations

We have not investigated the crossbreeding of antibiotic resistance with the usual antibiotic treatments for pigs. These data could have been provided to us by the breeders, however, our samples were rather taken from pig meat resellers who ensure that they do not subject the animals purchased to any antimicrobial treatment before their slaughter.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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