ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 11, pp.75621-75628, November, 2018

DOI: https://doi.org/10.24941/ijcr.33204.11.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANTIBACTERIAL RESISTANCE IN MAJOR BACTERIAL COMMUNITIES OF A FEW PIG FARMS AND POULTRIES OF CAMEROON: A GLANCE ON THE DIVERSITY OF PHENOTYPIC RELATED MECHANISMS

[1,2,3]Simo Louokdom Josué, [1,2,*]Fotsing Kwetché Pierre René, [1,2]Lettie Akum Mbaku, [1,2]Yawat Djogang Anselme Michel, [1,2]Gamwo Dongmo Sandrine, [1,2]Serge Honoré Tchoukoua, [1,2]Kouamouo Jonas, [4]Omer Njajou, [1,2]Kourouma Kaba, [3]Kuiaté Jules Roger and [1,2]Jean Michel Tekam

[1]Laboratory of Microbiology, Université des Montagnes Teaching Hospital; Bangangté, Cameroon
[2]School of Pharmacy, Higher Institute of Health Sciences, Université des Montagnes, Bangangté, Cameroon
[3]Faculty of Science, University of Dschang; Dschang, Cameroon
[4] School of Public Health, University of Minnesota Saint Paul, USA

ARTICLE INFO

Article History:
Received 02nd August, 2018
Received in revised form
20th September, 2018
Accepted 07th October, 2018
Published online 30th November, 2018

Key Words:

Resistance mechanisms,
Poultry and pig farm bacteria,
Phenotypes

**Corresponding author*

ABSTRACT

The main objective of the present study was to address phenotypic characterization of resistance mechanisms in bacteria with their trends in poultry and pig farms. More specifically, a few resistance mechanisms were investigated in bacteria isolated from farms in the Western Region of Cameroon. The target traits included extended spectrum beta-lactamase (ESBL), inducible cephalosporinase (IC), high- and low-level penicillinases (HLP and LLP, respectively), high- and low-level cephalosporinases (HLC and LLC, respectively) and inhibitor-resistant penicillinase (IRP) in Gram-negative bacteria. In Gram-positive, they were limited to IC and ESBL. All detections were conducted according to the disk diffusion principles (Kirby-Bauer) with antibiotics that are commonly used for phenotypic detection of resistant traits. A total of 624 isolates from farms in Bafoussam, Bafang, Bandjoun and Kweko (Western Region of Cameroon) underwent the tests. The most common bacteria isolates belonged to Gram-negative bacilli members of the *Enterobacteriaceae* family (70%). Resistance rates recorded were highest with Amoxicillin and Amoxicillin/Clavulanic-acid. In further details, seven resistance mechanisms were detected; with more than one in the same isolates in some cases. More subtle details highlighted that, their rates broadly varied from 3% with HLC through 67% with LLC, with higher diversity in pig farms. All *Serratia* spp. expressed LLP while the highest ESBL rate was observed in *Salmonella* (84.6%). *Staphylococci* were also found to express ESBL (44%). Overall, HLP, IRP and ESBL-expression appeared first, second and third most frequently detected (67%, 56%, and 50%, respectively). All *Staphylococci* expressed resistance to Oxacillin (100%) that otherwise reflects resistance to methicillin, while 26.2% of isolates showed resistance to Erythromycin and Clindamycin (indicating constitutive MLSB phenotypes). Altogether, these findings indicated that antibiotic therapy is seriously threatened in the settings; reiterating the need for routine phenotypic tests and to enforce an antibiotic resistance stewardship program in Cameroon.

Citation: Simo Louokdom Josué, FotsingKwetché Pierre René, Lettie Akum Mbaku, Yawat Djogang Anselme Michel et al. **2018.** "Antibacterial resistance in major bacterial communities of a few pig farms and poultries of cameroon: a glance on the diversity of phenotypic related mechanisms", *International Journal of Current Research*, 10, (11), 75621-75628.

INTRODUCTION

Infectious diseases (IDs) are leading causes of mortality and morbidity worldwide, especially in low-income communities. They have become difficult to conquer because of the increasing rates of microbial resistance. Antimicrobial resistance threatens effective prevention and treatment of an ever-increasing rate of infections caused by bacteria, parasites, viruses and fungi (Levy, 1998). Emerged in the 1945thresistance to antibiotics has become a very challenging issue nowadays because of its continuous and seemingly unstoppable surge (Cataño *et al*.,2012; Coates *et al*., 2011). In fact, bacterial resistance increases the length of hospital stay, cost of treatment and the rates of morbidity and mortality (Cataño *et al*., 2012). Infections caused by resistant bacterial strains (true pathogens and opportunistic pathogens) are as common as the causative agents express tolerance to several pharmacological classes of antibiotics (Levy, 1998; Cataño *et al*., 2012). This situation is made worst by the fact that there are limited number of novel classes of antibacterial agents on the market since most new antibiotics are analogues of existing

ones (Coates *et al*., 2011). According to several authors, misuse of antimicrobial in human medicine, animal husbandry, and crop production play critical role in the process of selection and dissemination of resistance traits that diffuse amongst mixed microbial populations (Levy, 1998; Zechini, 2009; Martins *et al*., 2013). Thousands of deaths caused by *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus* or *Mycobacterium tuberculosis* are due to resistance to chemotherapy (Martins *et al*., 2013). Bacterial resistance causes a large proportion of nosocomial infections in hospitals, especially in intensive care units that have become epicenters of life threatening infections (Weinstein, 1998; WHO, 2015). High vulnerability of patients, invasive procedures, large number and broad varieties of antibacterial agents prescribed, have collectively created appropriate environments for bacterial resistance genotype/phenotypes selection (Zechini, 2009), making of the antibiotic resistance a big public health issue throughout the world and especially in resource-limited countries like Cameroon where the negligible drug regulation comes along with counterfeit drugs, unrestricted access and careless prescription of antibiotics (Chevalier and Mallea, 2000; WHO, 2002).

Otherwise bacterial resistance is a worldwide phenomenon but their geographic distribution differs across regions and health facilities based on human behavior largely attributable to with variables like living standard, education, basic hygienic practices (Inweregbu *et al*.,2005) and improper combination therapy observed in all low-and-middle-income communities across the globe. Sets of holistic data that are necessary to advocate, design and enforce any stewardship strategy toward controlling resistance for better healthcare are limited in many settings. For this advocacy to be effective, knowledge on resistance trends and various resistance mechanisms expressed by bacteria is crucial. These are useful tools, for instance in implementing combination empiric therapy in emergency and in areas where susceptibility tests cannot be performed.

The present study aimed at investigating through major resistance mechanisms expressed by bacteria in poultry and pig farms and discussing likely connections with IDs caretaking in human. This pioneer work conducted on multidrug-resistant isolates recovered from farms in West-Cameroon was an attempt to initiate tracking resistance traits that disseminate from farms animals into human communities, in the global frame of the current One Health paradigm which targets reducing the burden of ID in the intermediate and long run.

MATERIAL AND METHODS

Ethical consideration, study site and population

The go-ahead for the present survey was obtained from the Head of the Université des Montagnes' Teaching Hospital under Ref: 2017/0105/CUM/ADM for specimen analyses that were performed in the premises of its Laboratory of Microbiology. The West Region of Cameroon is known as the most important basin for animal's farms (poultry and Pig) in Central Africa and often regarded as the country's reserve that supplies animal proteins needs throughout the country and across borders. This study was conducted in Bafoussam, Bandjoun, Bafang and Kweko. All are semi-urban areas in which population share sets of socio-economic determinants like beliefs agro-pastoral activities and trade.

Field data collection, sample collection and bacteria isolation

This was a descriptive study. From November 2017 to July 2018, it was conducted in Bafoussam, Bafang, Kweko and Bandjoun where sample collection took place. Subsequent to culture isolation and identification, bacterial isolates were cryo-preserved in Brain Heart Infusion Both (BHIB) with 20% glycerol for phenotypic characterization. Prior to phenotypic testing, purified isolates were reseeded on nutrient agar (Liofichem)® and incubated overnight (18-24hat37°C) to have the necessary fresh bacterial populations. From the resulting culture, a suspension density equivalent to 0.5 McFarland was prepared and adjusted to the final inoculum opacity required for standard susceptibility test by agar diffusion on Mueller Hinton agar. Reference bacterial strains used for quality control were *E. coli (*ATCC25922), *E. faecalis (*ATCC 29212) and *S. aureus (*QC1625). Phenotypic tests and result interpretations were guided lead according to the « Comité de l'Antibiogramme de la Société Française de Microbiologie CA-SFM, 2017 » plus the criteria listed in Table 1 as used in previous reports(CLSI, 2012;Gangoué Piéboji *et al*., 2004; Fick Hindler, 2007; Cockerill, 2011; Smith *et al*., 2013; Fotsing Kwetché *et al*., 2015). The target resistance mechanisms included inducible cephalosporinase (IC), Extended Beta-lactamases (ESBL) and Inducible Clindamycin (ICl) resistance in Gram-positive; ESBL, IC, High level penicillinase (HLP), Low level penicillinase (LLP), High level cephalisporinase (HLC), Low level cephalosporinase (LLC) and Inhibitor resistant penicillinase (IRP) in Gram-negative rods (Table 1). The antibiotics disks used included Penicillin P (10 µg), Cefotaxim (5 µg), Oxacillin (1 µg), Amoxicillin (30 µg), Amoxicillin/Clavulanic-acid (20/10 µg), Imipenem (10 µg), Cefuroxime (30 µg), Ceftriaxon (30µg), Ceftazidim (10 µg), Cefoxitin (30µg), Aztreonam (30 µg), Cotrimoxazole (25 µg), Gentamicin (120µg), Nalidixic acid (30 µg), Norfloxacin (30 µg), Levofloxacin (30 µg), Nitrofurantoin (300 µg), Ciprofloxacin (30 µg), Tetracycline (30 µg), Vancomycin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg), Cefazolin (30 µg), Piperacillin (100 µg), Ticarcillin (30 µg), Tazobactam/ Piperacillin (30 µg), Cefepime (30 µg) and Sulbactam/ Cefoperazone (105 µg). Data recorded were summarized in terms of rates of phenotypes per bacterial major types. Data entry and processing were done on the Excel 2016 spreadsheet, the statistical tests used were the Chi Square Test and the logistic regression performed with the Statistics softwares namely: R i386 3.4.3 and IBM SPSS Statistics version 23.

RESULTS

Bacterial population used in the study: From the original specimens that consisted of manure, animal food and drinking water, 624 isolates were recovered and used in the present study. The respective numbers of isolates in these animal production units were 311 and 313; distributed into 12 major bacterial types. Further pieces of related information were reorganized and displayed as shown in Table 2. Overall, the largest numbers were recovered from stool/manure (55% in poultries and 60%in pig farms); but 100% sterility could not be recorded in any specimen categories. Combined data also indicated that Gram-negative rods represented 36% of the total in the poultries and 62% in the pig farms. Moreover, bacteria from the *Enterobacteriaceae* family literally overwhelmed these proportions in both breeding environments; followed by

Table 1. Phenotype investigated per bacterial types (summary)

LLP: low level penicillinase; HLP: high level penicillinase; LLC: low level cephalosporinase; HLC: high level Cephalosporinase; IRP: in hibitorresistant penicillinase; ESBL: extendedspectrumbeta-lactamase; AMC: Amoxicillin/ clavulanicacid; CTX:

Bacterialgroup	Target phenotype	Antibiotic used and clinicalcategory
Enterobacteriaceae	LLP	AX*; AMC*;CEF**
	HLP	AX*; AMC*;PIPL*CEF*
	IRP	AX*;AMC*;PIPL*
	LLC	AX*; AMC*;CEF*;FOX*
	HLC	AX*;AMC*;PIPL*;CEF*;FOX*CTX**;CAZ**;ATM**
	ESBL	AX*; AMC*;PIPL*;CEF*;CTX*;CAZ*;ATM*(double disksynergy-confirmed by the AMC-TX/CAZ/ATM/CRO test)
Gramnegative-non fermentingrods	Inducible cephalosporinase	Double disk antagonismtest(IMP/FOX-CAZ)
	Penicillinase	PIPL*/**;CTX*/**;ATM*/**
	LLC	CTX*
	HLC	PIPL*;CTX*;ATM*;CAZ*
	Inducible Cephalosporinase	Doublediskantagonismtest(IMP/FOX-CAZ)
	ESBL	AX*; AMC*;PIPL*;CEF*;CTX*;CAZ*;ATM*(double disksynergy-confirmed by the AMC-TX/CAZ/ATM/CRO test)
	ESBL	AX*; AMC*;PIPL*;CEF*;CTX*;CAZ*;ATM*(double disksynergy-confirmedbytheAMC-TX/CAZ/ATM/CRO test)
	Inducible Cephalosporinase	Double disk antagonism test (IMP/FOX-CAZ)
Gram-positivecocci	Constitutive Clindamycin Resistance CCR	E^*, C^* uniform circular zones of inhibition for both disks
	InducibleClindamycin Resistance ICR	Er^* , Cl^s D-zone around clindamycin, circular zone of inhibition around Erythromycin (*Puttingerythromycin disk (15µg) in close proximity (15 mm edge to edge) to a clindamycin disk (2µg).*

Cefotaxime; CAZ: Ceftazidim; ATM: Aztreonam; CRO: Ceftriaxon; IMP: Imipenem; FOX: Cefoxitin; CEF: Cefazolin; AX: Amoxicillin; PIPL: Piperacillin; *:Resistant;**: moderatelyresistant;*/**: resistantormoderatelyresistant; CCR : constitutive Clindamycin resistance; ICl: Inducible clindamycin Resistance,; Cl^s: clindamycin susceptible; Cl^* :clindamycin resistant

Table 2. Specimen's rates of isolations per microbial type and per farm

Poultries					
Bacterial types	Food	Stool	Water	Total	%
Bacillus spp.	11	23	3	37	12
Citrobacterfreundi	0	7	2	9	3
Clostridium spp.	4	12	2	18	6
Enterobacter aerogenes	5	14	3	22	7
Enterobacter hafnia	27	27	12	66	21
Escherichia coli	7	5	2	14	4
*Proteus*spp.	5	9	11	25	8
Pseudomonas spp.	0	3	0	3	1
*Shigella*spp.	0	2	0	2	1
Staphylococcus aureus	20	62	17	99	32
Streptococcus spp.	7	8	1	16	5
TOTAL	86	172	53	311	100
Pigfarms					
Bacterial types	Food	Stool	Water	Total	%
Bacillus spp.	10	35	16	61	20
Citrobacterfreundi	7	69	6	82	26
Enterobacter spp.	0	4	3	7	2
Escherichia coli	2	5	2	9	3
*Proteus*spp	3	47	13	63	20
Pseudomonas spp.	0	3	0	3	1
Salmonella spp.	4	18	7	29	9
Serratia marcessens	0	1	0	1	0.32
Staphylococcus aureus	31	6	21	58	19
TOTAL	57	188	68	313	100

***Staphylococcus* in the pig farms and *Bacillus* in the poultries':** *Pseudomonas* was the least frequently isolated in both pig and poultry farms.

Resistance phenotypes rates and their distribution in poultry: All bacteria isolates expressed multiple-resistance. This could involve 75% of the antibacterial agents used. Out of these multidrug-resistance-positive isolates, the overwhelming proportion (≈70%) belonged to the *Enterobacteriaceae* family, followed by *Staphylococcus aureus*. Further characterizing resistant mechanisms led to the summarized pieces of information that were organized per bacterial types and presented in Table 3 for poultries'. All *Staphylococcus aureus* expressed resistance to Oxacillin.

The rates of LLP, HLP, and IRP were quite similar for all Gram-negative rods (overwhelmed by members of the *Enterobacteriaceae* family). The highest rates of ESBL were detected in Gram-positive cocci (*Staphylococcus*, *Bacillus*, and *Streptococcus*) and *Citrobacter* while unlike the two former genera; IC was most common in *Citrobacter*.

Resistance phenotypes rates and their distribution in pig farms: A similar analytical procedure on phenotypic characteristic in pig farms resulted in the production of more informative data summary presented in terms of frequency per bacterial group as displayed in Table 4.

Table 3. Phenotype rates distribution (%) in poultry farms

Bacterialcategories	Phenotypiccharacteristics							
	LLP (%)	HLP (%)	IRP (%)	LLC (%)	HLC (%)	ESBL (%)	IC (%)	MRSA (%)
*Pseudomonas*spp.	1(10)	0 (0)	0 (0)	1(6.7)	2(6.7)	2 (6.7)	1(6.7)	NA
*Shigella*spp.	1(10)	1(6.7)	1 (6.7)	1(6.7)	1(3.3)	3(10)	2(13.3)	NA
*Proteus*spp.	1(10)	1 (6.7)	1 (6.7)	1(6.7)	1 (3.3)	1(3.3)	1(6.7)	NA
E.coli	1(10)	1 (6.7)	1 (6.7)	1(6.7)	1 (3.3)	2(6.7)	0 (0)	NA
*Enterobacter*spp.	1(10)	1(6.7)	1 (6.7)	1(6.7)	2 (6.7)	4(13.3)	0(0)	NA
*Citrobacter*spp.	0 (0)	0 (0)	0 (0)	2 (12.3)	10(33.3)	14(46.7)	9(60)	NA
*Clostridium*spp.	2(20)	5(33.3)	5(33.3)	2 (13.3)	0 (0)	1 (3.3)	1(6.7)	NA
*Bacillus*spp.	0 (0)	4 (26.7)	4(26.7)	0 (0)	8(26.7)	12 (40)	0(0)	NA
*Streptococcus*spp.	NI	NI	NI	NI	NI	11(31.4)	3(20)	NA
*Staphylococcus*spp.	NI	NI	NI	NI	NI	14 (40)	5(33.3)	5(100)

NI: notinvestigated; NA: Not Applicable LLP: low level penicillinase; HLP: high levelpenicillinase; LLC: low level cephalosporinase; HLC: high level cephalosporinase; IRP:inhibitorresistantpenicillinase;ESBL:extendedspectrumbeta-lactamase

Table 4. Phenotype distribution rates (%) in pig Farms

Bacterialcategories	Phenotypiccharacteristics							
	LLP (%)	HLP (%)	IRP (%)	LLC (%)	HLC (%)	ESBL (%)	IC (%)	MRSA (%)
*Pseudomonas*spp.	1(25)	1(17)	1(17)	2(17)	2(17)	2 (17)	2(33)	NA
*Serratia*spp.	2(100)	2(67)	2 (67)	2(67)	2(33)	4 (67)	0 (0)	NA
*Proteus*spp.	6 (100)	6(67)	6 (67)	8(89)	8(44)	8 (44)	2(22)	NA
E.coli	6(100)	6(67)	6(67)	8 (89)	8(44)	10(56)	3 (33)	NA
*Enterobacter*spp.	4 (50)	4(33)	4(33)	5(42)	6(33)	6(33)	1(11)	NA
*Citrobacter*spp.	4 (50)	5(56)	5(56)	5(56)	5(28)	6 (33)	1(11)	NA
*Salmonella*spp.	4 (66.7)	5(56)	5(56)	5(56)	7(39)	9(50)	1 (11)	NA
*Bacillus*spp.	0 (0)	0 (0)	0(0)	0(0)	3 (17)	3(17)	3 (33)	NA
*Streptococcus*spp.	NI	NI	NI	NI	NI	0 (0)	0(0)	NA
*Staphylococcus*spp.	NI	NI	NI	NI	NI	7 (39)	4(44)	3(100)

NI: notinvestigated; NA: Not Applicable LLP: low level penicillinase; HLP: high level penicillinase;LLC: low level cephalosporinase; HLC: high level cephalosporinase; IRP:inhibitorresistantpenicillinase;ESBL:extendedspectrumbeta-lactamase

Table 5. Phenotypes rates (%) in pig and poultry farms

Bacterialcategories	Phenotypiccharacteristics							
	LLP (%)	HLP (%)	IRP (%)	LLC (%)	HLC (%)	ESBL (%)	IC (%)	MRSA (%)
*Citrobacter*spp.	4 (25)	5(21)	5(21)	5(21)	5(10)	6 (13)	10(42)	NA
E.coli	7(44)	7(29)	7(29)	9 (38)	9(19)	12(25)	3(13)	NA
*Enterobacter*spp.	5(38)	5(24)	5(24)	6(29)	8(19)	10(24)	1(5)	NA
*Proteus*spp.	7(44)	7(29)	7(29)	9(38)	9(19)	8(17)	3(14)	NA
*Salmonella*spp.	4 (67)	5(56)	5(56)	5(56)	7(39)	9(50)	1(11)	NA
*Serratia*spp.	2(100)	2(67)	2(67)	2(67)	2(33)	4 (67)	0(0)	NA
*Shigella*spp.	1(10)	1(7)	1(7)	1(7)	1(3)	3(10)	2(13)	NA
*Pseudomonas*spp.	2(17)	1(6)	1(6)	3(17)	4(11)	4(11)	3(17)	NA
*Bacillus*spp.	0 (0)	4 (17)	4(17)	0(0)	11(30)	15(31)	3(14)	NA
*Clostridium*spp.	2 (20)	5(33)	5 (33)	2(13)	0 (0)	1 (3)	1(5)	NA
*Streptococcus*spp.	NI	NI	NI	NI	NI	11 (23)	3(14)	NA
*Staphylococcus*spp	NI	NI	NI	NI	NI	21(44)	9(43)	8(100)

NI: notinvestigated; NA: Not Applicable; LLP: low level penicillinase; HLP: high level penicillinase; LLC: low level cephalosporinase; HL: high level cephalosporinase; IRP: inhibitorresistantpenicillinase; ESBL: extendedspectrumbeta-lactamase

The rates of LLP were highest in all *Enterobacteriaceae.* They ranged from 50% with *Enterobacter* through 100%with *Proteus*, *Serratia* and *E. coli.* Those of HLP, IRP and LLC were quite similar for all Gram-negative rods overwhelmed by isolates from the *Enterobacteriaceae* family. They broadly ranged from 33% in *Enterobacter* through 89% in *Proteus* and *E. coli*. In *Pseudomonas*, the rates were similar across for HLP, IRP, LLC, HLC and ESBL. ICs were most common in Gram-positive, precisely *Staphylococci* and *Bacillus*. Moreover, all *Staphylococci* expressed resistance to Oxacillin.

Frequency of phenotypes in both pig and poultry farms: Broadly summarized, resistance phenotype rates were then displayed as shown in Table 5 for the whole study. Amongst the outstanding findings, all *Serratia* expressed LLC. This phenotype was detected in 84.6% of *Staphylococcus* and *Enterobacter.* In *Serratia* ESBLs phenotype was highest (66.7% of the isolates). All *Staphylococcus aureus* expressed resistance to Oxacillin.

IC and ESBL were also expressed by large number of isolates. In several cases, resistance induction (by FOX/IMP test) against *third-generation cephalosporin* antibiotics was associated with reduced susceptibility to AMC while, about 40% of isolates from the *Enterobacteriaceae* family associated ESBL expression with IC-expression. With regards to inducible clindamycin (ICl), 31 isolates of *Staphylococcus* expressed resistance to Erythromycin and were subjected to the D-test. Predominantly, 23(54.8%) of these isolates were recovered from stools and 19(45.2%) from food and water. Out of this number 11(26.2%) expressed resistance to Clindamycin, implying constitutive Macrolides-Lincosamides-Streptogramin B (MLSB) resistance phenotype. Out of the 20 isolates that expressed susceptibility to Clindamycin, 8 (19.01 %) were positive for the D-zone test, indicating inducible MLSB-resistance.

Farm/resistance phenotypes associations: Farm connected phenotypes were further addressed and displayed as shown in Table 6.

Table 4. Rates of phenotypes associated to antibiotics in farms

PHENOTYPES	YES/ NO	[ALL] N=561	PIG N=252	POULTRY N=309	*p*	N
LLP_Amox/Clavul	NO	192 (58.0%)	68 (36.0%)	124 (87.3%)	<0.001	331
	YES	139 (42.0%)	121 (64.0%)	18 (12.7%)		
LLP_Amoxicillin	NO	133 (40.3%)	53 (28.0%)	80 (56.7%)	<0.001	330
	YES	197 (59.7%)	136 (72.0%)	61 (43.3%)		
HLP_Amox/Clavul	NO	192 (58.4%)	68 (36.0%)	124 (88.6%)	<0.001	329
	YES	137 (41.6%)	121 (64.0%)	16 (11.4%)		
HLP_Amoxicillin	NO	133 (40.3%)	53 (28.0%)	80 (56.7%)	<0.001	330
	YES	197 (59.7%)	136 (72.0%)	61 (43.3%)		
HLP_Piperacillin	NO	55 (40.4%)	14 (58.3%)	41 (36.6%)	0.082	136
	YES	81 (59.6%)	10 (41.7%)	71 (63.4%)		
IRP_Amox/Clavul	NO	192 (58.4%)	68 (36.0%)	124 (88.6%)	<0.001	329
	YES	137 (41.6%)	121 (64.0%)	16 (11.4%)		
IRP_Amoxicillin	NO	133 (40.3%)	53 (28.0%)	80 (56.7%)	<0.001	330
	YES	197 (59.7%)	136 (72.0%)	61 (43.3%)		
IRP_Piperacillin	NO	55 (40.4%)	14 (58.3%)	41 (36.6%)	0.082	136
	YES	81 (59.6%)	10 (41.7%)	71 (63.4%)		
HLC_Amox/Clavul	NO	192 (58.4%)	68 (36.0%)	124 (88.6%)	<0.001	329
	YES	137 (41.6%)	121 (64.0%)	16 (11.4%)		
HLC_Amoxicillin	NO	133 (40.3%)	53 (28.0%)	80 (56.7%)	<0.001	330
	YES	197 (59.7%)	136 (72.0%)	61 (43.3%)		
HLC_Piperacillin	NO	55 (40.4%)	14 (58.3%)	41 (36.6%)	0.082	136
	YES	81 (59.6%)	10 (41.7%)	71 (63.4%)		
HLC_Cefoxitime	NO	270 (81.8%)	144 (76.2%)	126 (89.4%)	0.003	330
	YES	60 (18.2%)	45 (23.8%)	15 (10.6%)		
HLC_Cefotaxime	NO	251 (93.3%)	121 (94.5%)	130 (92.2%)	0.603	269
	YES	18 (6.69%)	7 (5.47%)	11 (7.80%)		
HLC_Ceftazidime	NO	103 (50.0%)	51 (78.5%)	52 (36.9%)	<0.001	206
	YES	103 (50.0%)	14 (21.5%)	89 (63.1%)		
HLC_Aztreonam	NO	121 (45.3%)	72 (57.1%)	49 (34.8%)	<0.001	267
	YES	146 (54.7%)	54 (42.9%)	92 (65.2%)		
ESBL_Amox/Clavul	NO	229 (59.2%)	105 (42.5%)	124 (88.6%)	<0.001	387
	YES	158 (40.8%)	142 (57.5%)	16 (11.4%)		
ESBL_Amoxicillin	NO	176 (45.4%)	96 (38.9%)	80 (56.7%)	0.001	388
	YES	212 (54.6%)	151 (61.1%)	61 (43.3%)		
ESBL_Piperacillin	NO	55 (40.1%)	14 (56.0%)	41 (36.6%)	0.118	137
	YES	82 (59.9%)	11 (44.0%)	71 (63.4%)		
ESBL_Cefotaxime	NO	257 (93.5%)	127 (94.8%)	130 (92.2%)	0.535	275
	YES	18 (6.55%)	7 (5.22%)	11 (7.80%)		
ESBL_Ceftazidime	NO	109 (41.4%)	57 (46.7%)	52 (36.9%)	0.136	263
	YES	154 (58.6%)	65 (53.3%)	89 (63.1%)		
ESBL_Aztreonam	NO	122 (41.1%)	73 (46.8%)	49 (34.8%)	0.047	297
	YES	175 (58.9%)	83 (53.2%)	92 (65.2%)		
IC_Imipeneme	NO	289 (82.1%)	162 (76.8%)	127 (90.1%)	0.002	352
	YES	63 (17.9%)	49 (23.2%)	14 (9.93%)		
IC_Cefoxitin	NO	311 (80.4%)	185 (75.2%)	126 (89.4%)	0.001	387
	YES	76 (19.6%)	61 (24.8%)	15 (10.6%)		
IC_Ceftazidime	NO	109 (41.4%)	57 (46.7%)	52 (36.9%)	0.136	263
	YES	154 (58.6%)	65 (53.3%)	89 (63.1%)		

Overall, 75% of the phenotypes were associated with the farms. Moreover, they were often linked to the antibiotics used. This could be observed in ≈ 55% of cases.

DISCUSSION

The present study addressing phenotypic mechanisms of resistance in bacteria isolated from animal farms revealed a very high diversity which was, in some cases, associated with the farms. The 624 multidrug-resistant isolates submitted to the procedures were overwhelmed by members of the *Enterobacteriaceae* family (70%) *versus* 22% of Gram-positive cocci and 8% of non-fermenting Gram-negative rods. These rates were in agreement with previous reports and in connection with poor sanitation (Angulo *et al*., 2004; Souna, 2011). The *Enterobacteriaceae* family encompasses large groups of fermenting Gram-negative, non-spore-forming facultative anaerobes. In food and water microbiology their presence is associated with poor hygiene. In addition, they are potent IDs agents in farm animals.

Accordingly, the high rates in food and drinking water could help predict the roles they might play in animal health profile in these farms. Non-fermenting rods were almost limited to *Pseudomonas* (a strict aerobe) probably in connection with the restricted conditions for growth, unlike *Enterobacteriaceae*. As non-spore-forming Gram-negative bacteria however, moist environments are suitable for their survival and perpetuation, like *Enterobacteriaceae*. Obviously, environments in pig farms are moister than in poultries explaining, at least partially, the documented rates (62% and 36%, in pig farms and poultries, respectively). Gram-negative rods (*Pseudomonas* and *Enterobacteriaceae)* are known powerful engines for traits selection and dissemination that ensure fitness throughout the bacteria world (Bennett, 2008; Martínez and Baquero, 2014), consistent with their rates in the present survey, Bennett (2008) and, Martínez and Baquero (2014) about gene selection and spread which is favored by population density. These conditions are typically provided in farms and would theoretically therefore; facilitate genes transfer amongst both phylogenetically close and distant species which share similar or connected niches. In this regards, genotypes that

are selected in Gram-negative rods could easily disseminate not only to other Gram-negative, but also to Gram-positive bacteria which were predominated by *Staphylococci* and to a lesser extent *Streptococci* in the present investigation. The magnitude of how this extensive spread operates is yet to address, acknowledging that so far some phenotypes were thought to be restricted to genetically close bacteria (ESBL in *Enterobacteriaceae* and Methicillin resistance to *Staphylococcus*, for instance). Genus *Staphylococcus* is another large group of bacteria that encompasses both human and animal pathogens. Isolates were common from both pig farms and poultries, then, very likely to (in agreement with above discussions) have played key roles in resistance traits dissemination. This assertion is also consistent with the rates and diversity of phenotypic traits detected in the course of the present study, further supported by the fact that mobile genetic elements could carry gene sequences that encode several sets of phenotypic characteristics. This likely explains, therefore, not only advents of tolerance to several antibacterial agents conferring multiple resistance, but also expression of several mechanisms in the same isolate. According to previous findings (Pitout *etal*., 1998;Chong *et al*., 2011; Olowe *et al*., 2012; Singer *et al*., 2016) important factors such as pesticides in prophylaxis and antibiotics in growth supplementation in farms are thought to be critical in both cross- and co-resistance in line with related surveys (Simo Louokdom *et al*.,2018, Cheugoue Towo, 2018) on the use of antibacterial agents in these farms. Regardless of the amplitude, some other strains might play similar roles in line with the density-dependent gene transfer paradigm with the "tool set" theory, prevailing climatic conditions and human activities(Bennett, 2008; El Bakkouri *et al*., 2009; Martínez and Baquero, 2014; Spellberg *et al*., 2016). It is for these reasons that holistic epidemiology is necessary for the choice of appropriate-line antibacterial therapy. Or, in the context of the present work, probabilistic antibiotic therapy in animal and human typically builds on literature and recommendations of health authorities from other countries (Ministry of Health of the USA, CA-SFM, France, for instance) which hardly (if ever) match local realities, then likely to fail and exacerbate resistance selection.

Previous investigations demonstrated that segments of microbial DNA conferring resistance to drug could stochastically move amongst bacterial (Smith *et al*., 2013; Neyra *et al*., 2014). The most common bacteria isolates belonged to Gram-negative rods members of the *Enterobacteriaceae* family (70%) while two amongst the most commonly tolerated agents were Amoxicillin and Amoxicillin/Clavulanic-acid combination. These drugs and related ones were common in the study environment (Cheugoue Towo, 2018; Simo Louokdom*et al*., 2018). Seven resistance mechanisms were detected (several of which were expressed in the same isolate)at varying rates (3% with HLC through 67% with LLC), in agreement with the above one-step multiple gene-clusters transfer hypothesis. In this regards all *Serratia* expressed LLP while the highest ESBL rate was observed in *Salmonella* (84.6%). *Staphylococcus aureus* was also found to express ESBL (44%). Originally found in Gram-negative rods, ESBL phenotypes are more and more detected in this genus (Schaumburg *et al*., 2014; Fotsing Kwetché *et al*., 2015). Overall HLP, IRP and ESBL-expression appeared first, second and third most frequently detected (67%, 56%, and 50%, respectively). Previous studies (Lehner *et al.,* 2009) highlighted that beyond the higher risk of outbreaks, they are growing health challenges to clinicians who initiate patient caretaking, to scientists who are committed to developing cost-effective antimicrobial agents and the whole health system (Lehner *et al*., 2009). Nowadays, this challenge is exacerbated with globalization; substantiated in the present work by findings on *Staphylococcus* and Oxacillin (100% resistance). In fact, Oxacillin resistance infers resistance to several antibacterial agents and could be explained by the presence of several mechanisms in the same isolate as observed above. Induction of C_3G resistance by Imipenem/Cefoxitin was, for instance, sometimes associated with reduced Imipenem activity in the presence of AMC. In the same frame several isolates also associated ESBL expression and IC; further supporting the unpredictable cluster-gene transfer enabling the co- and cross-resistance. In *Enterobacteriaceae*, low level cephalosporinase expression was most frequently observed (56%) and might explain, at least in part, the high resistance rates to aminopenicillins' and to first-generation of cephalosporins. Also, IC/ESBL association was documented in 40% of Gram-negative rods that belonged to the *Enterobacteriaceae* group, in agreement with Fotsing Kwetché *et al*., (2015). Combination of mechanisms in the same isolate is a significant threat imposed by infectious agents as they are causes of the unpredictable therapeutic options faced by health authorities.

Moreover, the increasing high methicillin resistance rates in *Staphylococci* represent critical heal issues (Yilmaz *et al*., 2007). Therapeutic failure with Clindamycin is known to be in connection with multiple mechanisms that confer resistance to Macrolides, Lincosamides and Streptogramin B (MLSB) antibiotics. Findings from the present investigation indicated high D-test positivity that, in fact, also predicts higher likelihood of therapeutic failure with inappropriate combination therapy against *Staphylococcus* infections, just as the IC and ICl.

In line with the necessity to fight against resistance that may, not only cause heavy economic losses in farms but also spread in exposed human communities that are often characterized by resource limitation (Nguendo-Yongsi, 2011; Bhutta *et al*., 2013; Chopra *et al*., 2013; Kotloff *et al*., 2013; Walker *et al*., 2013), proper advocacy is needed for sustainable innovative initiatives towards achieving resistance stewardship in microorganisms. Efforts to prevent above threats build on the foundation of proven public health strategies that include immunization, infection control, protection of food and drug supply, effective caretaking by trained healthcare personnel and education on the critical related issues beyond antimicrobial resistance stewardship. The use of probiotics in animal husbandry should also be encouraged to limit that of antimicrobials in prophylaxis, therapy and growth promotion. This are key issues to address as the need is expressed from the current work's results in which almost all antibiotics used are associated with several resistance phenotypes. This should be in line with integrative policies and all stakeholders' commitment at both local and global levels in the current One Health spirit and principles. The main actors in this struggle will be the farmers because they are the primary beneficiaries. Future work will shade more light on how these mechanisms/phenotypes actually diffuse and affect caretaking in exposed human populations.

Conclusion

The present work revealed high bacterial diversity and high rates of bacterial resistance in animal farms; observed with

Gram-positive cocci, Gram-positive rods and Gram-negative rods. In addition, a large variety of resistance mechanisms were detected and thought to be connected with overall tolerance recorded. Altogether, these findings indicated that antibiotic therapy is seriously threatened and reiterated the need for integrative policies for advocacy and enforcement of antibiotic resistance stewardship program in animal husbandry.

Acknowledgements

This piece of work is a contribution to a large-scale program on food and water security thought and launched in farm by late Sitcheping Kuetche Claude, engineer in Water Facility to whom special tribute is paid here. The authors are also thankful to the "Association pour l'Education et le Développement (AED)" under of the UdM for logistic support through the Université des Montagnes.

Additional notice: *The authors declare that this work is devoid of any conflict of interest.*

REFERENCES

Angulo, F. J., Nargund, V. N., and Chiller, T. C. 2004. Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human Health consequences of such resistance. *Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health*, Vol. 51.Pp. 374–79.

Bennett, P. M. 2008. Plasmid encoded antibiotic resistance: Acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology,* Vol. 153: Pp. S347–57.

Bhutta Zulfiqar, A., Jai Das, K., Walker, N., Rizvi, A., Campbell, H., Rudan, I., *et al*. 2013. Interventions to address deaths from childhood pneumonia and diarrhoea equitably: what works and at what cost? *The Lancet*, 381(9875):1417–29.

Cataño, J. C., Echeverri, L. M., and Szela, C. 2012. Bacterial contamination of clothes and environmental items in a third-level Hospital in Colombia. *Interdisciplinary Perspectives on Infectious Diseases,* 1–6.

Cheugoue Towo, E. 2018. Etude de la résistance bactérienne aux antibiotiques dans les élevages de porcs et impact dans la communauté. [thèse]. Pharmacie. Université des Montagnes - Cameroun.

Chevalier, J., Mallea, M., Pagès, J. M. 2000. Comparative aspects of the diffusion of Norfloxacin, Cefepime and Spermine through the F Porin channel of *Enterobacter* Cloacae. *Biochem J.,* 348:223–27.

Chong Yong, Yoshikiyo Ito, and Tomohiko Kamimura. 2011. Genetic evolution and clinical impact in Extended-Spectrum β-Lactamase-producing Escherichia coli and *Klebsiella pneumoniae*. *Infection, Genetics and Evolution,* 11(7):1499–1504.

Chopra, M., Mason, E., Borrazzo, J., Campbell, H., Rudan, I., Liu, L., *et al*. 2013. Ending of preventable deaths from pneumonia and diarrhoea: An achievable goal. *The Lancet*, 381(9876):1499–1506.

CLSI. 2012. Clinical Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement.

Coates, Anthony Rm, Gerry Halls, and Yanmin Hu. 2011. Novel classes of antibiotics or more of the same? *British Journal of Pharmacology*, 163(1):184–94.

Cockerill, Franklin R. 2011. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement. Clinical and Laboratory Standards Institute (CLSI).

El Bakkouri, J., Belabbes, H., Zerouali, K., Belaiche, A., Messaouidi, D., Perrier Gros Claude J. D., *et al*. 2009. Résistance aux antibiotiques d*'Escherichia coli* uropathogène communautaire et consommation d'antibiotiques à Casablanca (Maroc). *European Journal of Scientific Research,* 36(1):49–55.

Fick Hindler, J., and Jorgensen, J. H. 2007. Antimicrobial Susceptibility Testing. In, Connie R. Mahon, Donald C. Textbook of Diagnostic Microbiology. 3rd edition. edited by G. M. Lehman. St. Louis: Saunders Elsevier.

Fotsing Kwetché, P. R., Simo Louokdom, J., Kamga. C., Kaba, K., and Kouamouo, J. 2015. Beta-Lactamase associated resistance phenotypes amongst multidrug resistant bacteria isolated in a school Hospital of West Cameroon*. Integrated Journal of British,* 2(8):1–70.

Inweregbu, K., Jayshree, D., and Pittard, A. 2005. Nosocomial Infections. Continuing Education in Anaesthesia, *Critical Care & Pain* 5(1):14–17.

Kotloff, K. L.,. Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., *et al.* 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, Case-Control Study. The Lancet, 382(9888):209–22.

Lehner, S., Grabein, B., Pfaller, P., and Kopp, R. 2009. Relevance of ESBL-Producing Pathogens for Clinical Surgery: Diagnostics, Therapy, and Prevention. Der Chirurg; Zeitschrift Fur Alle Gebiete Der Operativen Medizen, 80(6):527–36.

Levy, S. B. 1998. The Challenge of Antibiotic Resistance. *Scientific American*, 278(3):46–53.

Martínez, J. L. and Baquero, F. 2014. Emergence and spread of antibiotic resistance: setting a parameter space. *Upsala Journal of Medical Sciences*, 119(2):68–77.

Martins, Ana, Attila Hunyadi, and Leonard Amaral. 2013. Suppl 1: Mechanisms of resistance in bacteria: an evolutionary approach. *The Open Microbiology Journal*, 7:53.

Neyra, R. C., Frisancho, J. A., Jessica, L., Rinsky, C. R., Colleen Carroll, K., Rule, A. M., *et al.* 2014. Multidrug-resistant and Methicillin-resistant Staphylococcus aureus (MRSA) in hog slaughter and processing plant workers and their community in North Carolina (USA). *Environmental Health Perspectives,* 122(5):471.

Nguendo-Yongsi, H. B. 2011. Microbiological evaluation of drinking water in a Sub-saharan urban community (Yaoundé). *Am j Biochem and Mol Bio.,* 1(1):68–81.

Olowe, O. A., Oladipo, G. O., Makanjuola, O. A. and Olaitan, J. O. 2012. Prevalence of Extended Spectrum Beta-Lactamases (ESBL) Carrying Genes in *Klebsiella* spp from Clinical Samples at Ile-Ife, South Western Nigeria. *International Journal of Pharma & Bio Sciences,* 1(2).

Piéboji Gangoué, J., Koulla-Shiro, S., Ngassam, P., Adiogo, D., Njine, T., and Ndumbe, P. 2004. Antimicrobial resistance of Gram-negative *Bacilli* isolates from Inpatients and Outpatients at Yaoundé Central Hospital, Cameroon. *International Journal of Infectious Diseases,* 8(3):147–54.

Pitout, J. D. D, Thomson, K. S., Hanson, N. D., Ehrhardt, A. F., Moland E. S., and Sanders, C. C. 1998. β-Lactamases responsible for resistance to Expanded-spectrum Cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa., 42(6):1350–54.

Schaumburg, Frieder, Alabi,A. S., Frielinghaus, L., Grobusch, M. P., Köck, R., Karsten Becker, Saadou Issifou, Kremsner, P. G., Georg, P., and Mellmann, A. 2014. The risk to import ESBL-Producing *Enterobacteriaceae* and *Staphylococcus aureus* through chicken meat trade in Gabon. *BMC Microbiology*, 14(1):286.

Simo Louokdom, J., Fotsing Kwetché, P. R., Yawat Djogang, A. M., Gamwo Dongmo, S., Nankam Nguekap, W. L., Tchoukoua, S. H., *et al*. 2018. Antibiotic Susceptibility / Resistance profile of bacteria from farm wastes : findings in excreta from four poultries of West Cameroon. *World Journal of Advance Healthcare Research*, 2(4):213–21.

Singer, A. C., Shaw, H., Rhodes, V., and Hart, A. 2016. Review of antimicrobial resistance in the environment and Its relevance to environmental regulators. *Frontiers in Microbiology,* 7:1728.

Smith, T. C., Wondwossen Gebreyes, A., Abley, M. J., Harper, A. L., Forshey, B. M., Male, M. J., H. *et al.* 2013. Methicillin-resistant Staphylococcus aureus in Pigs and Farm Workers on conventional and antibiotic-free swine farms in the USA. *PLoS* One, 8(5):e63704.

Souna, D. 2011. Epidémiologie de la résistance aux antibiotiques des entérobactéries au niveau du CHU de Sidi Bel Abbes.

Spellberg, B., Hansen, G. R., Kar, A., Cordova, C. D., Price, L. B., and Johnson, J. R. 2016. Antibiotic Resistance in Humans and Animals. *National Academy of Medicine*,1-15.

Walker, C. L., Fischer, I. R., Li, L., Harish, N., Evropi, T., Zulfiqar, A. B., *et al.* 2013. Global Burden of Childhood Pneumonia and Diarrhoea. The Lancet 381(9875):1405–16.

Weinstein, R. A. 1998. Nosocomial Infection Update. *Emerging Infectious Diseases*, 4(3):416.

WHO. 2015. Antimicrobial Resistance Fact Sheet No. 194.

World Health Organization (WHO). 2002. WHO Traditional Medicine Strategy 2002-2005. *World Health Organisation* Geneva, 1–74.

Yilmaz, G., Aydin, K., Iskender,S., Caylan, R., and Koksal, I. 2007. Detection and Prevalence of Inducible Clindamycin Resistance in *Staphylococci*. *Journal of Medical Microbiology,* 56(3):342–45.

Zechini, B., Versace, I. 2009. Inhibitors of Multidrug Resistant Efflux Systems in Bacteria. *Recent Patents Anti-Infect Drug Discov.,* 4:37–50.

****