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RESEARCH ARTICLE

ANTIBIOTIC SUSCEPTIBILITY PROFILE OF BACTERIA FROM FARM WASTES: FINDINGS IN CHICKEN EXCRETA, FOOD AND WATER FROM FOUR POULTRIES *VERSUS* TREND IN A NON-EXPOSED COMMUNITY OF WEST CAMEROON

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ABSTRACT

The present survey addressed the trend of bacterial resistance to antibacterial agents in poultry and community connects. It was initiated to gather necessary information on bacterial communities in farm waste, animal food and water; as well as the types of antimicrobial agents used with susceptibility/resistance profile to common antibiotics. Chicken excreta, food and water collected from four farms in Bafoussam and Bandjoun (neighbourhoods in the Western Region of Cameroon) underwent microbiological analyses according to standard protocols. The overall picture indicated that all items submitted to laboratory screening were contaminated. Most common bacteria isolates belonged to the *Enterobacteriaceae* family, genera *Bacillus* and *Staphylococcus.* Members of these groups are known to be engines for resistance traits selection and dissemination and might become dreadful aetiologies of zoonotic infections. A closer look revealed that in 72% of cases, a variety of fluoroquinolones were used in the farms, contrasting with Tetracycline and Nitrofurans that were less common (14%, each). Tolerance was common with some antibacterial agents that belonged to the large groups of quinolones (Nalidixic acid), beta-lactams (Aztreonam, Amoxicillin, Oxacillin), Erythromycin and Co-trimoxazole. Further insight through data from farms and the community highlighted subtle difference amongst bacterial populations and resistance rates to fluoroquinolones ($P < 0.001$), just as site-specific tendency ($P < 0.05$). Interestingly, the farmers acknowledged the threat that resistance might pose to their activity. Both the isolation and resistance rates could help anticipate the heavy economic burden that farm-related infections might generate. Biological alternatives to antimicrobials in farms were therefore thought to be primordial and feasible with the farmers as the primary human resources for the task.

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INTRODUCTION

Infectious diseases are recognized leading causes of morbidity and mortality worldwide. They ravaged millions of lives across the globe prior Louis to Pasteur and Robert Koch works that disclosed and highlighted the connection between microorganisms and deadly infectious conditions in the 1865-1870s. Microbiology then emerged as scientific discipline and opened ways for investigations through etiologic and antimicrobial agents. The discovery of Penicillin by Fleming and its introduction in human medicine (1928-1939s) were criticalsteps in the control of diseases caused by microorganisms, especially bacteria.

This success was demonstrated during the Second World War (1939-1945) when its use reduced the number of related deaths amongst conflict's victims. This successful experience was also rapidly followed by detection of Penicillin-tolerant bacteria isolates and tolerance to other antibacterial agents that were developed and introduced in human medicine subsequently (Barber, 1961). Bacterial resistance developed and became a major public health threat in the 1980s when extended spectrum antibiotic-hydrolyzing enzymes (constitutively expressed or induced by other environmental factors) were described. Extended spectrum beta-lactamases were then thought to be associated with Gram-negative rods,

especially from the *Enterobacteriaceae* family of bacteria (Aubry-Damon *et al.,* 2005; Simo Louokdom *et al*., 2016). Soon after they were shown to encompass several variants which could undergo horizontal transfer amongst phylogenetically close and distant bacteria species (Schaumburg *et al.,* 2014; Fotsing Kwetché *et al*., 2015), including non-pathogens that may become dreadful resistant opportunistic; then serious threat to human and animal health. Accordingly, current major threats associated with infectious disease appear to have deviated from the inherent ability of microorganisms to cause disease (as professional pathogens do), to become, in line with investigations through drug-resistance, that of larger variants of microorganisms most of which are typically opportunistic. Nowadays, bacterial infections are difficult to control because they are costly and diversified, in connection with the bacterial species and strains in cause.

This is further exacerbated by the flexibility of the bacterial genome and the use of several human-driven engines of resistance genotypes/phenotypes selection and dissemination. These phenomena are favoured by many factors that include international travelling and aggravated poverty observed in several parts ofthe world. These factors often appear as causes and consequences of war, famine and unfair national and international trade agreements that come with globalization and other complex enabling factors (Perugini *et al*., 2005; Planta, 2007). Otherwise, low living standards and poor hygiene are paramount determinants for stochastic changes that occur amongst bacteria in mixed microbial populations in all ecosystems (World Health Organization, 2000). Antimicrobial resistance is a natural phenomenon that develops to improve the microbial population fitness. It had, however, been exacerbated by human activities related with human, animal, plant health and industrialization (Aubry-Damon *et al*., 2005; Perugini *et al*., 2005; Planta, 2007; Courvalin, 2008; Segerman, 2012; Magill *et al*., 2015). The role of animal husbandry in microbial resistance growth is consistently alleged (Aubry-Damon *et al*., 2005; Zhou *et al*., 2015), but poorly investigated in resource-limited areas, though attributed to inappropriate use of anti-infectious agents in human medicine (Schaumburg *et al.,* 2014, Fotsing Kwetché *et al*., 2015, Simo Louokdom *et al*., 2016). Its real impact would help appreciate the amplitude of the threat in order to enforce necessary policies in infection prevention and management. The present survey was initiated to appreciate the extend of bacterial resistance that develops in poultry and the potential role of antimicrobial agents used by farmers. Investigations provided pieces of information on bacterial communities in a few farms, the types of antimicrobial agents used and the trends of bacterial susceptibility/resistance to antibacterial agents in farm compared with the one recorded in a remote human community. In the short run, these findings could help appreciate the risk linked to the use of antimicrobial agents in animal breeding environments, and anticipate ABR-mitigation policies in the global policy which aims to optimize production outputs in farms. In the intermediate and long run, they will serve in the on-going ABR stewardship program in the frame of the global struggle against drug-resistant infections burden and in poverty alleviation.

MATERIAL AND METHODS

Study Site, study population and ethical consideration: The West Region of Cameroon is the most important basin for poultry activity in the Central Africa sub-region. Also regarded as one of the most densely populated area in Cameroon, its population is firmly attached to wide ranges of agro-pastoral activities. Sampling was performed in Bafoussam and Bandjoun; two semi-urban neighbourhoods in which inhabitants share socio-economic determinants like beliefs, individual and other intercommunity social values. Laboratory screening was conducted in the Laboratory of Microbiology of the Université des Montagne's Teaching Hospital under authorization reference N°: 2017/0104/CUM/ADM issued by the institution Head.

Field data collection, specimen collection and bacteria isolation

From December 27th, 2017 through March 15th, 2018, the present descriptive experimental study was conducted in Bandjoun and Bafoussam were chicken excreta (manure), animal drinking water; food were collected in four poultry farms. Through questionnaires, several pieces of information on farm management were recorded after the farmer's consent. All specimens were collected according to standard procedures in small, clean, sterile plastic bags, and then conveyed immediately to the Laboratory for investigations. Two series of fingerprinting were also performed. The first involved farmers, their co-workers and members of the farm vicinity. The second was done in a remote human community that obviously did not interact directly with farm environments for primary profile comparison. Five fingers of each hand were printed on culture media in the 60 mm-Petri dishes prepared on the eve. All cultures, isolations and identifications were conducted according to standard guidelines (REMIC, 2017). Briefly, isolation was performed on Liofilchem® agar namely Mannitolsalt, McConkey, Hektoen, Muller Hinton with 5% sheep blood. Additional identifications procedures were completed according to standard morphological, biochemical and enzymatic profile guidelines as recommended by the same reference repository (REMIC, 2017).

Antibacterial Susceptibility profile investigation

Bacterial Susceptibility/resistance profiles screening were performed by disk diffusion (Kirby-Bauer) with 22 conventional antibacterial agents chosen from drug families amongst the most commonly used in Cameroon in both human medicine and animal husbandry. The tests were conducted on 24 h bacterial pure culture obtained by streaking isolates on fresh nutrient agar and incubating the preparation at 37 °C. From the resulting bacterial population, a suspension to the density of a McFarland 0.5 turbidity standard prepared in 0.9% physiological saline was adjusted to the final opacity recommended for susceptibility tests by agar diffusion technique on Mueller Hinton agar. All test procedures and interpretations were performed according to the standard guidelines recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM, 2016). The antibiotic disks tested included: Penicillin (10µg), Imipenem (10µg), Cefotaxime (5µg), Amoxicillin/clavulanic acid (20/10µg), Amoxicillin (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Nitrofurantoin (300µg), Cefoxitin (30µg), Cefuroxime (30µg), Gentamicin (120µg), Vancomycin (30µg), Ciprofloxacin (30µg), Nalidixic acid (30µg), Trimethoprim/ Sulfamethoxazole (1.25/23.75 µg), Oxacillin (1µg), Erythromycin(15µg), Norfloxacin (30 µg), Levofloxacin (30µg), Tetracycline (30µg), Aztreonam (30µg) and Clindamycin (2µg). Reference bacterial strains used in quality

control for identification and susceptibility tests were *Staphylococcus aureus* QC1625, *Escherichia coli* ATCC 25922 and *E. faecalis* ATCC 29212.

RESULTS

From January 3rd through April 7th 2018, 306 specimens of chicken excreta (manure), chicken food and drinking water were collected from four farms. The farmers and their collaborators on the spot also filled four related survey forms. In the farms and their neighbourhood (nearby-communities), 123 worker's finger printing were conducted *versus* 80 in a remote community. An insight through questionnaire data indicated that 75% of responders were male. All of these had attended university studies. On the other hand, 25% were female whose highest education level was the secondary school. All farmers used water, Cresyl bleach and a variety of antiseptics solutions for routine disinfection at the entry of the breeding perimeters. For infectious disease prevention in the farm, they also adopted routine cleaning and antimicrobials (antibiotics 80%; antiseptic 20%). The use of these antimicrobials was 50% of the times done by direct on-farm spraying, 50% as cleaning solutions and, 100% in all farms by direct administration to animals through beverages. The antibiotics used included Flumequine, Furaltadone and Oxytetracycline (systematically used by all farmers); Norfloxan, Enrofloxacin, Ciprofloxacin and Norfloxon (used as the second-line in case of outbreak), administered in animal's drinking water for three to five days depending on the intended goals (prevention or outbreak management).

In all farms, animal selection was performed by the clients who otherwise had free access into the breeding areas, then in direct contact with resident animals. The main sources of water used were either the wells or pipes. On the role of antimicrobial agents in farms, 75% farm staff acknowledged its benefits in animal protection against out breaks but also recognized that microbial resistance development might be due to inappropriate drug use. They were also aware of the danger the poor use of antibacterial agents might represent for the farm animals and human communities that depend on the products and services from the farms. Further details on antibacterial agents used indicated that they belonged to three pharmaceutical categories (Table I). A closer look revealed that in 72% of cases, a variety of fluoroquinolones was used, contrasting with Tetracycline and Nitrofurans that wereless common (14% of times, each).

Specimens and contamination rates in farms

Specimens in farms

Specimens subjected to laboratory screening comprised 237 excreta, 49 foods, 20 drinking water and 123 fingerprinting performed in the farm vicinity. Further related pieces of information were reorganized as displayed in Table 2. Globally, manure overwhelmed the material submitted to laboratory analyses (55%), followed by fingerprinting and animal food. Most specimens were collected from Bandjoun 1.

Contamination rates and bacterial communities in farms

None of the specimens was sterile at all points of view. Further pieces of information on contamination rates recorded from screening of the 429 products were reorganized and displayed as shown in Table 3. Overall, in farms 394 bacterial isolates were recovered at various rates. The highest onewas that of the Gram-positive bacteria (62%) that included primarily *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp. and *Clostridium* spp.; while Gram-negative consisted of members of the *Enterobacteriaceae* family and *Pseudomonas.* Moreover, in decreasing order, excreta and fingerprinting analyses yielded the highest isolation rates. Overall and regardless of the locations/farms outstanding picture revealed that *Bacillus, Citrobacter, Enterobacter, Proteus, Staphylococcus* were most common.

Table 1. Antibacterial agents used in farms

Antibiotics types	Pharmacological category
Furaltadone	Nitrofuran
Oxytetracycline	Tetracycline
Flumequin	Fluoroquinolone
Norfloxan	Fluoroquinolone
Enrofloxacin	Fluoroquinolone
Ciprofloxacin	Fluoroquinolone
Norfloxon	Fluoroquinolone

Table 2. Distribution of specimens per type and per farm

Location	Specimen type N (%)				Total
	Feeds	Drinking water	Finger printing	Excreta	
BAFOUSSAM 1	25 (24)	10 (9)	24 (23)	47 (44)	106
BAFOUSSAM 2	1 (1)	0 (0)	27 (24)	83 (75)	111
BANDJOUN 1	23 (13)	10 (6)	47 (28)	92 (53)	172
BANDJOUN 2	0(0)	0 (0)	25 (62)	15 (38)	40
Total	49 (11)	20 (5)	123 (29)	237 (55)	429

Frequency of contamination and bacterial community in the test remote community

Specimen's analysis from the 80 participants enrolled in the test community for fingerprinting resulted in 52 positive cultures (65 %contamination rate) (Table 4). The overall picture disclosed seven bacterial types categorized into three major groups: Gram-negative rod, Gram-positive rod and Gram-positive cocci. The most diversified group was that of gram-negative rods, encompassing essentially members of the *Enterobacteriaceae* family of bacteria (35%), while Gram-positive cocci (47%) were overwhelmed by *S. Aureus* (35%).

Table 3. Bacterial type per location and specimen

Location	Bacterial type	Specimen type N (%)				Total
		Feeds	Drinking water	Finger Printing	Stools	
BAFOUSSAM 1	*Bacillus* spp	2 (20)	1 (10)	5 (50)	2 (20)	10
	Clostridium spp	0 (0)	0 (0)	2 (67)	1 (33)	3
	Enterobacter aerogenes	1 (14)	3 (43)	0 (0)	3 (43)	7
	Enterobacter hafnia	11 (41)	6 (22)	0 (0)	10 (37)	27
	Escherichia coli	3 (50)	0 (0)	0 (0)	3 (50)	6
	Proteus spp	4 (67)	0 (0)	0 (0)	2 (33)	6
	Shigella spp	0 (0)	0 (0)	0 (0)	3 (100)	3
	Staphylococcus aureus	4 (13)	0 (0)	14 (44)	14 (44)	32
	Streptococcus spp	0 (0)	0 (0)	3 (60)	2 (40)	5
	Total	25 (25)	10 (10)	24 (24)	40 (40)	99
BAFOUSSAM 2	*Bacillus* spp	0 (0)	0 (0)	4 (29)	10 (71)	14
	Citrobacterfreundi	0 (0)	0 (0)	0	4(100)	4
	Clostridium spp	0 (0)	0 (0)	3 (60)	2 (40)	5
	Enterobacter aerogenes	0 (0)	0 (0)	0 (0)	3 (100)	3
	Enterobacter hafnia	1 (9)	0 (0)	0 (0)	10 (91)	11
	Escherichia coli	0 (0)	0 (0)	0 (0)	3 (100)	3
	*Proteus*spp	0 (0)	0 (0)	0 (0)	11(100)	11
	Pseudomonas spp	0 (0)	0 (0)	0 (0)	1 (100)	1
	*Shigella s*pp	0 (0)	0 (0)	0 (0)	1(100)	1
	Staphylococcus aureus	0 (0)	0 (0)	15 (39)	24 (61)	39
	Streptococcus spp	0 (0)	0 (0)	5 (71)	2 (29)	7
	Total	1 (1)	0 (0)	27 (27)	71 (72)	99
BANDJOUN 1	*Bacillus* spp	0 (0)	0 (0)	14 (70)	6 (30)	20
	Citrobacter freundi	4 (44)	1 (11)	0 (0)	4 (44)	9
	Clostridium spp	0 (0)	0 (0)	6 (75)	2 (25)	8
	Enterobacter aerogenes	10 (59)	0 (0)	0 (0)	7 (41)	17
	Enterobacter hafnia	6 (33)	1 (6)	0 (0)	11 (61)	18
	Escherichia coli	1 (13)	2 (25)	0 (0)	5 (63)	8
	Proteus spp	0 (0)	5 (33)	0 (0)	10 (67)	15
	Staphylococcus aureus	2 (4)	1 (2)	17 (33)	31 (61)	51
	Streptococcus spp	0 (0)	0 (0)	10 (83)	2 (17)	12
	Total	23 (15)	10 (6)	47 (30)	78 (49)	158
BANDJOUN 2	*Bacillus* spp	0 (0)	0 (0)	10 (83)	2 (17)	12
	Citrobacter freundi	0 (0)	0 (0)	0 (0)	2 (100)	2
	Clostridium spp	0 (0)	0 (0)	2 (50)	2 (50)	4
	Staphylococcus aureus	0 (0)	0 (0)	8 (53)	7 (47)	15
	Streptococcus spp	0 (0)	0 (0)	5 (100)	0 (0)	5
	Total	0 (0)	0 (0)	25 (66)	13 (34)	38

Table 4. Distribution of isolates recovered from fingerprinting in the community

Bacterial type	Number of isolates	(%)
Bacillus spp	10	19
Citrobacter freundii	2	4
Enterobacter aerogenes	8	15
Enterobacter hafnia	6	12
Proteus spp.	2	4
Staphylococcus aureus	18	35
Streptococcus spp	6	12
Total	52	100

Antibiotic susceptibility profile

Susceptibility profile in farm's and farm vicinity's isolates

Susceptibility/resistance profiles per major bacteria groups and isolation sites were displayed as shown in Table 5. Overall, the susceptibility rates were very low with drugs from the pharmacological groups of quinolones (namely Ciprofloxacin, Norfloxacin, Nalidixicacid, Levofloxacin) in all farms. These overall low susceptibility rates were also recorded with Tetracycline, Co-trimoxazole, Penicillin, Cefuroxime and Oxacillin. Thoroughly closer rate values were also observed from one farm to the other with the same antibiotics. Susceptibility to Amoxicillin/clavulanic acid, Levofloxacin, Imipenem was globally high.

Susceptibility profile of bacterial isolates from worker's hands

Focus on susceptibility profiles of bacteria from the hand of workers at farm entries and exits further yielded data summarized in Table 6.

Typically, data recorded revealed very low susceptibility rates with some antibiotics namely Co-trimoxazole, Nalidixic acid, Ceftriaxone, Ceftazidime and Oxacillin. Concerning Norfloxacin, Clindamycin, and Levofloxacin these rates were not as low, while improved susceptibility was observed with Gentamicin, Amoxicillin-Clavulanic acid, Imipenem, Ciprofloxacin and Cefoxitin. Slight variation could be observed between entries and exits. With a few exceptions, fingerprinting's in/out data revealed overall similar pictures, however. The most frequent intermediate categories were obtained with Vancomycin in Bandjoun for both Gram-positive cocci and Gram-positive rods.

Bacteria susceptibility/resistance in the test community

The 52 isolates recovered and categorized as Gram-negative rods (≈35%), Gram-positive rods (≈19%) and Gram-positive cocci (≈46%) tested as done above with isolates from the farm yielded the trends summarized as shown in Table 7.

Table 5. Susceptibility/resistance profile of isolates from Bafoussam and Bandjoun

Antibiotics	Phenotype	BAFOUSSAM 1			BAFOUSSAM 2			BANDJOUN 1			BANDJOUN 2		
		Isolates (%)			Isolated (%)			Isolated strains (%)			Isolated strains (%)		
		GN rods	GP rods	GP cocci	GN rods	GP rods	GP cocci	GN rods	GP rods	GP cocci	GN rods	GP rods	GP cocci
Vancomycine	I	0	20	47	0	42	60	0	25	31	20	25	29
	R	100	40	32	0	16	24	0	25	20	50	50	42
	S	0	40	21	0	42	16	100	50	49	30	25	29
Gentamicine	I	22	17	10	3	17	8	8	25	17	0	0	0
	R	18	0	35	15	17	8	25	50	33	0	0	29
	S	60	83	55	82	66	84	67	25	50	100	100	71
Ciprofloxacin	I	2	0	10	15	0	0	11	0	0	0	0	0
	R	41	50	70	44	58	65	36	50	69	100	50	86
	S	57	50	20	41	42	35	53	50	31	0	50	14
Trimethoprim/sulfa methoxazole	I	0	0	0	0	0	6	0	0	3	0	0	0
	R	71	25	82	45	50	64	60	75	92	100	25	100
	S	29	75	18	55	50	30	40	25	5	0	75	0
Nalidixic acid	I	10	17	0	6	8	3	0	12	8	0	0	0
	R	45	50	65	50	50	70	60	63	75	100	25	100
	S	45	33	35	44	42	27	40	25	17	0	75	0
Norfloxacin	I	6	0	0	0	0	0	19	0	0	0	0	0
	R	31	50	79	50	42	62	30	50	88	100	75	86
	S	63	50	21	50	58	38	51	50	12	0	25	14
Erythromycin	I	11	0	30	12	0	0	2	0	0	0	0	0
	R	78	100	0	38	67	33	77	0	100	100	0	0
	S	11	0	70	50	33	67	21	100	0	0	0	0
Clindamycin	I	14	0	33	0	22	25	0	0	0	15	0	0
	R	57	50	45	0	22	17	50	86	52	58	50	71
	S	29	50	22	100	56	58	50	14	48	27	50	29
Nitrofurantoin	R	19	0	0	41	0	13	66	0	50	0	0	0
	S	81	100	1	59	0	87	34	100	50	100	0	0
Levofloxacin	I	23	0	0	NT	NT	NT	7	0	0	NT	NT	NT
	R	10	25	67	NT	NT	NT	43	75	53	NT	NT	NT
	S	67	75	33	NT	NT	NT	50	25	47	NT	NT	NT
Amoxicilline/ clavulanic acid	I	0	17	5	0	0	0	0	0	6	0	25	0
	R	57	0	5	15	0	0	43	12	6	0	25	14
	S	43	83	90	85	100	100	57	88	88	100	50	86
Imipenem	I	0	0	0	NT	NT	NT	3	0	0	NT	NT	NT
	R	0	0	0	NT	NT	NT	55	14	36	NT	NT	NT
	S	100	100	100	NT	NT	NT	42	86	64	NT	NT	NT
Oxacillin	R	100	100	100	100	83	96	86	100	91	0	100	100
	S	0	0	0	0	17	4	14	0	9	0	0	0
Penicillin G	I	NA	0	0	NA	0	0	NA	0	0	NA	0	0
	R	NA	75	83	NA	67	48	NA	86	47	NA	100	43
	S	NA	25	17	NA	33	52	NA	14	53	NA	0	57
Cefoxitine	I	6	0	0	0	100	4	0	0	0	0	0	0
	R	33	0	6	19	8	4	49	86	21	0	0	14
	S	61	100	94	81	92	92	51	14	79	100	100	86
Aztreonam	I	0	0	0	67	0	0	38	25	14	NT	NT	NT
	R	46	0	100	33	0	0	12	75	72	NT	NT	NT
	S	54	100	0	6	0	100	50	0	14	NT	NT	NT
Cefuroxime	I	31	20	50	6	100	50	16	50	20	0	0	70
	R	59	50	0	58	0	0	59	0	50	100	100	10
	S	10	30	50	36	0	50	25	50	30	0	0	20
Ceftriaxone	I	0	0	0	0	0	6	8	0	0	0	0	0
	R	67	100	76	87	100	94	52	86	85	100	100	100
	S	33	0	24	13	0	0	40	14	15	0	0	0
Amoxicilline	I	17	33	40	0	0	38	1300	50	33	0	100	33
	R	70	34	0	32	0	12	73	0	50	100	0	50
	S	13	33	60	68	0	50	14	50	17	0	0	17
Ceftazidime	I	3	25	11	100	0	17	0	0	0	0	0	0
	R	22	75	68	0	100	83	54	86	85	100	100	100
	S	75	0	21	0	0	0	46	14	15	0	0	0
Tetracycline	I	0	0	0	6	8	7	0	0	0	0	0	0
	R	53	80	85	72	67	78	80	100	100	100	100	100
	S	47	20	15	22	25	15	20	0	0	0	0	0
Cefotaxime	I	0	0	0	6	10	10	0	0	0	10	0	0
	R	0	0	36	6	10	0	0	0	10	10	10	10
	S	100	100	64	88	80	90	100	100	90	80	90	90

GN rods: Gram-negative rods (*Enterobacteriaceae* and *Pseudomonas);* GP rods: Gram-positive rods (*Clostridium* and *Bacillus)*; GP Cocci: Gram-positive Cocci (*Staphylococcus* and *Streptococcus)* NT: Not tested; NA: Not applicable

Globally, these data indicated low susceptibility for some antibacterial agents that belonged to the large groups of quinolones (Nalidixic acid), beta-lactams (Aztreonam, Amoxicillin, Oxacillin), Erythromycin and Co-trimoxazole.

In the same vein, improved susceptibility was recorded with Gentamicin, Amoxicillin-Clavulanic Acid combination, Imipenem, Ciprofloxacin and Norfloxacin. Further insight through data from farms and the community highlighted subtle differences between the two settings.

Table 6. Susceptibility profile of bacterial isolates from worker's hands

Antibiotics	Phenotypes	BAFOUSSAM 1 Way in/Out		BAFOUSSAM 2 Way in/Out		BANDJOUN 1 Way in/Out		BANDJOUN 2 Way in/Out	
		Isolated strains (%)		Isolated strains (%)		Isolated strains (%)		Isolated strains (%)	
		GP rods	GPcocci	GP rods	GP cocci	GP rods	GP cocci	GP rods	GPcocci
Vancomycine	I	0/40	22/63	50/40	31/86	71/31	81/9	80/71	63/60
	R	50/20	44/0	0/20	23/14	29/23	13/18	20/14	25/40
	S	50/40	33/37	50/40	46/0	0/46	6/73	0/14	13/0
Gentamicine	I	50/20	11/0	50/20	38/0	0/0	6/9	0/29	0/0
	R	0/40	22/12	0/0	23/0	14/8	0/0	20/29	0/20
	S	50/40	67/88	50/80	38/100	86/92	94/91	80/42	100/80
Ciprofloxacin	R	0/60	78/75	0/0	77/14	14/0	6/9	40/71	25/60
	S	100/40	22/25	100/100	23/86	86/100	94/91	60/29	75/40
Trimethoprim/sulfamethoxaz	I	0/0	11/0	0/0	0/0	0/0	0/0	0/0	0/0
ole	R	50/100	67/75	50/60	85/43	100/77	69/82	100/57	75/80
	S	50/0	22/25	50/40	15/57	0/23	31/18	0/43	25/20
Nalidixic acid	I	50/0	22/13	50/20	8/0	0/0	0/0	0/14	13/0
	R	50/100	67/63	50/60	69/100	100/92	81/73	100/57	75/100
	S	0	11/25	0/20	23/0	0/8	19/27	0/29	13/0
Norfloxacin	R	50/40	44/25	50/0	69/14	29/31	25/27	40/43	25/60
	S	50/60	56/75	50/100	31/86	71/69	75/73	60/57	75/40
Erythromycin	R	NT/NT	15/0	70/60	85/86	90/100	100/100	NT/NT	NT/NT
	S	NT/NT	15/100	30/40	15/14	10/0	0/0	NT/NT	NT/NT
Clindamycin	I	0/0	0/0	0/0	0/0	14/15	0/0	0/29	13/20
	R	100/40	44/25	0/60	54/14	43/38	56/64	60/14	50/20
	S	0/60	56/75	100/40	46/86	43/42	44/36	40/57	38/60
Levofloxacin	R	100/20	33/75	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
	S	0/80	67/25	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT	NT/NT
Amoxicilline/ clavulanic	I	0/0	11/0	0/0	0/29	0/0	0/0	0/0	0/0
acid	R	0/0	0/12	0/40	15/0	29/23	19/0	0/0	13/0
	S	100/100	89/88	100/60	85/71	71/77	81/100	100/100	88/100
Imipenem	I	0/0	0/0	0/20	0/29	0/0	0/0	0/0	0/0
	R	50/0	0/0	50/20	46/0	0/0	6/9	0/29	13/60
	S	50/100	100/100	50/60	54/71	100/100	94/91	100/71	88/40
Oxacillin	R	100/80	89/75	100/60	92/57	100/100	100/100	100/71	88/80
	S	0/20	11/25	0/40	8/43	0/0	0/0	0/29	12/20
Penicillin G	R	100/40	56/50	50/60	31/57	57/54	69/82	40/71	75/60
	S	0/60	44/50	50/40	69/43	43/46	31/18	60/29	25/40
Cefoxitine	R	50/0	11/12	50/60	38/43	29/15	13/18	0/14	25/0
	S	50/100	89/88	50/40	62/57	71/85	88/82	100/86	75/100
Aztreonam	I	5/0	0/0	0/0	0/0	0/23	0/9	0/NT	0/0
	R	85/80	89/88	50/60	50/71	100/69	88/73	60/NT	66/100
	S	10/20	11/12	50/40	50/29	0/8	13/18	40/NT	34/0
Cefuroxime	I	NT/NT	NT/NT	0/40	26/70	10/15	100/18	NT/NT	0/7
	R	NT/NT	NT/NT	50/60	26/30	70/77	0/82	NT/NT	100/80
	S	NT/NT	NT/NT	50/0	48/0	20/8	0/0	NT/NT	0/13
Ceftriaxone	I	0/0	0/0	50/20	0/14	14/0	6/0	0/0	0/20
	R	50/100	78/75	50/60	69/86	86/100	94/100	100/86	88/80
	S	50/0	22/25	0/20	31/0	0/0	0/0	0/14	12/0
Amoxicilline	R	NT/NT	100/100	70/80	50/29	88/92	76/82	NT/NT	0/10
	S	NT/NT	0/0	30/20	50/71	12/8	24/18	NT/NT	100/90
Ceftazidime	I	0/40	22/12	50/20	0/29	14/23	19/9	0/5	12/0
	R	100/60	78/63	50/60	92/42	86/77	81/91	80/80	88/100
	S	0/0	0/25	0/20	8/29	0/0	0/0	20/15	0/0
Tetracycline	I	50/62	NT/NT	0/20	8/14	NT/NT	0/0	20/14	5/0
	R	50/38	NT/NT	50/80	46/43	NT/NT	92/100	75/86	82/80
	S	0/NT	NT/NT	50/0	46/43	NT/NT	8/0	5/0	13/20
Cefotaxime	I	10/7	7/5	400/150	10/10	14/4	6/0	0/4	0/10
	R	50/80	80/70	50/75	72/85	80/96	90/100	95/86	80/80
	S	40/13	13/25	10/10	18/5	6/0	4/0	5/10	20/10

GP rods: Gram-positive rods grouping *Clostridium* and *Bacillus*. GP Cocci: Gram-positive Cocci grouping *Staphylococcus* and *Streptococcus*

First, the significant difference amongst bacterial populations ($P < 0.001$); second the resistance rates shown not to be site-specific (location-specific) ($P < 0.05$), and widely spread to fluoroquinolones (Ciprofloxacin and Norfloxacin) differed in magnitudes for both settings ($P < 0.001$).

DISCUSSION

The core focuses of the present survey were to describe the susceptibility/resistance profile of bacteria in poultry, assess its likely connection to the human communities profile and identify what antimicrobial agents are used in farms. Data analyses revealed high levels of contaminants in manures, food and animal drinking water.

If the presence of bacteria in manure could be anticipated as partly made of the contents of the animal guts, recording high rates in animal drinking water and food represents a critical health threat for humans, animals and the hosting environment. In fact, ingestion of bacteria could result in disease outbreaks in the farm. More likely, therefore, enforcing frequent outbreak prevention initiatives would explain the use of wide ranges of antibiotics recorded during the present investigation. Most common antibacterial agents included Oxytetracycline, Furaltadone and Flumequine. Previous work conducted in Khartoum Sudan (Sirdar *et al*., 2012) on the use of antimicrobials in farm disclosed that Oxytetracycline, Colistin, Tylosin and Enrofloxacin were very common in poultry (Ogunleye *et al*., 2008; Sirdar *et al*., 2012).

Table 7. Antibiotic susceptibility profile from the test community

Antibiotic	Isolate type (%)			
	Phenotype	GN rods (Nb)	GP rods (Nb)	GP cocci
Vancomycine	I	NA	40	67
	R	NA	0	17
	S	NA	60	17
Gentamicine	I	0	0	8
	R	11	0	0
	S	89	100	92
Ciprofloxacin	R	11	0	0
	S	89	100	100
Trimethoprim/sulfamethoxazole	R	44	80	50
	S	56	20	50
Nalidixic acid	I	0	20	0
	R	50	80	92
	S	50	0	8
Norfloxacin	R	0	0	8
	S	100	100	92
Erythromycin	I	0	0	8
	R	67	60	75
	S	33	40	17
Clindamycin	I	11	0	0
	R	78	40	42
	S	11	60	58
Nitrofurantoin	R	33	60	17
	S	67	40	83
Amoxicilline/ clavulanic acid	R	0	20	0
	S	100	80	50
Imipenem	I	0	0	25
	R	0	0	17
	S	0	100	58
Oxacillin	R	NA	80	50
	S	NA	20	0
Penicillin G	R	NA	80	42
	S	NA	20	8
Cefoxitine	R	10	20	58
	S	90	80	42
Aztreonam	I	0	0	8
	R	20	0	33
	S	80	100	59
Cefuroxime	I	90	60	33
	R	10	40	17
Ceftriaxone	I	0	0	17
	R	100	100	75
	S	0	0	8
Amoxicilline	R	40	60	58
	S	60	40	42
Ceftazidime	I	0	0	33
	R	100	100	42
	S	0	0	25

As it was the case from the findings of the present study, drugs were often administered through drinking water. Subsequent to laboratory analyses, isolates recovered consisted in decreasing order of Gram-negative rods (*Enterobacteriaceae,* 43%), Gram-positive cocci (40%),and Gram-positive rods (18%). Similar rates were reported in clinical settings in Cameroon (Gangoué-Piéboji *et al*., 2004; Fotsing Kwetché*et al*., 2015) and in Iran(Zangeneh *et al*., 2009). An overview of antimicrobial agentsindicated that antibiotics were generally usedfor anti-infectious prophylaxis then, at sub-therapeutic doses. It is also recognized that low doses of stressors are potent selectors for adaptive genetic traits including antibacterial resistance(Ngoune *et al*., 2010; Gondam Kamini *et al*., 2016; Guetiya Wadoum *et al*., 2016; Rabenirina 2016; Rahmatallah *et al*., 2016). This could also justify the high rates of resistance observed duringthe present survey. The highest resistance rates in their work were substantiated with quinolones (Nalidixic acid, Ciprofloxacin and Norfloxacin), Cyclins and Sulphonamides. These antibiotics were also found to be frequent amongst drug regimens that were used by farmers in their investigation areas (Ogunleye *et al*., 2008; Alo and Ojo, 2008; Ngoune *et al*., 2010; Gondam Kamini *et al*., 2016; Guetiya Wadoum *et al*., 2016).

Other studies by Bogaard *et al*. (2000) and, Kolar *et al*. (2002) reported similar findings. According to the authors of these latter two researches, amplified use of antibacterial agents affects selection of resistance in both pathogenic bacteria and endogenous microflora of humans and animals through direct and indirect contacts. Moreover, subtle data analysis from the present work indicated that bacteria of the external environment very likely become affected, and subsequently represent aetiologies of resistant human and animal infections. One of the likely efficient and common dissemination pathways would therefore be colonizing host gut and transfer resistance genes to endogenous microbial populations (Bogaard and Stobberingh 2000; Kolar *et al*., 2002) which, when passed out to the external environment exacerbate contamination likelihood in unhygienic contexts like one in which this work was conducted. Similar scenarios could be pictured in all low-and-middle-income settings in developing countries throughout the world. In connection with the findings from the present survey and, built on the diversity of antimicrobial agents identified, advents of cross-resistance could easily be predicted. Though pieces of information that could guide alleging co-resistance were very limited, ruling out this possibility would not be reasonable as co-selection that

amplifies tolerance through acquisition of mobile genetic determinants like plasmids, integrons and transposons or through mutation on the inherent nucleosides/nucleotides is also very likely in the study environments. This was substantiated by resistance rates observed with macrolides (Erythromycin and Clindamycin) and beta-lactams (Oxacillin, Penicillin G, Cefuroxime and Ceftriaxone) while antibiotics from these groups were not used. Otherwise, these findings imply that unrelated drugs co-selected the related traits as already observed in other reports (Robicsek *et al*., 2006; Marcusson *et al*., 2009; Tagajdid *et al*., 2010; Cantón and Ruiz-Garbajosa 2011). In addition, these selective agents' spectra might be broader beyond current understanding, because a comprehensive list cannot be made in a given ecological niche and primarily, in those with inappropriate sanitation. As highlighted earlier in the present discussion, these resistant isolates are likely to colonize poultry farmers and the neighbouring human populations (Liazid, 2012; Kouamouo *et al*., 2013; Benfreha-Temmouri, 2014); consistent with findings from the couple farmer/neighbouring population fingerprinting analysis in which resistance profiles were slightly similar to farm specimen data, but significantly different between the farm and the remote test community. Members of the *Enterobacteriaceae* family overwhelmed the isolation before *Staphylococcus* and *Bacillus*. These three groups of bacteria are effective in selecting adaptive genetic traits, likely to play critical roles in trait selection amongst phylogenetically close and distant species (Bennett, 2008; Martínez and Baquero, 2014; Maguire and Maguire, 2017) owing to global relatedness in DNA composition.

According to related principles, high isolation rates correlate the high density that in turn favours selection of advantageous traits in all mixed microbial populations (Martínez and Baquero, 2014). Density and the related selection ability of these major bacteria groups are also associated with ubiquity, consistent with molecular oxygen affinity (non-fastidious and facultative anaerobes) in all groups. However, *Enterobacteriaceae* adapt less well than *Staphylococcus* and *Bacillus.* Strains from these two latter genera resist drought-related stresses than *Enterobacteriaceae.* That might explain as least in part the lower isolation rates of Gram-negative rods from fingerprinting compared with those recorded in water and food analyses. Coliforms are reliable indicators of low hygiene (of recent contamination) than *Staphylococcus* and *Bacillus.* This assumption builds on the limited time they would survive in such stressful environment as hands, for instance (Livermore, 2002). In addition to the abilities shared with *Enterobacteriaceae* and *Staphylococcus*, *Bacillus* is endospore-forming; then more resistant to drought than *Staphylococcus*. Otherwise, member of this genus would stand harsher environmental conditions than Gram-negative rod and Gram-positive cocci.

In that vein, previous works consistently suggest that they could be used in antibiotic susceptibility / resistance stewardship in health facilities (Simo Louokdom *et al*., 2016; Noukela Noumi *et al*., 2017; Tchapdie Ngassam *et al*., 2017). Findings from the present survey further indicated that for at least three reasons, they could also be considered infarms for similar purposes: 1- the isolation rates would be high enough to allow robust statistics, them more reliable; 2-they are rarely (if ever) aetiologies of infectious diseases in immune-competent hosts, then easy to manipulate; 3-they are ubiquitous and non-fastidious, then easy to grow.

The high resistance recorded with some antibacterial agents in the community were consistent with the above development and closely related with low hygiene (Van Den Bogaard and Stobberingh, 2000). If the resistance trends observed in human medicine are commonly attributed to misuse of antibiotics in hospitals and communities, the present work addresses the necessity to redefine both the types of traits and amplitudes of their selection and diffusion from farms into exposed human communities. Data from the present study did not generate comprehensive information related to that issue; but future surveys should address such a crucial One Health concern that emerges as global threat. Otherwise, subsequent works will inform better on amplitude of interfaces. With a subtle glance at the cost of resistance and its association with human an animal welfare, antibiotics listed in the present investigation are drugs of choice in the caretaking of animal and human bacterial infections (Oluwasile *et al*., 2014). The cost of resistance could be high for at least three reasons: 1-contamination of animal food and drinking water might cause diseases that require costly drugs in case of resistance to available ones; 2-these resistant bacteria could be linked to higher morbidity and mortality; as well as heavy economic losses in the farms; 3-once these resistant bacteria are transmitted to humans, the indirect losses are added to higher mortality and morbidity which further mastermind the vicious cycle of poverty. Improving hygienic conditions infarm s would increase production and thereby the Gross National Product. These goals are hard to reach but possible to achieve based on the educational background of the farmers. Other alternatives to antibiotic in their various roles are under investigation (Maguire and Maguire, 2017) and could be critical in farms. These include the use of probiotics with organisms like *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcu*s and *Saccharomyces* (Simon*et al*., 2001). This requires, however, important human and financial resources that could only be mobilized with an overall political will.

Conclusion

The present work indicated that bacterial types known to be potent vectors of gene transfer and aetiologies of infectious diseases in animals and humans (*Enterobacteriaceae* and *Bacillus* and *Staphylococci*) were recovered frequently. In addition, resistance rates were invariably high in all settings; most likely in connection with the drugs used by farmers for different purposes. Though yet to be addressed, both the isolation and resistance rates could help anticipate the heavy economic burden that farm-related infections might generate. Biological alternatives to antimicrobials in farms were, therefore, thought to be primordial, feasible, and then suggested.

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