Antibiotics resistance and abundance of some bacteria from agricultural and non-agricultural soil in one of the most agriculturally-active settings in West Cameroon

ABSTRACT

Background: Agriculture is also concerned with the problem of bacterial resistance because agricultural soils are reservoirs of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes. **Objective:** An investigation about ARB was carried out on agricultural soils in Mangoum, a neighborhood of the Foumbot municipality (Noun division, West Cameroon). **Method:** It was conducted as a cross-sectional descriptive study with a total of 46 soil specimens collected from plant farms and a control plot. Isolation, enumeration and antibiotic susceptibility tests were performed according to standard protocols. **Results:** The bacteria recovered included *Aeromonas* spp., *Chryseobacterium* spp., *Pseudomonas* spp., *Staphylococcus* spp., and Gram-positive rods. Their loads in the farmland soils were significantly lower than in the control plot. Overall, susceptibility tests performed with 169 bacterial colony morphotypes revealed high resistance rates. Also, most of the isolates expressed multidrug-resistance to the antibiotics used, while highest resistance rates were recorded with isolates form agricultural plots. Levofloxacin, Imipenem, Gentamicin and Ciprofloxacin were globally the most effective. **Conclusion:** Agreeing with previous surveys conducted on animal farms, these findings could provide support to the sustainable orientation of policies regarding the control of antimicrobial resistance in Cameroon from the One Health perspective.

Keywords: Agricultural soils, Antibiotic, Resistant Bacteria, Bacterial Abundance

1. INTRODUCTION

One of the most significant advances made in health throughout the 20th century was the development of antimicrobial agents used to control microorganisms, which are often etiologies of health disorders in humans, animals and plants. These advances in health-related microbiology resulted in major achievements in connection with alleviated morbidity and mortality due to infectious diseases across global human populations. The recorded achievements and further anticipations were, however, undermined by microbial resistance expressed against previously effective drugs [1-3]. The emergence and spread of antibiotic-resistance bacteria (ARB) are currently known to result from selective pressure exerted by diverse engines like antibacterial agents or other antimicrobials that promote the activity of mobile genetic determinants harboring resistance traits, useful in supporting population fitness in adverse environmental conditions [4,5].

Agricultural soils are known as reservoirs for ARB and antibiotic-resistant genes (ARG) [6,7]. Some mobiles recognized as factors for the selection and dissemination of resistance traits in plant farms include the use of pesticides and manure in crop production [3,5], though their contribution is yet to be accurately assessed because of the versatility in compositions and application. Antibiotics used as food supplements in farm animals are consistently pointed out as a major cause of selection for resistant phenotypes that diffuse throughout diverse close and remote bacterial populations, as well as animal feces, vehicle of residual or non-metabolized antibiotics in exposed environments. Consequently, since animal manure (made from animal feces) serves as soil fertilizers in crop production, the likelihood for selection and spread of ARB and ARG in vulnerable environmental settings is high [8]. Like other selective agents, biocides used to improve agricultural yields can also cause pressure that co-selects antibiotic-resistant bacterial populations [9,10]. Selection in all environments is facilitated by the high flexibility of soil prokaryotic microorganisms' genomes that explain their rapid adaptation to new environments, and to the newly introduced chemical compounds [5].

These pieces of information support all investigations in the framework of AMR in agriculture, acknowledging that altered microbial populations will not only affect the quality of soils and the quality of crops that can effectively grow, but also expose humans and animals as well. In a larger project aiming at assessing AMR and contributing factors, the present investigation was conducted in order to investigate ARB in farmland soils of one of the most important crop production basins in West Cameroon. Together with related findings in animal and

plant farms, recovered information will guide decision-makers in developing suitable policies that will advocate and support sustainably the antimicrobial resistance stewardship in plant farms, aligning with those in connection with human and animal health according to the One Health principles.

2. MATERIAL AND METHODS

2.1 Study design

Data collection in the present cross-sectional study was conductedon agricultural farms located in Mangoum, a neighborhood of the Foumbot municipality (Noun division, West Cameroon). Thereafter, specimen analyses were performed in the Laboratory of Microbiology at the "Université des Montagnes" Teaching Hospital (UdMTH). This work was conducted between September and October, 2020.

Before field work, administrative approvals were obtained from legal authorities. Authorizations were provided by the Foumbot sub-divisional officer and the division Head for the Ministry of Agriculture and Rural Development. Also, before field sample collection, all farm owners signed a voluntary written informed consent to authorize the research to be carried out on their lands. For the present study, ethical approval was not required because humans were not the subjects of interest. The work was carried out only on environmental samples, particularly soil specimens; farmers were not at risk.

Laboratory screening was performed under authorization reference N° 2020/176/AED/UDM/CUM delivered by the UdMTH General Administration. Specimen collection was performed in the farms in which the owner was affiliated and recorded in the local divisional headquarter for the Ministry of Agriculture and Rural Development.

2.2Data collection

An adapted data collection sheet was used to gather relevant pieces of information in connection with the investigation goals. These pieces of information included the type of manure used, source of manure, frequency of manure use, most recent date of manure application, pesticide use, frequency of pesticide use, biosecurity-biosafety practices, and training in agricultural activities.

2.3Samples collection and transport

In line with biosafety and biosecurity rules, portions of approximately 50 g of soil were randomly collected with sterile spatula at different locations in each farm, and preserved separately in labelled sterile containers. The same procedure was used to collect soil specimens from a nearby virgin piece of land (referred as a "control plot") characterized by wild vegetation, no visible chemical or manure application or no other visible anthropogenic activity. Relatively close to each other, all the farms and the control plot were located in the same area. Samples were stored in refrigerated containers and conveyed to the laboratory for screening that was performed within the 24 hours post-collection.

2.4 Samples analysis (Bacterial screening)

This screening was performed according the principles of standard protocols [11].

2.4.1 Culture

At the laboratory, each portion of the soil was thoroughly mixed to homogeneity. Then, 5 g of the resulting preparation was added to, and thoroughly mixed with 45 mL of sterile physiological saline. Thereafter, a series of successive decimal dilutions were made in sterile physiological saline. From each suspension (diluted and undiluted), 50 µL of the inoculum was spread over the entire surface of McConkey and Mannitol Salt isolation agars with a sterile Pasteur pipette rake. The inoculated agar plates were then incubated overnight (for 24 hours) at 37°C.

2.4.2 Identification I (macroscopy screening) and enumeration

After incubation, bacterial growth on each culture medium was assessed and those on which bacterial growth was recorded were characterized. The emerging characteristics included colonies' descriptions (according to their shape, size, consistency, color, surface and opacity). According to morphotypes, differential enumeration of the colonies was carried out. Only plates on which colony count varied between 30 and 300 were used in this exercise. For each soil sample, the bacterial load (*N*) expressed in terms of Colony Forming Units per gram of soil (CFU/g of soil) was calculated according to the formula $N = 900 \times m \times 10^{d} / 5$ (where 900- is the ratio of the volume of the initial suspension to the volume of the inoculum, *m*- the number of Colony Forming Units (CFU) per Petri dish, 10^{d} -the dilution factor, *d*- the dilution number, 1/5- the conversion factor from the number of CFU in 5 g of soil sample to the number of CFU in 1 g of soil).

2.4.2 Identification II: microscopy and bio-enzymatic identification

After enumeration, bio-enzymatic identification steps were conducted on distinct colony types. Gram stain was then followed by an exploration of bacterial metabolism according to the target bacterial group. This exploration was carried out with a series of identification parameters including the oxidase test, tests on KliglerHajna agar (glucose and lactose fermentation, gas and hydrogen sulfide production), tests on Mannitol-Mobility medium (mannitol fermentation, bacteria mobility, nitrate reductase production), urease, indole, TDA, gelatinase, Voges-Prokauer, decarboxylase (ADH, ODC, LDC), catalase, free coagulase and DNAase tests for Gram-negative rods and Gram-positive cocci. Concerning Gram-positive rods, identification was limited to microscopy on Gram-stained smears.

2.5 Antibiotic susceptibility tests

Susceptibility tests were carried out by standard disk diffusion according to the "Comité de l'Antibiogramme de la SociétéFrançaise de Microbiologie, EUCAST" (CASFM) [12]. All tests were conducted with 24 h pure subculture grown on nutritive agar from colonies that were randomly isolated as representative of each colony morphotype in all samples. A total of 14 antibacterial agents commonly used in bacterial infection control in Cameroon were used in subjected bacterial pools. Namely, they were Amoxicillin (20 or 25 µg), Amoxicillin/Clavulanic acid (20/10 µg), Aztreonam (30 µg), Cefepime (30 µg), Cefoxitin (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Norfloxacin (10 µg), Penicillin G (10 U), Tetracycline (30 µg), Trimethoprim/Sulfamethoxazole (1.75/23.25 µg). For the clinical categorization of GPR isolates and the Penicillin G (10 U) testing, the 2013 recommendation of CASFM was used [13]. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were the reference bacterial strains used for quality control throughout the process.

2.6 Data analysis

Data recorded included pieces of information collected from farm owners, types of bacteria recovered, bacterial loads and clinical categories (susceptible-susceptible at high posology-resistant) of studied isolates. These data were recorded and processed with Microsoft Excel 2013 and analyzed with tools provided by IBM SPSS statistic version 20. In this paper, bacterial loads were presented for each sample. Regarding the clinical categories, results were presented in terms of frequencies per bacterial type and antibacterial agents. Linear regression tests were performed to assess the association between bacterial loads and agricultural plot characteristic(that is plot undergoing agricultural transformations and treatments). Significant results were admitted for p-values (*P*) less than 0.05.

3. RESULTS

3.1 Survey results

In this work, six agricultural farms were enrolled. Data analysis from the survey sheet revealed that all farm owners have been trained in agriculture. However, there wereweaknesses in biosafety and biosecurity (partial hygiene, which was limited to hand washing after farming) and an absence of knowledge related to antibiotic resistance. Pesticides and animal manure were used on all farms. From swine and poultry farms, manure was spread before plowing and seeding. The latest application was done between 2 and 6 weeks (depending on the farm) before the present study was initiated. The pesticide application was carried out at 3, 4 or 5-day intervals (in 3, 1 and 2 farms, respectively). No specific reference existed to guide farmers' practices. Gloves and boots were also rarely used.

3.2Bacterial diversity and loads

From a total of 46 soil samples collected (Control plot: 5, Farm 1: 5, Farm 2: 6, Farm 3: 6, Farm 4: 8, Farm 5: 9, Farm 6: 7), bacteria recovered included *Aeromonas* spp., *Chryseobacterium* spp., *Pseudomonas* spp., *Staphylococcus* spp., and Gram-positive rods (GPR). *Chryseobacterium* spp., were the least frequently isolated. In addition, bacterial loads in farm specimens were significantly lower than those recorded from the control plot specimens (Tables 1 and 2). The highest bacterial densities were obtained with Gram-positive rods, *Aeromonas* and *Pseudomonas* (Table 2). The linear regression tests indicated that the total bacterial loads and those for each bacterial group were associated with the characteristic of "agricultural plot" (for total bacterial loads: $P<0.001$; for *Staphylococcus* spp. and *Pseudomonas* spp. loads: $P = 0.004$; for *Aeromonas* spp. and GPR loads: $P<0.001$).

Table 1. Total loads (CFU/g of soil) of bacteria

Samples	Control plot	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Sample 1	1.0062×10^{7}	2.178×10^{4}	1.656×10^{4}	2.34×10^{4}	1.6452×10^{5}	2.5488×10^{6}	3.7548×10^{5}
Sample 2	3.888×10^{7}	2.934×10^{4}	5.598×10^{4}	3.492×10^{4}	8.802×10^{4}	2.3454×10^{6}	4.14×10^{4}
Sample 3	6.84×10^{6}	1.8162×10^{5}	5.328×10^{5}	4.662×10^{4}	1.5282×10^{6}	2.106×10^{4}	1.3608×10^{5}
Sample 4	4.662×10^{6}	4.374×10^{4}	1.206×10^{5}	3.78×10^{6}	8.568×10^{5}	2.7432×10^{6}	1.2942×10^{5}
Sample 5	7.704×10^{6}	2.538×10^{4}	2.556×10^{4}	5.526×10^{4}	1.557×10^{5}	2.6586×10^{6}	2.934×10^{4}
Sample 6	-	-	1.026×10^{4}	5.922×10^{4}	4.41×10^{4}	5.922×10^{4}	4.446×10^{4}
Sample 7	-	-	-	-	2.916×10^{4}	4.392×10^{4}	1.251×10^{5}
Sample 8	-	-	-	-	1.05732×10^{6}	6.498×10^{4}	-
Sample 9	-	-	-	-	-	1.5012×10^{6}	-

CFU: Colony Forming Units, -: absence of visible bacterial growth

Table 2. Loads (CFU/g of soil) of various bacterial groups

Samples	Control plot	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6	Control plot	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
	***Aeromonas* spp.**							***Pseudomonas* spp.**						
Sample 1	8.496×10^{6}	7.56×10^{3}	-	3.96×10^{3}	-	-	4.878×10^{4}	1.152×10^{6}	-	-	3.24×10^{3}	1.3176×10^{5}	-	1.656×10^{4}
Sample 2	1.476×10^{7}	1.062×10^{4}	2.16×10^{3}	8.28×10^{3}	1.8×10^{4}	-	1.656×10^{4}	2.25×10^{7}	1.8×10^{3}	-	1.134×10^{4}	5.04×10^{3}	4.878×10^{5}	2.484×10^{4}
Sample 3	5.76×10^{5}	1.008×10^{5}	4.86×10^{5}	9.72×10^{3}	2.232×10^{5}	2.106×10^{4}	1.368×10^{4}	3.78×10^{5}	-	-	1.116×10^{4}	-	-	4.104×10^{4}
Sample 4	1.314×10^{6}	1.494×10^{4}	5.22×10^{4}	8.424×10^{5}	1.44×10^{4}	-	4.14×10^{3}	2.07×10^{6}	7.56×10^{3}	-	-	1.548×10^{5}	-	5.94×10^{3}
Sample 5	1.584×10^{6}	4.14×10^{3}	8.28×10^{3}	3.708×10^{4}	3.024×10^{4}	-	9.54×10^{3}	-	-	-	-	-	2.6316×10^{6}	4.86×10^{3}
Sample 6	-	-	-	-	-	4.554×10^{4}	-	-	-	-	3.492×10^{4}	2.16×10^{3}	1.044×10^{4}	4.68×10^{3}
Sample 7	-	-	-	-	1.98×10^{3}	2.61×10^{4}	6.48×10^{3}	-	-	-	-	7.02×10^{3}	-	6.768×10^{4}
Sample 8	-	-	-	-	1.566×10^{4}	1.926×10^{4}	-	-	-	-	-	1.98×10^{3}	1.296×10^{4}	-
Sample 9	-	-	-	-	-	1.08×10^{4}	-	-	-	-	-	-	5.994×10^{5}	-
	GPR							***Staphylococcus* spp.**						
Sample 1	4.14×10^{5}	1.422×10^{4}	1.656×10^{4}	1.62×10^{4}	3.276×10^{4}	2.5488×10^{6}	-	-	-	-	-	-	-	3.1014×10^{5}
Sample 2	-	1.098×10^{4}	5.004×10^{4}	1.53×10^{4}	6.498×10^{4}	-	-	1.62×10^{6}	-	-	-	-	1.8576×10^{6}	-
Sample 3	-	8.82×10^{3}	2.88×10^{4}	9.18×10^{3}	9.216×10^{5}	-	-	5.886×10^{6}	-	-	1.656×10^{4}	-	-	8.136×10^{4}
Sample 4	-	1.26×10^{4}	4.5×10^{4}	-	6.876×10^{5}	2.7432×10^{6}	8.982×10^{4}	1.278×10^{6}	-	-	2.9376×10^{6}	-	-	-
Sample 5	6.12×10^{6}	1.62×10^{3}	5.76×10^{3}	1.818×10^{4}	-	-	1.494×10^{4}	-	7.74×10^{3}	1.152×10^{4}	-	9.09×10^{4}	2.7×10^{4}	-
Sample 6	-	-	6.66×10^{3}	3.42×10^{3}	3.492×10^{4}	3.24×10^{3}	3.348×10^{4}	-	-	3.6×10^{3}	2.088×10^{4}	-	-	-
Sample 7	-	-	-	-	1.602×10^{4}	-	4.68×10^{4}	-	-	-	-	4.14×10^{3}	4.86×10^{3}	-
Sample 8	-	-	-	-	-	2.772×10^{4}	-	-	-	-	-	1.03968×10^{6}	5.04×10^{3}	-
Sample 9	-	-	-	-	-	4.824×10^{5}	-	-	-	-	-	-	4.086×10^{5}	-
	***Chryseobacterium* spp.**													
Sample 1	-	-	-	-	-	-	-							
Sample 2	-	5.94×10^{3}	3.78×10^{3}	-	-	-	-							
Sample 3	-	7.2×10^{4}	1.8×10^{4}	-	3.834×10^{5}	-	-							
Sample 4	-	8.64×10^{3}	2.34×10^{4}	-	-	-	2.952×10^{4}							
Sample 5	-	1.188×10^{4}	-	-	3.456×10^{4}	-	-							
Sample 6	-	-	-	-	7.02×10^{3}	-	6.3×10^{3}							
Sample 7	-	-	-	-	-	1.296×10^{4}	4.14×10^{3}							
Sample 8	-	-	-	-	-	-	-							
Sample 9	-	-	-	-	-	-	-							

GPR: Gram-positive rods, CFU: Colony Forming Units; -: absence of visible bacterial growth

3.3 Bacteria susceptibility to antibiotics

Susceptibility tests to antibiotics were performed with 169 bacterial colony morphotypes (the distribution of numbers of bacterial colony morphotypes found is presented in table 3). These tests revealed several cases of antibiotic multidrugresistance, with isolates from agricultural plots more frequently expressing resistant phenotypes than those from the control plot (Table 4). The most effective antibiotics were Levofloxacin, Imipenem, Gentamicin and Ciprofloxacin.

Table 3. Number of bacterial colony morphotypes found

Bacterial group	Control plot	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
***Aeromonas*spp.**	6	6	4	6	8	8	7
***Chryseobacterium*spp.**	-	4	3	-	3	1	3
***Pseudomonas*spp.**	4	2	-	5	7	7	7
***Staphylococcus*spp.**	5	1	2	4	4	5	2
Gram-positive rods	3	9	6	11	10	8	8

Table 4.Bacterialsusceptibility to antibiotics profile

Antibiotics	Farm plots															Control plot											
	Aeromonas spp.			*Chryseobacterium* spp.			*Pseudomonas* spp.			*Staphylococcus* spp.			GPR			*Aeromonas* spp.			*Pseudomonas* spp.			*Staphylococcus* spp.			GPR		
	S	SHP	R	S	SHP	R	S	SHP	R	S	SHP	R	S	SHP	R	S	SHP	R	S	SHP	R	S	SHP	R	S	SHP	R
AMX	26	0	74	14	0	86	-	-	-	-	-	-	56	6	38	17	0	83	-	-	-	-	-	-	0	0	100
AMC	41	0	59	36	0	64	-	-	-	-	-	-	35	0	65	83	0	17	-	-	-	-	-	-	67	0	33
FOX	46	18	36	57	7	36	-	-	-	17	0	83	73	13	13	50	0	50	-	-	-	40	0	60	67	0	33
CRO	15	3	82	21	0	79	-	-	-	-	-	-	23	0	77	83	0	17	-	-	-	-	-	-	67	0	33
FEP	5	5	90	21	0	79	21	0	79	-	-	-	19	0	81	17	17	67	50	25	25	-	-	-	100	0	0
ATM	21	5	74	29	0	71	25	0	75	-	-	-	-	-	-	83	0	17	50	0	50	-	-	-	-	-	-
IMP	62	3	36	64	7	29	50	4	46	-	-	-	29	2	69	83	0	17	75	0	25	-	-	-	100	0	0
CIP	62	15	23	71	0	29	50	14	36	6	61	33	69	10	21	100	0	0	100	0	0	20	80	0	33	67	0
NOR	54	0	46	57	0	43	-	-	-	50	0	50	-	-	-	100	0	0	-	-	-	100	0	0	-	-	-
LEV	54	18	28	64	7	29	68	7	25	78	11	11	92	4	4	100	0	0	100	0	0	80	20	0	67	33	0
GEN	46	0	54	43	0	57	-	-	-	78	0	22	-	-	-	50	0	50	-	-	-	100	0	0	-	-	-
SXT	0	10	90	0	14	86	-	-	-	6	6	89	15	0	85	67	0	33	-	-	-	40	20	40	33	0	67
TET	-	-	-	-	-	-	-	-	-	28	11	61	48	10	42	-	-	-	-	-	-	80	20	0	67	33	0
PEN	-	-	-	-	-	-	-	-	-	0	0	100	-	-	-	-	-	-	-	-	-	0	0	100	-	-	-

GPR: Gram-positive rods, S: frequencies of susceptible isolates, SHP: frequencies of isolates susceptible at high posology, R: frequencies of resistant isolates, AMX: Amoxicillin (20 or 25 µg), AMC: Amoxicillin/Clavulanic acid (20/10 µg), ATM: Aztreonam (30 µg), CIP: Ciprofloxacin (5 µg), CRO: Ceftriaxone (30 µg), FEP: Cefepime (30 µg), FOX: Cefoxitin (30 µg), GEN: Gentamicin (10 µg), IMP: Imipenem (10 µg), LEV: Levofloxacin (5 µg), NOR: Norfloxacin (10 µg), PEN: Penicillin G (10 U), TET: Tetracycline (30 µg), SXT: Trimethoprim/Sulfamethoxazole (1.75/23.25 µg); -:not tested

4. DISCUSSION

Data analysis from the present survey conducted in Mangoum's farmlands, a neighborhood of the Foumbot municipality (Noun division, West Cameroon), primarily revealed that all farmers used animal manure and pesticides in crop production. Pesticides were applied every 3, 4 or 5 days, depending on the farmer's will. Manures originated from avian and swine farms and were applied before soil plowing and seeding. The most recent application dates before the present study were found between 2 and 6 weeks. This overall tendency to use fertilizers and pesticides resides in the logic that targets sustainable higher-level production of good-quality crops, in line with the ever-growing population demands for better welfare that should couple with economic benefits for farmers. This could explain the relentless efforts in plant protection against pests and invaders, in addition to plant growth supplements that are common in the study area. However, these practices correlate with a higher risk of generating factors that are likely to promote the selection of antibacterial resistance (ABR) and dissemination of antimicrobial resistance genes (ARG) [6-10]. The phenomena of ARG selection and spread, more obvious with bacteria may concern other prokaryotes within and amongst ecological niches, in connection with the microbial genome flexibility. Acknowledging that the genetic code is not only universal but degenerated as well, anticipating adverse effects on exposed eukaryotes is reasonable.

Major bacteria recovered were Gram-positive rods, *Aeromonas* spp., *Chryseobacterium* spp., *Pseudomonas* spp., and *Staphylococcus* spp., in subjected soil specimens. Known as endogenous environmental bacteria flora, their loads in the farmland soils were significantly lower than in the control plot. Since the perceptible difference between the control and farm soils is alleged through the absence or the presence of transformations and treatments made on these soils, data analysis further highlights that microbial populations are adversely affected by human activities, consistent with previous reports on agropastoral activities and microbial populations [14]. Otherwise, although useful for crop protection, pesticides do affect soil microflora beyond expectation ranges. Their repeated use maintains pressure that might cause an evolution in the niche microbiota, altering thereby the inherent microbial soil characteristics in types and diversity [15,16]. These events are likely to affect in the long run the target soil's mineralogy and the overall ''soil health'' subsequent to population evolution. If human activities can explain the difference in bacteria loads between the farms and the control plot, inherent specificities of practices on each agricultural plot could explain certain differences in bacterial loads between the target agricultural plots. These specificities could have created variations in the effect of treatments and activities on microbial populations, consistent with the above development on the risk of microbiota alteration that may evolve irreversibly with sustainable pressure, and affect any future agricultural project. Other factors like the chemical and organic composition of soils, soil characteristics, and contextual abiotic and biotic factors might also explain these plot-specific variations in the recorded bacterial loads [17-20] in line with varied practices. Otherwise, harmonized agricultural policies guided by local-accompanying trained leaders are necessary. The present investigation revealed that the application of manure and/or pesticides depended on the farmer's will, without any reference, though they claimed to be trained in agriculture. For instance, gloves and boots were rarely used (if ever), in addition to thelack of clean water for onsitebaths.

The paucity of biosafety and biosecurity amenities and the absence of knowledge regarding antimicrobial resistance further emphasize the need for capacity building. This should stand as a pre-requisite for a safer application of pesticides and animal manure, consistent with their likely adverse impact on exposed human and animal populations beyond alteration of the microbial flora.

With a glance on bacterial susceptibility, a reduced number of antibacterial agents used was effective on bacteria isolated from agricultural soils compared to those from the control plot. Globally, fluoroquinolones, Imipenem and Gentamicin were the most effective on bacterial isolates. Like bacterial loads, this difference further highlights the impact that soil transformations and treatments can have on endogenous microbial populations.

Two factors could be pointed out as very likely to have contributed to the higher resistance rates in farmlands. The first one is the use of manure. On the farm soils investigated, the manure applied consisted of feces of farm animals that are known as reservoirs of antibiotic residues, antibiotic-resistant germs and mobile genetic elements [21]. Accordingly, in the presence of manure, soil bacteria are exposed to selective pressures caused by antibiotic-resistant strains and genes that have been selected in animal digestive tracts and/or in the farm environment on one hand, and to the pressure caused by antibiotic residues contained in it on the other. These multivariate stresses combine and exacerbate acquisition rates of resistance genes and thus, the emergence of more resistant bacteria populations [21-24]. This development agrees with findings from previous surveys

conducted in animal farms in Cameroon (West [25,26], Littoral [27], South [28]), which reported high levels of antibiotic-resistant bacteria in animal feces and in some items within the farm premises like animal feed and drinking water. Also, the clinical category profiles of isolates from these previous studies overlap with those recorded during the present one. This overlapping tendency might highlight the links between animal farms and plant farms. This view should be taken into account in all initiatives that focus accurately on antimicrobial resistance according to the One Health principles (multisite, multidisciplinary, gender-related, holistic view, to name a few) in Cameroon.

Pesticide application is the second engine that likely contributed to antibiotic resistance selection. Bacteria can express resistance to antibiotics when they are exposed to other biocides by cross-selection, co-selection, and phenotype change. Also, indirect promotion of less susceptible microbial sub-population upon exposure to biocides occurs through several mechanisms including activation of *SOS* response,DNA repair,and other induced alterations, which ultimately result in microbial fitness [29] alongside with altered environmental microbial diversity.

Additionally, the results recorded indicate that these farmers are exposed to a diversity of multidrug-resistant bacteria, likely etiologies of infections. Consequently, they could be acting as disseminators of these bacteria, driving therefore, the spread of ARB and ARG into their communities. The paucity of biosecurity andbiosafety further exacerbates this transporter role. Based on current data and findings, however, it is not possible to anticipate which driver (manure or pesticides) was most effective in selecting resistance traits, and to what extent.

Higher rates of ARB in farm soils justify the need for amplified investigations on ARG in crop production environments in order to track and control ARB and ARG dissemination in vulnerable settings and communities in Cameroon. The present survey was conducted during the rainy season. The rainfall was identified by previous authors as a mobile reservoir facilitating the transmission and proliferation of ARG, and enhancing bacterial resistance and the abundance of ARG in soils [30]. Then, future investigations should include meteorological parameters such as seasonal changes in the study design to better track the selection and diffusion of ARB and ARG in Cameroon's agricultural sector. Furthermore, since antimicrobial concentrations and drug residues also influence resistance selection (consistent with the above arguments), the identification of antibiotic residues that are present in the soil, different types of pesticides used and their respective concentrations in the soil would, in future surveys, strengthen the understanding of the development and spread of microbial resistance on farms.

These links bringing together West Cameroon's agricultural farms, animal farms and exposed human communities further reinforce the need to emphasize the One Health paradigm from a more holistic point of view. In this, current data provide support to advocate One Health activities such as raising farmers' awareness and improving policies that focus on issues associated with antimicrobial resistance for global welfare.

5. CONCLUSION

The present investigation revealed that the agricultural practices greatlyaffectthe microbial flora on farms in Mangoum, West Cameroon. The application of pesticides and manure in plant farms was very likely associated with the selection of antibiotic-resistant bacterial strains. Rates of antibiotic-resistant bacteria were higher in agricultural farm soils. Also, based on current data analysis, it wasn't possible to specify which substrate (manure or pesticides) was more effective in selecting the resistance traits observed. Overall, the most effective antibiotics were Levofloxacin, Imipenem, Gentamicin, and Ciprofloxacin. Agreeing with previous surveys conducted on animal farms with similar environmental conditions, these findings could provide support for the sustainable orientation of policies regarding the control of antimicrobial resistance in Cameroon from the One Health perspective.

Disclaimer

This paper is an extended version of a preprint document of the same author.

The preprint document is available in this link: https://www.biorxiv.org/content/10.1101/2024.08.27.609998v1

[As per journal policy, preprint article can be published as a journal article, provided it is not published in any other journal]

DATA AVAILABILITY

Data associated with this work were not deposited into a publicly available repository. All the data of this work are present in this paper.

ARTIFICIAL INTELLIGENCE DISCLAIMER

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

1. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet. 2022; 399(10325): 629-55. DOI: https://doi.org/10.1016/S0140-6736(21)02724-0
2. Jonas OB, Irwin A, Berthe FCJ, Le Gall FG and Marquez PV. Drug-resistant infections: a threat to our economic future (Vol. 2): final report (English). HNP/Agriculture Global Antimicrobial Resistance Initiative Washington, D.C.: World Bank Group. 2017; 172p. Available from: http://documents.worldbank.org/curated/en/323311493396993758/final-report
3. Iwu CD, Korsten L and Okoh AI. The incidence of antibiotic resistance within and beyond the agricultural ecosystem: A concern for public health. Microbiology Open. 2020; 9(9):e1035. DOI: https://doi.org/10.1002%2Fmbo3.1035
4. Carle S. la résistance aux antibiotiques : un enjeu de santé publique important!.Pharmactuel. 2009 ; 42 : 6-21. French
5. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB *et al*. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. J Infect Public Health. 2021; 14(12): 1750 – 66. DOI: https://doi.org/10.1016/j.jiph.2021.10.020
6. Wang F, Fu YH, Sheng HJ, Topp E, Jiang X, Zhu YG *et al*. Antibiotic resistance in the soil ecosystem: A One Health perspective. CurrOpin Environ Sci Health. 2021; 20: 100230. DOI: https://doi.org/10.1016/j.coesh.2021.100230
7. Igbinosa EO, Beshiru A, Igbinosa IH, Cho GS and Franz CMAP. Multidrug-resistant extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* from farm produce and agricultural environments in Edo State, Nigeria. PLoS ONE. 2023; 18(3): e0282835. DOI: https://doi.org/10.1371/journal.pone.0282835
8. Lima T, Domingues S and Da Silva GJ. Manure as a Potential Hotspot for Antibiotic Resistance Dissemination by Horizontal Gene Transfer Events. Vet Sci. 2020; 7(3): 110. DOI: https://doi.org/10.3390%2Fvetsci7030110
9. Malagón-Rojas JN, Parra Barrera EL and Lagos L. From environment to clinic: the role of pesticides in antimicrobial resistance. Rev PanamSaludPublica. 2020; 44: e44. DOI: https://doi.org/10.26633%2FRPSP.2020.44
10. Qiu D, Ke M, Zhang Q, Zhang F, Lu T, Sun L *et al*. Response of microbial antibiotic resistance to pesticides: An emerging health threat. Sci Total Environ. 2022; 850: 158057. DOI: https://doi.org/10.1016/j.scitotenv.2022.158057
11. Denis F, Ploy MC, Martin C, Bingen E and Quentin R. Bactériologie médicale, Techniques usuelles. 2nd edition. Paris : Elsevier Masson SAS, 2011. French.
12. Comité de l'antibiogramme de la Société Française de Microbiologie, CASFM / EUCAST. Recommandations 2020 V.1.2 Octobre. SociétéFrançaise de Microbiologie. 2020. 181p. French.
13. Comité de l'antibiogramme de la Société Française de Microbiologie. Recommandations 2013. SociétéFrançaise de Microbiologie. 2013. 60p. French.

14. Gupta A, Singh UB, Sahu PK, Paul S, Kumar A, Malviya D *et al*. Linking Soil Microbial Diversity to Modern Agriculture Practices: A Review. Int J Environ Res Public Health. 2022; 19(5): 3141. DOI: https://doi.org/10.3390%2Fijerph19053141.

15. Steiner M, Falquet L, Fragnière AL, Brown A and Bacher S. Effects of pesticides on soil bacterial, fungal and protist communities, soil functions and grape quality in vineyards. EcolSolutEvid. 2024; 5: e12327. DOI: https://doi.org/10.1002/2688-8319.12327

16. Jeyaseelan A, Murugesan K, Thayanithi S and Palanisamy SB. A review of the impact of herbicides and insecticides on the microbial communities. Environ Res. 2024; 245: 118020. DOI: https://doi.org/10.1016/j.envres.2023.118020

17. Nam S, Alday JG, Kim M, Kim H, Kim Y, Park T *et al*. The relationships of present vegetation, bacteria, and soil properties with soil organic matter characteristics in moist acidic tundra in Alaska. Sci Total Environ. 2021; 772: 145386. DOI: https://doi.org/10.1016/j.scitotenv.2021.145386

18. Bystrianský L, Štofik M and Gryndler M. Soil-derived organic particles and their effects on the community of culturable microorganisms. Folia Microbiol (Praha). 2018; 63(1): 69-72. DOI: https://doi.org/10.1007/s12223-017-0537-4

19. Wu N, Li Z, Meng S and Wu F. Soil properties and microbial community in the rhizosphere of *Populus alba* var. *pyramidalis* along a chronosequence. Microbiol Res. 2021; 250: 126812. DOI: https://doi.org/10.1016/j.micres.2021.126812

20. Chamard J, Faticov M, Blanchet FG, Chagnon PL and Laforest-Lapointe I. Interplay of biotic and abiotic factors shapes tree seedling growth and root-associated microbial communities. Commun Biol. 2024; 7(1):360. DOI: https://doi.org/10.1038/s42003-024-06042-7

21. Xie WY, Shen Q and Zhao FJ. Antibiotics and antibiotic resistance from animal manures to soil: a review. Eur J Soil Sci. 2018; 69: 181-95. DOI: https://doi.org/10.1111/ejss.12494

22. Huang J, Mi J, Yan Q, Wen X, Zhou S, Wang Y *et al*. Animal manures application increases the abundances of antibiotic resistance genes in soil-lettuce system associated with shared bacterial distributions. Sci Total Environ. 2021; 787: 147667. DOI: https://doi.org/10.1016/j.scitotenv.2021.147667

23. Rahube TO and Yost CK. Characterization of a mobile and multiple resistance plasmid isolated from swine manure and its detection in soil after manure application. J ApplMicrobiol. 2012; 112(6): 1123-33. DOI: https://doi.org/10.1111/j.1365-2672.2012.05301.x

24. Heuer H, Schmitt H and Smalla K. Antibiotic resistance gene spread due to manure application on agricultural fields. CurrOpinMicrobiol. 2011; 14(3): 236-43. DOI: https://doi.org/10.1016/j.mib.2011.04.009

25. YawatDjogang AM, FotsingKwetché PR, Simo Louokdom J, GamwoDongmo S, NankamNguekap WL, Tchoukoua SH *et al.* Antibiotic susceptibility profile of bacteria from farm wastes: findings in chicken excreta, food and water from four poultries versus trend in a non-exposed exposed community of West Cameroon. Int J Curr Res. 2018; 10(11): 75629-38.

26. Mbognou LS, TamatchoKweyang BP, YawatDjogang AM, Youté OD, Tchoukoua SH and FotsingKwetche PR. Tackling bacteria resistance in farms: Focus on fluoroquinolones, beta-lactams and cyclins in poultry farms of the Bamboutos division, West-Cameroon. WJAHR. 2024. 8(4): 134-42

27. NgandjuiYonga C, NankamChimi R, FotsingKwetché PR, KouengouaKouengoua PA, NjayouNgapagna A, YawatDjogang AM *et al*. Antibacterial resistance: Trend in a few poultry farms of the Wouri division, Littoral Cameroon. World J Pharm Pharm Sci. 2021. 10(4):275-88.

28. FotsingKwetché PR, Well à Well à Koul PB, Kengne A, TamatchoKweyang BP, YawatDjogang AM, Youté OD *et al*. Investigating through cross-adulteration and antimicrobial resistance in animal farms: focus on Poultries in South Cameroon. World J Pharm Res. 2021. 10(2): 1296-312.

29. Paul D, Chakraborty R and Mandal SM. Biocides and health-care agents are more than just antibiotics: Inducing cross to co-resistance in microbes. Ecotoxicol Environ Saf. 2019; 174: 601-10. DOI: https://doi.org/10.1016/j.ecoenv.2019.02.083

30. Wang Q, Guo S, Hou Z, Lin H, Liang H, Wang L *et al*. Rainfall facilitates the transmission and proliferation of antibiotic resistance genes from ambient air to soil. Sci Total Environ. 2021; 799: 149260. DOI: https://doi.org/10.1016/j.scitotenv.2021.149260