



**SPREADING BACTERIA AND RESISTANCE FROM FARMS INTO HUMAN
COMMUNITY: A GLANCE ON THE ROLE OF POULTRIES' FARMING IN SOUTH
CAMEROON**

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ABSTRACT

Antimicrobial resistance has become a major global health concern for its alarming growth which couples with emergence of life-threatening microbial conditions requiring antimicrobial agents for their control. The present work conducted in southern Cameroon focused on antibacterial agents used in poultry farms, the susceptibility profiles of isolates from these farms and their connections with findings in the neighboring human community's bacterial populations. Each participant signed an informed consent prior to questionnaire filling and specimen collection. Specimen collection was conducted by fingerprinting in farmers and in community members. Isolation, identification and susceptibility tests were carried out according to standard guidelines (REMIC 2018 and CA-SFM 2019). Data analysis revealed that most common antibacterial agents used in the farms belonged to the groups of beta-lactams (40%) and quinolones (20%). In addition, all farm respondents were aware of the role that antimicrobial agents could play in infection control, acknowledged that their misuse could reduce their effectiveness; but could not state any connection with human health. A total of 342 bacterial isolates were recovered from 250 specimens (154 from farms and 188 from the neighboring community). Most common isolates were members of the *Staphylococcaceae* family and more precisely, from the genus *Staphylococcus* (49% from farms and 81% from surrounding populations), followed by *Enterobacteriaceae* (42% from farms and 19% from surrounding populations). Invariably, highest resistance rates were recorded with common drugs: Co-trimoxazole, Ceftriaxone, Amoxicillin/Clavulanic acid (100%, each), Ceftriaxone (90%) and Tetracycline (88%). Combined data analysis further highlighted putative germs communication from farms into the neighborhood, favored by the low hygiene standards that obviously facilitated the spread of resistant bacteria from the farms into the human communities (primarily Gram-positive's), and misuse of engines for resistance selections. Collectively, these findings stressed urgent needs for better farm management with refreshed human resources.

KEYWORDS: Bacteria susceptibility profile, spread, farm, Human community.

INTRODUCTION

Microbial resistance (MR) became a global health challenge for infectious diseases management by the 1980s with increased rates of drug-resistant bacteria primarily recoded with beta-lactam antibiotics. Its impacts and amplitudes vary from one setting to the other, in connection with contextual determinants that include the general population welfare, hygiene and related living standards, educational background and other direct and indirect resources for health. Overall,

MR refers to the ability of microorganisms to perpetuate and guarantee fitness in the presence of deleterious agents. This inherent phenomenon has been exacerbated by anthropogenic factors that eventually made drugs ineffective in the caretaking of infections in several instances.^[1] For instance, misuse of antibiotics generates selection pressure in professional and opportunistic infection etiologies which are, for the very large majority members of host's endogenous microbial flora. These organisms generally take advantage of the

reduced host immunity to cause what is referred to as infectious diseases (IDs).^[2] The WHO's Global Antimicrobial Resistance Surveillance System (GLASS) observed that the most common resistant bacteria in human infectious disorders included primarily *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* and *Salmonella*.^[1] Reporting the high resistance rates to several bacterial infections in high- and low-income countries, the GLASS admitted that antibiotic resistance was a widespread issue as it affects 500,000 people throughout 22 countries in which antibacterial agents have been used to fight against bacterial infections since the 1950s.

Beyond healthcare facilities where antibacterial agents are commonly used for IDs therapy and prophylaxis^[3], the use of antibiotics in animal feed/food has also developed considerably for growth supplementation, though the real role is yet to address comprehensively beside the virtues of hygiene in animal farms.^[4,5] Nowadays also, investigation interests into crop production with antimicrobial agents might provide powerful arguments that could be used to discuss and better address MR at the global scale. The current One Health's paradigm that encompasses humans, animals and the environment offers subtle but precious opportunities to understanding the impacts and sequences of events in each environmental unit on the others. Otherwise, resistance growth in animal and plant farms would have significant impacts on human both in community and hospital acquired bacterial infections.^[4,6,7] More and more in fact, animal farms are recognized as sources of resistant bacteria that affect human populations. Accordingly, the development and spread of resistance from animal husbandry into human populations could be real sources of concern in human medicine.

The increasing rates of antibiotic-resistant infections that couple with reduced opportunities for new antibiotics accentuate the global public health threat posed by the growing number of exposed peoples. Hence, innovating approaches to reduce the current trends of antibiotic resistance is necessary at both local and global scales. Such initiatives are primordial in that, the growing threats associated with antimicrobial resistance (AMR) emerge and spread beyond borders and predictions. One such threat is the current COVID-19 global crisis that requires the use of antibiotics in the drug regimens used in infection control; in connection with the putative role that endogenous opportunistic bacteria play in disease progression. Others such infections are those caused by influenza virus (N1H1) and the range of others etiologies of upper and lower respiratory tract infections around the world.

In Cameroon, previous investigations on antibacterial resistance (ABR) globally focused on hospital and farms.^[3,5] The authors observed that selection potential of farms was high and that improved hygiene and appropriate use of antibiotic could mitigate the selection pressure

and dissemination of resistant strains. The present survey was carried out as a continuation of a series of studies that will elucidate major segments in germ transmission from farm to human communities. Key variables were based on resistotyping and relied on susceptibility profile from farmers *versus* susceptibility profile from the neighborhood human community.

MATERIAL AND METHODS

Type, location of study and ethical consideration

This experimental survey was conducted from January 17th through April 19th, 2019. To get started, an ethical document was delivered by the institutional ethical committee of the "Université des Montagnes" (Reference N°: 2019/180/UdM/PR/CIE). The technical research authorization was also obtained from the Head of the "Université des Montagnes" Teaching Hospital (Reference N°: 2019/083/AED/CUM) for the activities to be carried out in the premises of its Laboratory of Microbiology. The voluntary and informed consent of each participant was obtained upon arrival at the farms and the community.

Specimen collection and laboratory analyses

The specimens submitted to screening originated from poultry farms in South Cameroon. After the voluntary and informed consent from each participant, the farmers had to fill out a questionnaire, which provided pieces of information on the management of their farms.

For the farmers, specimens were taken in the evening at the end of the activities (when they left the farm premises) by fingerprinting performed on MacConkey and Mannitol Salt agar (Liofichem®) in Petri dishes. Then, the preparations were transported in refrigerated containers ($\approx 4-8^{\circ}\text{C}$) to the Laboratory of Microbiology of the "Université des Montagnes" Teaching Hospital for further steps in the screening process.

In the laboratory, the streaked Petri dishes were incubated aerobically for 24 hours at 37°C . Upon completion of incubation, colonies isolation followed as recommended by standard protocols.^[20] Briefly for each colony to be identified, a macroscopic examination was made subsequent to Gram stain. Gram-negative rods (GNR) were further characterized with macro-gallery tests that included those revealed by Hajna Kligler (glucose and lactose fermentation, gas production, and hydrogen sulphide), Mannitol-mobility-nitrate medium, urea-indole-tryptophan deaminase, gelatin hydrolysis and, Voges-Proskauer tests. Identification of Gram-positive cocci (GPC) was carried out using the catalase, free coagulase, and DNase tests. For Gram-positive rods, identification was limited to Gram staining based on bacterial shape and spore types.

Antibiotic susceptibility tests

These tests were performed by the solid media diffusion method (Kirby-Bauer) with fresh (24 hours) pure subcultures performed on nutrient agar. For this purpose,

the bacterial suspension prepared in 0.9% sterile physiological saline was adjusted to the final density of 0.5 McFarland opacity scale, as recommended for susceptibility tests. All test procedures and interpretations were performed as recommended by the standard guidelines (“Comité de l'Antibiogramme de la Société Française de Microbiologie, CA-SFM, EUCAST, 2019”). The antibiotics tested were members of the families of drugs that are commonly used in humans and animals, provided by (Liofichem®). Namely, they were Penicillin G (10 µg), Imipenem (10 µg), Amoxicillin/Clavulanic Acid (20/10 µg), Amoxicillin (30 µg), Cefazidime (30 µg), Ceftriaxone (30 µg), Nitrofurantoin (300 µg), Cefuroxime (30 µg), Gentamicin (15 µg), Vancomycin (30 µg), Ciprofloxacin (05 µg), Nalidixic Acid (30 µg), Trimethoprim/Sulfamethoxazol (1.25/23.75 µg), Oxacillin (01 µg), Erythromycin (15 µg), Norfloxacin (5 µg), Levofloxacin (5 µg), Tetracycline (30 µg), Aztreonam (30 µg) And Clindamycin (2 µg).

For identification and susceptibility testing, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were used as reference strains for quality control.

Data Analysis

Data were recorded in Microsoft Excel 2010, while their analyses were conducted with Stview software tools. The focus were basically on the distribution of antibiotics and antibiotic susceptibility test profiles of bacterial isolated from farm workers, and antibiotic susceptibility test profiles of bacterial populations isolated from the neighborhood human community.

RESULTS

Farmers' knowledge, attitudes and practices

Subsequent to data collection, overall analyses indicated that the average age of the farmers was 38 years, with the range documented between 31 and 45 years.

Table I: Bacteria from farmers' and neighboring community members.

| Bacterial type | Fingerprinting | |
|-------------------------------|-------------------|-----------------------------|
| | Farmers (n = 188) | Community members (n = 154) |
| | Number (%) | Number (%) |
| <i>Enterobacter aerogenes</i> | 00 (00) | 06 (03) |
| <i>Enterobacter cloacae</i> | 21 (14) | 16 (09) |
| <i>Escherichia coli</i> | 30 (20) | 08 (04) |
| <i>Proteus spp.</i> | 00 (00) | 02 (01) |
| <i>Salmonella spp.</i> | 14 (09) | 04 (02) |
| GPR | 14 (09) | 00 (00) |
| CNS | 53 (34) | 70 (37) |
| <i>Staphylococcus aureus</i> | 22 (14) | 82 (44) |
| Total | 154 (100) | 188 (100) |

GPR: Gram-positive rod; CNS: coagulase-negative *Staphylococcus*

The overall picture revealed relatively diversified bacterial populations. Higher diversity was recorded in the neighboring community. More specifically, Gram-negative rods (GNR) were more frequently isolated from

A focus on sanitation, health management and biosecurity on farms identified antimicrobial agents like bleach and/or TH4 disinfectant and/or cresyl that were used for decontamination and cleaning. None of the farms had a sanitary SAS for disinfection prior to farm entry but, a disinfection tank was available at the entrance everywhere. For water provision the available system in all farms was associated with well resources.

Closed to 60% of antibiotics belonged to beta-lactams (40%) and fluoroquinolones (20%). Cyclins, furans, aminosids and macrolids accounted for 10% each. Basically, antibacterial agents that were commonly used included Oxytetracyclin, Ciprofloxacin, Flumequine, Furaltadone, Gentamicin, Erythromycin, Penicillin, Oxacillin, Ceftriaxone and Amoxicillin. The administration frequencies and posology varied from one farm to the other. But most commonly, antibiotics were passed through animal drinking water and food. All respondents were aware of the role that antimicrobial agents could play as growth could play as growth supplements and anti-infectious agents in microbial diseases prevention and/or control. They also recognized that the overuse of these agents could reduce drug effectiveness. None had ever undergone refreshment courses for competence improvement.

Bacterial populations and susceptibility profiles

The total of 250 specimens was collected (112 from farmers and 138 from the neighboring human community's members). From these specimens, 342 bacterial isolates were recovered and identified. Further pieces of information on the distribution of bacterial populations were summarized and displayed in table I.

farms specimens and GPC from the community. In both contexts, however, GPC overwhelmed the overall isolation rates. Other details indicated that GNR diversity was higher in the community.

Susceptibility profile trends

The susceptibility profiles recorded in both areas were summarized as shown in table II.

Table II: Susceptibility profile of bacteria from farmers and from community members.

| Antibiotics | Fingerprinting | | | | | | | | | | | | | | |
|------------------------|----------------|-----|----|---------------|-----|----|---------------|-----|----|---------------|-----|-----|---------------|-----|----|
| | Farmers | | | | | | | | | Community | | | | | |
| | Bacteria types | | | | | | | | | | | | | | |
| | GNR (n=65) | | | GPC (n=75) | | | GPR (n=14) | | | GNR (n=36) | | | GPC (n=152) | | |
| | Phenotype (%) | | | Phenotype (%) | | | Phenotype (%) | | | Phenotype (%) | | | Phenotype (%) | | |
| I | R | S | I | R | S | I | R | S | I | R | S | I | R | S | |
| Amoxicillin (30 µg) | 26 | 66 | 08 | NT | NT | NT | NT | NT | NT | 37 | 00 | 63 | NT | NT | NT |
| AMC (20/10 µg) | 13 | 26 | 61 | 26 | 29 | 45 | 13 | 26 | 61 | 62 | 00 | 30 | 27 | 11 | 62 |
| Aztreonam (30 µg) | NT | NT | NT | 07 | 92 | 01 | 10 | 81 | 09 | NT | NT | NT | 02 | 94 | 04 |
| Ceftazidime (30 µg) | 13 | 86 | 01 | 06 | 84 | 10 | 10 | 90 | 00 | 00 | 100 | 00 | 01 | 99 | 00 |
| Ceftriaxone (30 µg) | 08 | 91 | 01 | 03 | 95 | 02 | 00 | 100 | 00 | 19 | 81 | 00 | 00 | 100 | 00 |
| Cefuroxime (30 µg) | 28 | 71 | 01 | 00 | 100 | 00 | 00 | 100 | 00 | 75 | 25 | 00 | 20 | 78 | 02 |
| Ciprofloxacin (5 µg) | 16 | 79 | 07 | NT | NT | NT | NT | NT | NT | 14 | 00 | 86 | NT | NT | NT |
| Clindamycin (2 µg) | NT | NT | NT | 25 | 64 | 11 | 00 | 75 | 25 | NT | NT | NT | 38 | 27 | 35 |
| Erythromycin (15 µg) | 06 | 91 | 03 | 05 | 89 | 06 | 00 | 93 | 06 | 00 | 00 | 100 | 01 | 99 | 00 |
| Gentamicin (15 µg) | 50 | 10 | 40 | 37 | 13 | 50 | 35 | 29 | 36 | 33 | 22 | 45 | 57 | 11 | 32 |
| Imipenem (10 µg) | 29 | 64 | 07 | 35 | 50 | 15 | 32 | 45 | 23 | 35 | 18 | 47 | 60 | 17 | 23 |
| Levofloxacin (5 µg) | 00 | 100 | 00 | 13 | 83 | 04 | 10 | 81 | 09 | 15 | 80 | 05 | 48 | 20 | 32 |
| Nalidixic acid (30 µg) | 34 | 65 | 01 | NT | NT | NT | NT | NT | NT | 50 | 00 | 50 | NT | NT | NT |
| Nitrofurantoin 300 µg) | 35 | 61 | 05 | NT | N | NT | NT | NT | NT | 19 | 00 | 81 | NT | NT | NT |
| Norfloxacin (10 µg) | 12 | 85 | 03 | 27 | 57 | 16 | 39 | 45 | 16 | 47 | 53 | 00 | 38 | 44 | 56 |
| Oxacillin (1 µg) | NT | NT | NT | 20 | 27 | 53 | 29 | 26 | 45 | NT | NT | NT | 06 | 13 | 81 |
| Penicillin G (10 U) | NT | NT | NT | 23 | 15 | 62 | 23 | 13 | 64 | NT | NT | NT | 16 | 10 | 74 |
| Tetracycline (30 µg) | 15 | 82 | 03 | 18 | 62 | 19 | 50 | 50 | 00 | 50 | 50 | 00 | 10 | 85 | 05 |
| SXT (1.25/23.75 µg) | 16 | 82 | 02 | 00 | 97 | 03 | 03 | 94 | 03 | 00 | 100 | 00 | 18 | 81 | 01 |
| Vancomycin (30 µg) | NT | NT | NT | 42 | 34 | 24 | 35 | 29 | 36 | NT | NT | NT | 57 | 11 | 32 |

AMC: Amoxicillin/clavulanic acid; SXT: Trimethoprim/Sulfamethoxazol; GNR: Gram-negative rods; GPR: Gram-positive rods; GPC: Gram-positive cocci; S: susceptibility; R: resistant; I: intermediate; NT: Not tested

A glance throughout data indicated that the overall rates of resistant GNR isolates against beta-lactam, macrolides, quinolones, cyclins and sulfonamides were higher in farms than in the community. Among GPC, rates of resistance were higher in the community than in farms, typically with beta-lactams, macrolides and cyclins.

Furthermore, resistance rates larger than 50% were observed for 87% of the antibiotics tested on GNR isolated from farmers while in the community, this rate was 46%. Similarly for GPC, those above 50% were recorded for 62.5% of the antibiotics from farms *versus* 46.6% of the antibacterial drugs in the community.

DISCUSSION

The present survey on farm and community bacteria disclosed relevant facts on species diversity and antimicrobial susceptibility profiles. Relative modification in bacterial populations between both settings could be in connection with the quantitative and qualitative role played by biotic and abiotic features of the target ecosystems. Otherwise, the impact of selection engines as previously anticipated by Rreese *et al.*^[8], who observed that environmental stresses could be strongly associated with or, greatly influence microbial diversity.

No comprehensive list could be made of these engines of selection, but at first glance, they might primarily include disinfectants that were found at the entry of each farm and antimicrobial drugs used in animal food and drinking water. Putative inhibitory properties exerted by better fitted microorganisms on the extinction of other which are not well adapted should not be ruled out, however, as some might influence the growth of the others just as antibacterial agents do.^[9] Based on the number and types of antibiotics used in these farms therefore, this development could reasonably be predicted.

Antibiotics used in farms belonged to several therapeutic groups amongst which some were more common than the others. In this regards, beta-lactams and quinolones accounted for 60% of the total, quite the similar rate reported in human medicine. This commonness is likely consistent with enabling determinants like their availability and affordability, and in line with data from other farms and authors.^[4,7] In many developing countries like Cameroon, the use of antibacterial agents in animal breeding and crop production is not regulated. If in human medicine a few embryos of regulation tools exist (though not really followed-up) like initiatives are

yet to be undertaken in animal husbandry and crop production.

Further details indicated that other antibacterial agents like cyclins, furans, aminoglycosides and macrolids accounted for 10% each, with relevant potential at larger scale since each therapeutic group can, theoretically affects one or more aspects of bacterial physiology, and that antibiotics are commonly used either for prophylaxis or as growth supplement at lower doses in farms. The action of antibiotics on bacterial physiology could be testified by the resistance rates recorded in the present study.

As in humans^[10,11] arguments corroborating the frequent use of beta-lactam antibiotics in veterinary medicine include (but not limited to) variables like diversity, spectrum of action, availability, affordability, mode of administration and toxicity. The use of Cyclin (Oxytetracyclin in the present study) is consistent with the related arguments on drug availability and affordability.^[7] Antibacterial agents from this group are broad spectrum antibiotics, very common in infection control in farms. Unlike cyclins, furans are less available probably because of the ban that is in force in food-producing animals like poultry in many countries around the world.^[10] Like developments have previously been documented.^[4,12]

Overall, *Staphylococcus* rates were highest (49% in farm workers and 81% within the surrounding populations), followed by *Enterobacteriaceae* (42%, and 19%, respectively). This site-dependent distribution appeared to be in line with specific ecologic parameters as introduced above. This profile diversity could be associated with farm characteristics which are also activity-dependent. In fact, farmers are exposed to larger amounts of animals' wastes which are suitable environments for microbial growth, profile selection and diversification.^[13,14,15] The rates of Gram-positive cocci in both settings could therefore, be inherent to their ability to tolerate harsher environmental conditions like drought.

This ability is chiefly provided by the overall cellular organization that consists of a thicker cell wall, the ability to form resistance spores in some cases and the affinity they express with molecular oxygen^[13-15] consistent with ubiquity. Often associated with poor sanitation in poultries, this potential of Gram-positive bacteria was also documented in Morocco when El Ouahabi^[16] isolated and identified *S. aureus* in the excreta of broilers reared on farms with substandard hygiene. In addition to the typical Gram-positive envelope, *Bacillus* spp., are spore-forming and can express higher level of resistance to harsher environmental stresses than *Staphylococcaceae* and *Enterobacteriaceae* do, in line with previous reports.^[12,15]

Bacillus would, otherwise, be expected to overwhelm *Staphylococcus* populations in this instance. The reason

why this was not observed remains obscure (providing that some *Bacillus* can produce antibacterial agents that inhibit the growth of other bacterial populations) but could be strongly related to the "allocation cost theory" of resistance that rather resulted in population decrease.^[9] The lower bacterial diversity in farms could therefore be justified, at least partially, by the negative selection pressure imposed by antimicrobial agents, but the inhibition potentials of other bacterial types like *Bacillus* could also be safely be pointed out. The presence of *Enterobacteriaceae* in both groups of participants actually corroborates the low hygiene standards, testified by the presence of fecal coliforms. One consequence of low hygiene is, for instance, the salient rates of *Salmonella* (9% for farm workers and 2% for the neighborhood population). As important zoonotic disease etiologies, *Salmonella* infection can be associated with heavy economic burden in farms and in exposed human populations. A previous survey in Senegal reported 70% of *Salmonella*.^[10] Together and in connection with contextual implementation of minimal biosafety and biosecurity principles, these findings stress the need for better farm management. Otherwise, pathogens like *Salmonella* in farmers do eventually disseminate throughout exposed human communities. That of non-pathogens should also be scrutinized as they might revert into opportunistic disease etiologies in contexts of immune-depression, into true pathogens upon mutation and/or horizontal transfer of genes, or serve as vectors of resistance gene transfer in microbial populations.^[14,15]

With regards to susceptibility trends, resistance rates were higher in isolates from farms. These rates were similar to those documented from farm wastes in West Cameroon.^[4] Theoretically (though the rates slightly differed in the presence study between the two settings), this finding predicts greater likelihood for resistant traits transfer from the farm premises through farmers into the community, also consistent with previous findings^[2,17], but not sufficient to rule out that the resistance recorded in the neighboring human community originated exclusively from farms. In fact, and though not addressed in the present survey, community members use antimicrobials that could cause, at varied amplitude like effects. Overall, in instance of poor sanitation, animal husbandry contributes to selection of adaptive traits like virulence and resistance that eventually spread and undermine infection control in animals in humans. Evidences arise from the higher resistance rates recorded with most common antibiotics in both groups during the present survey. Other levels of resistance could be justified by co-resistance and/or cross-resistance phenomena. Previous studies in hospital environment^[3,18] revealed that resistant bacteria likely colonize the hospital environment and healthcare personnel. The same scenario could be observed on farms and amongst farmers with varying degrees of exposure.^[4,5]

Current trends globally reveal alarming resistance rates amongst bacteria and stress urgent implementation of mitigation policies. Primarily these include implementation of resistance selection mitigation policies on farms, the development of contextual barriers to the spread of resistant microorganisms into human communities. The paramount importance of this assertion is based on the growing zoonotic microbial threats related to influenza and corona virus infections that could be regarded as pressing arguments.^[6] Respiratory tract infections caused by these viral particles require antibacterial agents in the disease control therapeutic arsenal used to prevent complications due to their life-threatening potential in connection with super-infection by resistant opportunistic bacteria.

In general and taking together humans, animals and their environment, through the “One Health” concept^[6], farmers' knowledge, attitudes and practices should be strengthened mainly on hygiene based on the observance of biosafety and biosecurity guidance to mitigate exposure to occupational and opportunistic hazards like true pathogens, but more importantly like resistant opportunistic disease etiologies, acknowledging that resistance genes could be transmitted to phylogenetically distant species.^[13,14,18,19]

CONCLUSION

The present investigation disclosed relevant facts on species diversity and antimicrobial susceptibility profiles in both areas. Most frequent bacterial types were Gram-positive while beta-lactams and quinolones accounted for 60% of the antibiotics frequently used by farmers. These higher rates of Gram-positive bacteria were thought to be in connection with their inherent ability to tolerate harsher environmental conditions like drought and to produce resistance spores. Resistance rates in both settings were also invariably high, regardless invariable high, regardless of bacterial types. Furthermore, isolation of fecal coliforms in both settings typically corroborates the low hygiene standards that likely favored the spread of resistant potential disease etiologies from farms into human communities; primarily Gram-positive's and stresses urgent needs for better farm management.

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