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Impact of Host and Risk Exposures on the Prevalence of Resistance in Enterobacteriaceae: The Case of Humans and Broilers in the West Region of Cameroon

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Abstract

The "One Health" concept holds that human health is closely linked to that of the environment. For this reason, the fight against health problems such as antibiotic resistance must have a holistic approach involving human health as well as animal and environmental health. This work aimed to compare the impact of host and selected risk factors on the prevalence of resistance and resistance gene carriage in Enterobacteriaceae isolates from broilers, a key source of proteins, and from human subjects, and determine the genetic relatedness of the isolates as a zoonotic link. From this analysis, it was seen that the host type was a determinant in the resistance outcomes, with higher prevalences of resistance to quinolones and aminoglycosides and plasmid-borne resistance gene carriage in broiler isolates. However, clinical isolates showed a higher prevalence of extended spectrum beta lactamase production and multidrug resistance. Genotyping of E. coli showed genetic relatedness between human and poultry isolates, a proof of zoonotic circulation of the bacteria, thus the need to combat antibiotic resistance from a One Health perspective.

Keywords: Enterobacteriaceae, Host, Antibiotic Resistance, Genetic Relatedness and One Health

Introduction

The "One Health" concept of the World Health Organization (WHO) Food and Agricultural Organisation (FAO) and World Organisation for Animal Health (WOAH) holds that human health is closely linked to that of the environment, including animals used as food. As such, solutions to health concerns such as antibiotic resistance must have a holistic approach involving human health as well as animal and environmental health [1, 2].

The development of resistance can be an adaptation to environmental factors, including stress from bactericidal elements like antibiotics as well as horizontal gene transfer in luxuriant microbial communities. In animal farms, the emergence of antibiotic resistance involves mainly the misuse of antibiotics and conditions of insalubrity [3-6].

The abusive use of antibiotics drives positive selection for resistant mutants. These antibiotics end up in farm waste in sub-lethal doses, which expose bacteria in the waste to stress, thereby inducing shuffling, which activates new resistance genes [3, 7]. Insalubrity in farmhouses promotes extensive infection of the animals, enabling horizontal gene transfer by means of mobile genetic elements like plasmids [4, 8].

In humans, the development of antibiotic resistance is driven by the misuse of antibiotics due to overprescription, undermedication, and self-medication [9]. Resistant strains from animal farms can be transmitted to humans by several means, including direct contact, contact during evisceration and cleaning abattoirs, improperly cooked animal products, or when farm waste is used in gardening to grow vegetables [10, 11].

On the other hand, humans can also pass resistant strains to animals, mainly through contaminated water and feed [12]. Enterobacteriaceae is a family of clinically important bacteria, accounting for approximately 80% of Gram-negative bacilli-related diseases and 50% of bacterial diseases in clinical settings [13]. Members have a broad ability to develop ABR due to a plethora of plasmid-mediated resistance genes and a high capacity for mutation during environmental stress, such as antibiotic exposure at sub-lethal doses [7, 14].

These have made Enterobacteriaceae a serious threat to public health due to the association of different resistance mechanisms. For example, Escherichia coli and Klebsiella pneumoniae were the 1st and 3rd leading deadly pathogens, respectively, among the six leading mortal pathogens associated with resistance in 2019 [15, 16].

These leave us to postulate that the same bacterium in the system of a farm animal and in that of a human may be subjected to varied conditions that may drive differential acquisition of resistance. It is still undetermined whether these pathogens with the potential to circulate are the same strains developing in humans and broilers. We sought to evaluate the impact of selected factors on the prevalence of antibiotic resistance in Enterobacteriaceae and resistance gene carriage in broilers, a key source of proteins, and in humans, as well as the genetic relatedness of human and broiler isolates and the possibility of zoonotic transmission of resistant bacteria in this family.

Materials and Methods

Study Subjects and Site

This study was done using information from a database on Enterobacteriaceae isolates obtained from two studies, one on broilers and the other on humans carried out in the same geographical location and the same time frame. The study on broilers involved the molecular profiling of antibiotic resistance in Enterobacteriaceae from cloacal swabs, while the study on humans involved the molecular epidemiology of resistance genes in Enterobacteriaceae isolated from stool samples of febrile patients. Both studies were carried out in the West Region of Cameroon; sampling was done between the years 2019 and 2020. Parts of these studies have been published [17, 18].

The West Region is the smallest of the ten Regions in Cameroon but has the highest population density and is located in the north-western part of the country. Trading, agriculture, and animal husbandry are the main economic activities, with animal droppings from farms systematically used as manure. The region is also a cultural hotspot, with regular traditional and cultural celebrations, including funeral celebrations, always associated with mass catering.

Selection of Isolates

We randomly selected two groups each of 300 Enterobacteriaceae isolates, obtained from broiler cloacal swabs and human stool samples. The number of isolates selected for each genus was proportional to the total isolates for the genus. The human isolates were gotten from 278 patients, while the broiler isolates were gotten from 227 healthy broilers. These selected isolates were distributed as shown in Table 1.

Table 1: Distribution of selected Enterobacteriaceae isolates

Genus	Isolates from Broilers	Clinical isolates
Escherichia coli	63	66
Salmonella spp	58	65
Klebsiella spp	30	20
Proteus spp	30	50
Citrobacter spp	26	34
Enterobacter spp	24	10
Providencia spp	22	18
Hafnia spp	15	0
Shigella spp	12	11
Raoultella spp	11	0
Yersinia spp	10	0
Serratia spp	0	13
Edwarsiella spp	0	13
Total	300	300

Characteristics of the Isolates

The studies providing the isolates evaluated risk exposure in subjects and the antibiotic susceptibility patterns of these bacteria against the main antibiotic families used in the treatment of Enterobacteriaceae-related diseases, including quinolones (ciprofloxacin and levofloxacin) and aminoglycosides (gentamicin and amikacin).

The antibiotic susceptibility tests were performed by the disc diffusion method. Extended-spectrum beta lactamase (ESBL) production was determined by a double-disc synergy test using the antibiotics amoxicillin/clavulanic acid, ceftriaxone, cefotaxime, and ceftazidime. Multidrug resistance (MDR) was defined as resistance to antibiotics in two or more families of the antibiotics studied.

The resistant isolates were analysed for the carriage of plasmid-borne resistance genes by polymerase chain reaction (PCR), including the ESBL genes blaTEM, blaTEM-1, blaTEM-2, blaCTX-M, blaSHV-1, and blaKPC, the plasmid-mediated quinolone resistance (PMQR) genes qnrA, qnrS, qnrB, aac(6)-IB-CR, and qepA, and the plasmid-mediated aminoglycoside resistance (PMAR) genes aph(3′)-IA, ant(2′)-IA, and aac(6′)-IB.

Comparisons of Risks Versus Resistance outcomes and Resistance Gene Carriage

Comparisons of frequencies were done overall across the family without going to the genus or species so as to get the general trends of variations of the various factors evaluated across the family. We selected risk factors with relative comparison that could bring out useful relationships in correlation with the resistance outcomes in isolates. Some risks are a combination of several risks, with a subject considered exposed if s/he or it has at least an exposure to one, as described as:

- **Unsure water:** Any water source apart from pipe-borne water and commercial bottled water.
- **Use of antibiotics:** Self-medication or prescribed use in the last month for humans and use in feed for broilers.
- **Contact with faeces:** Humans with no latrine or toilet who do not wash their hands after visiting the toilet.
- **Poor sanitation:** Conditions of the environment such as litter, stagnant sewage, nature of latrine, rearing of animals around the residence.
- **Poor food hygiene:** Risky food habits like eating raw or undercooked food, eating outdoors, improper or no washing of vegetables and salads, non-regular washing of feeders, and evacuation of flow materials.
- **Age:** youths ≤18 years and adults >18 years for humans; ≤30 days and >30 days for chicken – long rearing periods.

The risks were correlated with five resistance outcomes, including resistance to at least one family of antibiotics ("Resistance"), MDR, ESBL production, resistance to Quinolones, and Aminoglycosides. We proceeded to compare the prevalences of plasmid-borne resistance genes in human and broiler isolates.

Evaluation of the Genetic Relatedness of Isolates

For the above comparisons to be useful from a One Health perspective, we proceeded to determine the genetic relatedness of the human and broiler Enterobacteriaceae isolates. To do this, we chose E. coli, a well-representative member of the Enterobacteriaceae and a WHO priority pathogen [19]. The phylogenetic relationship was studied by the technique of Enterobacteriaceae intergenic consensus polymerase chain reaction (ERIC-PCR).

Though this technique is not the gold standard for genotyping, it has been proven to be effective, repetitive, and cost-effective [20, 21]. The identity of E. coli isolates was confirmed by PCR using genus-specific primers for the 16S rRNA gene. The oligonucleotides for the amplification of the 16S rRNA gene were ECP79-F: GAAGCTTGCTTCTTTGCT and ECR620-R: GAGCCCGGGGATTTCACAT amplifying at an amplicon size of 544 base pairs (Sabat et al., 2000). ERIC sequences were amplified using the oligonucleotides ERIC-F: ATGTAAGCTCCTGGGGATTCAC and ERIC-R: AAGTAAGTGACTGGGGTGAGCG [22, 23].

DNA was extracted from fresh overnight colonies by heat shock [18]. The composition of the reaction mix for the identification of E. coli by PCR and ERIC-PCR was adapted from procedures described by Leinyuy et al. (2022) and Alsultan and Elhadi (2022), as shown in Table 2. The DNA amplicons were migrated, resolved on a 1.5% agarose gel, and visualized using UV light.

Table 2: Composition of PCR reaction mix

Reagent	Final volume = 25 µl			
	Identification of E. coli		ERIC-PCR	
	Volume	Final concentration	Volume	Final concentration
PCR grade water	14.9 µl	-	13.6 µl	-
1 X standard Taq buffer solution with 2.5mM MgCl2	2.5 µl	1X	2.5 µl	1X
Forward primer	1 µl	0.4 µM	1.5 µl	0.6 µM
Reverse primer	1 µl	0.4 µM	1.5 µl	0.6 µM
DNTP mix	0.5 µl	200 µM	0.7 µl	280 µM
Taq polymerase	0.1 µl	0.2 Units/µl	0.2 µl	0.4 Units/µl
DNA solution,	5 µl	-	5 µl	-

Statistical operations were carried out using IBM SPSS Statistics 20®. P-values were considered significant at p≤0.05 with a 95% confidence interval (CI). Proportions were considered to be different for z and t scores with magnitudes above 1.96. ERIC-PCR gel images were analysed using Gel Analyser. Hierarchical cluster analysis was done by Ward's method.

Results

Comparative Exposures of Subjects to Risk Factors

The comparative exposures to the risk factors evaluated in human and broiler subjects are given in Table 3.

Table 3: Comparative Exposure of Subjects to Risk Factors

Evaluated risk	Number (N) of subjects		Percentage of subjects exposed	
	Human subjects	Chicken subjects	Human subjects n (%)	Chicken subjects n (%)
Unsure water	278	227	185 (66.55)	160 (70.48)
Use of antibiotics			91 (32.73)	195 (85.90)
Contact with faeces			59 (21.22)	227 (100.00)
Poor Sanitation			147 (52.88)	207 (91.19)
Poor Food hygiene			238 (85.61)	227 (100.00)
Age			251 (90.29) – Adults	116 (51.10) – Old

n = number of subjects exposed to a particular risk

The practices in poultry farms involving episodic evacuation of floor materials have made it such that the birds are always in contact with their faeces, which constantly mix with feed. Thus, these risks become constant for poultry. There was extensive use of antibiotics in farmhouses.

Comparison of Patterns of Resistance in Isolates

The comparison of resistance trends against the various antibiotic families and MDR is given in Table 4.

Table 4: Comparison of Resistance Patterns in Isolates

	Resistance		N	Isolates from human subjects n (%)	Isolates from chicken subjects n (%)	z score	p-value
1.	Resistance to Quinolones	General		107 (35.67)	131 (43.67)	-2.0028	0.02275
		Ciprofloxacin		100 (33.33)	108 (36.00)	-0.6863	0.2451
		Levofloxacin		59 (19.67)	94 (31.33)	-3.2783	0.00052
2.	Resistance to Aminoglycosides	General		32 (10.67)	49 (16.33)	-2.0309	0.02118
		Gentamicin		28 (9.33)	44 (14.67)	-2.0101	0.02222
		Amikacin		31 (10.33)	09 (3.00)	3.6006	0.00016
3.	ESBL production			89 (29.67)	63 (21.00)	2.4406	0.00734
4.	Resistant to at least 1 antibiotic class			97 (32.33)	156 (52.00)	-4.8776	<0.00001
5.	MDR			167 (55.67)	139 (46.33)	2.2866	0.01101

N = total number of isolates, n = number of resistant isolates.

Table 4 shows a higher resistance rate to quinolones and aminoglycosides in broiler isolates than in clinical isolates. On the contrary, ESBL production and MDR were higher in clinical isolates than broiler isolates. The z score and p-values show significant differences between the prevalences of resistance outcomes.

Correlations between Risk Factors in Subjects and Resistance outcomes in Enterobacteriaceae Isolates

The correlation between the evaluated risks and the five resistance outcomes is presented in Table 5.

Table 5: Correlations between Risk Factors in Subjects and Resistance outcomes in Enterobacteriaceae isolates

1. Resistance to at least one family of antibiotics				
	Human subjects		Chicken subjects	
	Odds ratios	p-value	Odds ratios	p-value
Unsure water	0.790 (0.470 - 1.326)	0.373	1.650 (1.001 - 2.718)	0.049
Use of antibiotics	5.856 (3.414 - 10.045)	≤0.0001	1.74 (0.901 – 3.346)	0.097
Contact with faeces	2.647 (1.493 - 4.693)	≤0.0001	No statistics are computed because contact with faeces is a constant.	
Poor Sanitation	1.488 (0.759 - 2.919)	0.644	(0.366 (0.158 - 0.850)	0.016
Poor Food hygiene	0.905 (0.441 1.855)	0.786	No statistics are computed because poor food hygiene is a constant.	
Age	0.931 (0.406 2.132)	0.866	11.792 (6.792-20.474)	≤0.0001

2. MDR				
	Human subjects		Chicken subjects	
	Odds ratios	p-value	Odds ratios	p-value
Unsure water	1.228 (0.611 – 2.644)	0.565	2.102 (1.255 - 3.520)	0.004
Use of antibiotics	5.924 (2.965 – 11.839)	≤0.0001	1.666 (0.847 - 3.277)	0.138
Contact with faeces	5.095 (2.588 -10.030)	0.023	No statistics are computed because Contact with faeces is a constant.	
Poor Sanitation	0.749 (0.391 – 1.433)	0.383	3.144 (1.225 - 8.070)	0.013
Poor Food hygiene	1.156 (0.479 – 2.791)	0.748	No statistics are computed because poor food hygiene is a constant.	
Age	1.082 (0.358 – 3.276)	0.889	8.110 (4.817- 13.655)	≤0.0001
3. ESBL production				
	Human subjects		Chicken subjects	
	Odds ratios	p-value	Odds ratios	p-value
Unsure water	0.617 (0.361 – 1.054)	0.076	0.883 (0.485 - 1.609)	0.686
Use of antibiotics	6.214 (3.542 – 10.903)	≤0.0001	1.151 (0.504 - 2.630)	0.739
Contact with faeces	2.268 (1.259 – 4.088)	0.006	No statistics are computed because Contact with faeces is a constant.	
Poor Sanitation	0.920 (0.547 – 1.547)	0.754	0.876 (0.336 - 2.282)	0.786
Poor Food hygiene	0.623 (0.275 – 1.409)	0.253	No statistics are computed because poor food hygiene is a constant.	
Age	1.095 (0.448 – 2.673)	0.843	2.674 (1.474 - 4.850)	0.001
4. Resistance to Quinolones				
	Human subjects		Chicken subjects	
	Odds ratios	p-value	Odds ratios	p-value
Unsure water	0.868 (0.460 – 1.637)	0.663	2.584 (1.513 - 4.414)	0.000
Use of antibiotics	2.425 (1.307 – 4.497)	0.004	2.140 (1.049 - 4.366)	0.033
Contact with faeces	2.495 (1.289 – 4.827)	0.005	No statistics are computed because Contact with faeces is a constant.	
Poor Sanitation	1.272 (0.692 – 2.336)	0.439	4.752 (1.595 - 14.154)	0.002
Poor Food hygiene	0.968 (0.404 – 2.318)	0.942	No statistics are computed because poor food hygiene is a constant.	
Age	1.278 (0.424 – 3.847)	0.663	5.992 (3.605 - 9.961)	≤0.0001
5. Resistance to Aminoglycosides				
	Human subjects		Chicken subjects	
	Odds ratios	p-value	Odds ratios	p-value
Unsure water	1.657 (0.801 – 3.428)	0.171	0.846 (0.439 - 1.630)	0.618
Use of antibiotics	3.116 (1.631 – 5.956)	≤0.0001	1.200 (0.476 - 3.024)	0.700
Contact with faeces	2.379 (1.198 – 4.727)	0.011	No statistics are computed because Contact with faeces is a constant.	
Poor Sanitation	0.797 (0.420 – 1.512)	0.488	0.803 (0.288 - 2.244)	0.677
Poor Food hygiene	1.922 (0.870 – 4.245)	0.102	No statistics are computed because poor food hygiene is a constant.	
Age	1.590 (0.460 – 5.490)	0.462	2.773 (1.423 - 5.406)	0.002

Use of antibiotics (self-medication) and contact with faeces were strong risk factors.

They correlated significantly with all resistance outcomes for clinical isolates. Age tended to correlate with resistance outcomes in broilers. In broilers, age has a short time frame of between forty-five and sixty days; as such, variations in exposure to age as a risk tend to have an effect on resistance outcomes.

Evaluation of the Significance of Differences between Risk Variables and Resistance outcomes

The paired sample t test performed to evaluate the significance of differences between frequencies of exposure to the risk factors and resistance outcomes in human and broiler isolates gave the results in Table 6.

Table 6: Evaluation of the Significance of differences between Risk Factors and Resistance outcomes

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			Sig. (2-tailed)
					Lower	Upper		
Outcomes								
Pair 1	QR1 – QR2	0.270	0.615	0.036	0.200	0.340	7.603	0.000
Pair 2	Resistant1 - Resistant2	0.273	0.663	0.038	0.198	0.349	7.136	0.000
Pair 3	ESBL1 – ESBL2	-0.047	0.588	0.034	-0.113	0.020	-1.375	0.170
Pair 4	MDR1 – MDR2	0.317	0.620	0.036	0.246	0.387	8.847	0.000
Pair 5	AR1 - AR2	0.013	0.530	0.031	-0.047	0.074	0.436	0.663
Risk factors								
Pair 6	Antibiotics use1 - Antibiotic use2	0.533	0.569	0.033	0.469	0.598	16.246	0.000
Pair 7	Age1 – Age2	-0.390	0.588	0.034	-0.457	-0.323	-11.489	0.000
Pair 8	Poor sanitation1 – Sanitation2	0.443	0.584	0.034	0.377	0.510	13.145	0.000
Pair 9	Unsure water1 - Water source2	0.037	0.651	0.038	-0.037	0.111	0.976	0.330
Pair 10	Food hygiene1 - Food Hygiene2	-0.143	0.351	0.020	-0.183	-0.103	-7.073	0.000
Pair 11	Contact with faeces1 - Contact with faeces2	0.787	0.410	0.024	0.740	0.833	33.205	0.000

The subscript 1 represents the risk or resistance outcome in human and human isolates and 2 represents broilers and broiler isolates.

The paired sample t test shows that the frequencies of exposure to the risk factors and resistance outcomes were significantly different between human and broiler isolates except for the outcomes of resistance to at least one antibiotic, ESBL production, resistance to aminoglycosides, and the risk of unsure water.

Comparison of the Prevalence of Plasmid-Borne Resistance Genes

The comparison of the prevalences of plasmid-borne resistance genes, including ESBL genes, PMQR genes, and PMAR genes, is given in Table 7. The 2-proportion z scores show the significance of the differences between the prevalences in clinical isolates and in broiler isolates.

Table 7: Comparison of the prevalences of plasmid-borne resistance genes

Gene family	Gene	Number of human isolates	Prevalence in human isolates	Number of broiler isolates	Prevalence in broiler isolates	z score	p-value
ESBL genes	blaTEM	111	06 (05.41)	76	24 (31.58)	-4.7901	<0.00001
	blaTEM-1		29 (26.13)		53 (69.74)	-5.9031	<0.00001
	blaTEM-2		16 (14.41)		14 (18.42)	-0.7333	0.2327
	blaCTX-M		55 (49.55)		17 (22.37)	3.7518	0.00009
	BlaSHV-1		02 (01.80)		9 (11.84)	-2.8660	0.00205
	blaKPC		05 (04.50)		27 (35.52)	-5.5324	<0.00001
PMQR genes	qnrA	123	7 (05.69)	143	57 (39.86)	-6.5002	<0.00001
	qnrS		38 (30.89)		74 (51.74)	-3.4246	0.00013
	qnrB		19 (15.45)		29 (20.28)	-1.0219	0.15386
	aac(6')-IB-CR		47 (38.21)		84 (58.74)	-3.3392	0.00042
	qepA		12 (09.76)		28 (19.58)	-2.2350	0.01255
PMAR genes	aph(3′)-IA	32	09 (28.13)	52	18 (34.62)	-0.6185	0.26763
	ant(2′)-IA		05 (15.63)		11 (21.15)	-0.6267	0.26435
	aac(6′)-IB		08 (25.00)		36 (69.23)	-3.9417	0.00004

Globally, the prevalence of the genes across the three families was significantly different except for the blaTEM-2 ESBL gene, the qnrB PMQR gene, and the aph(3′)-IA and ant(2′)-IA PMAR genes. These genes generally had higher prevalences in chicken isolates than in human isolates.

Genetic Relatedness of Isolates

Sixty (60) and 53 E. coli isolates from broilers and humans, respectively, were identified by PCR. The amplification of ERIC sequences in these isolates showed varied sequences of bands of various sizes for each sample, from 88 base pairs to 1400 base pairs, as shown in Figure 1.

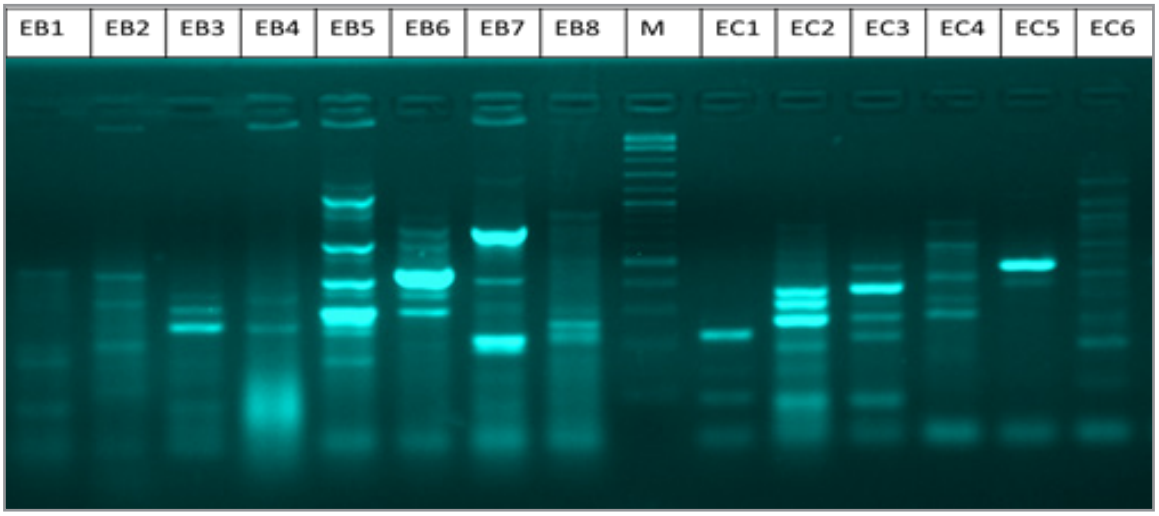

Figure 1: Gel Image Showing the Amplification of E. coli ERIC Sequences.

EB = E. coli from broilers; EC = E. coli from humans; M = molecular weight marker

Hierarchical cluster analysis of the length polymorphism gave the following dendrogram, Figure 2.

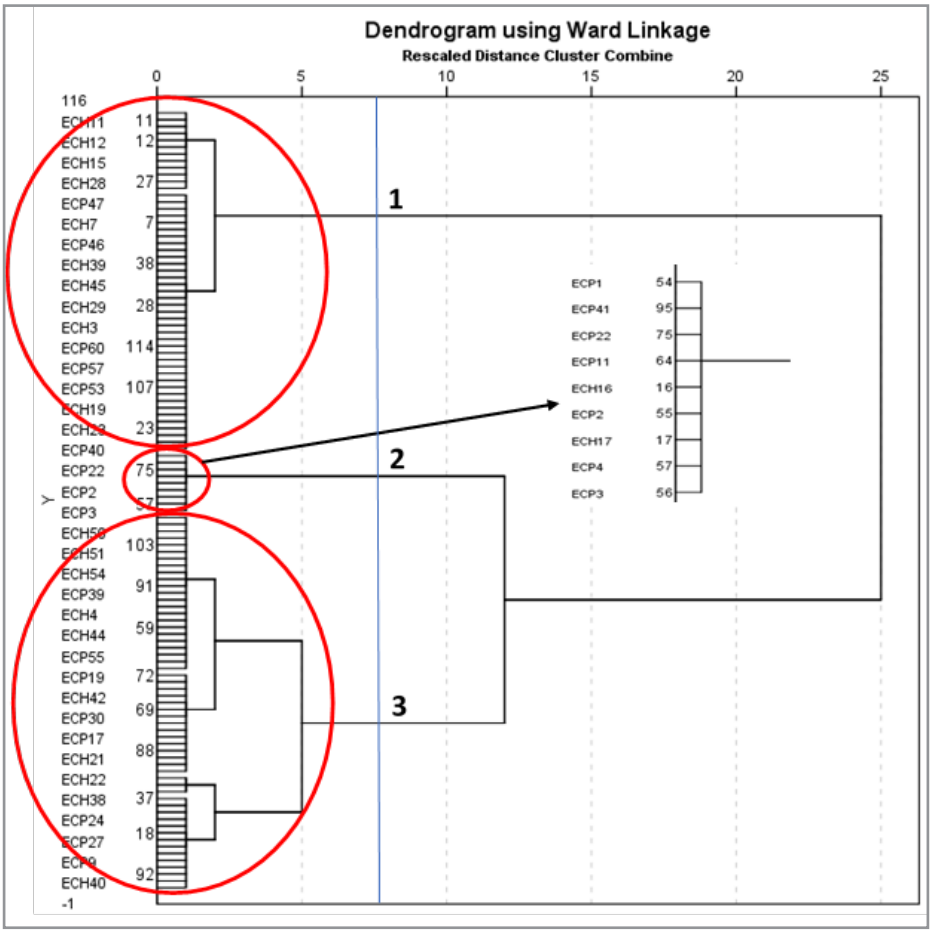

Figure 2: Dendrogram Showing the Genetic Relatedness of Clinical and Poultry E. coli isolates.

The red circles enclose the various clusters. The scale at the top indicates the Euclidean distances between clusters. Cluster 2 is expanded to show the constituent isolates.

The dendrogram shows an estimated three main clusters. Clusters 2 and 3, however, are part of a much larger cluster, with the Euclidean distance separating them large enough for them to be considered separate clusters. A detailed look at the three clusters shows that they all contain isolates from both humans and poultry. This shows that the isolates from both sources share a common genetic makeup. However, a basic look shows seven clusters, with two small clusters with only human isolates.

Discussion

This study sought to compare the effects of risk factors on resistance outcomes in Enterobacteriaceae isolates from broiler and human subjects, the prevalences of plasmid-mediated genes, and the genetic relatedness of the isolates as proof of the possibility of zoonotic sharing of resistant strains.

Use of antibiotics (self-medication) ($p \leq 0.0001$) and contact with faeces ($p \leq 0.0001$) were strong risk factors for the development of resistance in human isolates; they correlated significantly with all resistance outcomes. Self-medication usually involves practices where the patients practice undermedication, abandoning the antibiotic when there is relief without completing the dose [24].

This results in partial killing of even the susceptible isolates, exposing them to stress conditions under which they can reshuffle genes to resist the antibiotic as a survival strategy. It also involves overmedication with indiscriminate use of antibiotics at slight discomfort. In addition to stressing the bacteria without killing them, these practices also drive positive selection for resistant mutants [14, 25].

Contact with faeces increases the colonization of the gut, which allows gene sharing. Age is correlated with resistance outcomes in broilers. Here, age is interpreted as rearing periods, and thus old refers to long rearing periods. The correlation of the resistance outcomes with long rearing periods (>30 days) could be explained by the long duration of exposure to the risks, the time to get infected, or co-infection to allow horizontal gene transfers (Bolotin & Hershberg, 2017; Blumenthal, 2001). In broilers, age has a short time frame of between forty-five and sixty days; as such, variations in age as a risk tend to have an effect on resistance outcomes, unlike in humans, where old and young are a matter of decades.

A comparison of the frequencies of resistance outcomes showed a significantly higher resistance rate in broiler isolates against quinolones (43.67 against 35.67%, $p = 0.02275$) and aminoglycosides (16.33 against 10.67%, $p = 0.02118$). On the contrary, ESBL production (29.67 against 21.00%, $p = 0.00734$) and MDR (55.67 against 46.33%, $p = 0.01101$) were higher in clinical isolates. More broiler isolates showed resistance to at least one family of antibiotics (52.00 against 32.33%, $p = <0.00001$). It should be noted that quinolone and aminoglycoside resistance are largely mediated by plasmids, which are mobile genetic elements easily shared between species in a luxuriant community like the gut microbiota. Given that the broiler subjects were subjected to conditions favouring infection, they harbour extensive bacteria favouring horizontal gene transfer. In addition, antibiotic abuse, as noted in poultry farms, can drive positive selection for resistant mutants, making them more representative in the community [14, 25, 26].

It should be underscored that ESBL production is some sort of multi-resistance to the beta-lactams, a large family of antibiotics including, among others, penicillins and cephalosporins. Its co-development with MDR in clinical isolates is a dangerous melange that can complicate treatment of diseases caused by Enterobacteriaceae, especially in immunocompromised and critically ill patients [27].

Research has shown that these resistance outcomes—ESBL production and MDR—tend to occur frequently in leading nosocomial pathogens owing to high selection pressure for resistance genes in humans due mainly to antibiotic abuse [28, 29].

The z-score p-values showed significant differences between the prevalences of resistance outcomes except for overall resistance against aminoglycosides. This demonstrates that the host and exposure influence the development of resistance outcomes.

The paired sample t test comparing the risks and resistance outcomes in humans and broilers showed that the frequencies of exposure to the risk factors and resistance outcomes were significantly different between human and broiler isolates, save for the outcomes 'resistance to at least one antibiotic', ESBL production, resistance to aminoglycosides, and the risk-unsure water. The general practices in poultry farms involving episodic evacuation of flow materials have made it such that the birds are always in contact with their faeces, which constantly mix with feed [30].

Thus, these risks become constant for poultry. The use of antibiotics in farmhouses was systematic, explaining the high exposure in chicken subjects. This shows that the difference in the rate of exposure of the host to risk factors has an impact on the development of resistance in the isolates. It is a further indication that a clean environment and proper use of antibiotics in therapy are necessary in the fight against antibiotic resistance.

Globally, the prevalence of the various genes across the three families was significantly different except for the blaTEM-2 ESBL gene, the qnrB PMQR gene, and the aph (3′)-IA and ant (2′)-IA PMAR genes. These genes generally had higher prevalences in chicken isolates than in human isolates. It should be noted that these genes are present in isolates from apparently healthy subjects, and in addition, the expression of resistance is correlated with long periods of rearing corresponding to the age of mature broilers that are put out for consumption. There is thus a greater risk of poultry serving as a source of resistant bacteria for humans. With these outcomes, it seems broilers (and, by extension, animals) tend to withstand infection without developing diseases more than humans; this is yet to be verified in further studies.

To make genetic comparison, it is necessary to ascertain that the bacteria being compared belong to the same species. This justifies the molecular identification of E. coli isolates before phylo-

genetic analysis given that phenotypic identification is based on probability. ERIC sequences are 127-bp imperfect palindromes with multiple and variable repeats in the genomes of enteric bacteria and vibrios. They are highly variable among strains and species, well conserved, and the length polymorphism of these sequences due to variable repeats provides a genetic fingerprint useful in phylogenetic comparison of Enterobacteriaceae [20, 21].

Hierarchical cluster analysis of the amplified E. coli ERIC sequences produced a dendrogram with three main clusters each containing isolates from both humans and poultry. This shows that the isolates from both sources share a common genetic makeup. This is proof of zoonotic circulation of resistant strains given that the links of transmission between humans and poultry are well evident [12, 11].

However, a complete breakdown of the dendrogram shows a total of seven clusters, of which there are two tiny clusters made up only of clinical isolates. This is an indication that humans may tend to harbour unique strains of E. coli probably the pathogenic types that are involved in pathogenesis given that these were clinical isolates.

It is well established that humans can transmit these resistant strains to animals and vice versa [12, 11]. However, in the West Region of Cameroon, there is a far greater possibility of broilers serving as a source of contamination for humans with resistant strains than in the opposite scenario. This is because the population regularly consumes chicken at home, at roadside barbecues, and at mass catering events, which may be improperly prepared and thus carry viable bacteria. This population also uses broiler droppings in farms and water that can possibly be contaminated with bacteria by droppings used in farms and poultry farm waste.

Conversely, there are fewer possibilities of human-to-broiler contact because relatively few people work in poultry farms and are in direct contact with the birds to transmit the bacteria to the birds. Therefore, the high resistance rate in isolates from poultry means the poultry is probably serving as a source of resistant strains for the human population in the West Region [10, 17, 18].

Conclusion

Host type was a determinant in the resistance outcomes, with higher prevalences of resistance to quinolones and aminoglycosides and plasmid-borne resistance gene carriage in poultry isolates. MDR and ESBL production were higher in human isolates. These frequencies were, in general, significantly different, showing that risk exposures and resistance outcomes were host dependent. Genotyping of E. coli, a representative member of the Enterobacteriaceae, showed that the human and poultry isolates were genetically linked, a proof of the possibilities of zoonotic circulation of the bacteria and, by extension, the circulation of members of the whole family. This emphasizes the need for the fight against antibiotic resistance to be taken from a one-health perspective.

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