Antimicrobial use and resistance in Escherichia coli from healthy food-producing animals in Guadeloupe

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Abstract

Background

Selection pressure exerted by overuse of antibiotics in both human and veterinary medicine is responsible for increasing resistance to antibiotics. The objectives of this study were *(i)* to better understand antimicrobial use in pigs, beef cattle, and poultry on farms of Guadeloupe, French West Indies, and *(ii)* to acquire data on antimicrobial resistance in *Escherichia coli* in these food-producing animals. A cross-sectional survey was conducted in 45 farms on Guadeloupe. Practical use of antimicrobials was documented in declarative interviews between March and July 2018. Fecal samples were collected from 216 pigs, beef cattle, and broiler chickens between January 2018 and May 2019. The samples were cultured for bacterial isolation and identification, antimicrobial testing, and screening for bla_{CTX-M}, bla_{TEM}, and *tet*A resistance genes by PCR on extracted genomic DNA.

Results

The study shows rational use of antimicrobials consisting of occasional use for curative treatment by veterinary prescription. Tetracycline was the most commonly used antimicrobial, but this was not correlated to *E. coli* resistance. Extended spectrum β-lactamase (ESBL) *E. coli* isolates were detected in 7.3% of pigs, 14.7% of beef cattle and 35.3% of broilers. $bla_{CTX-M-1}$ was the predominant gene found in ESBL *E. coli* isolates (68.8%), followed by $bla_{CTX-M-15}$ (31.3%).

Conclusion

Despite rational use of antimicrobials, the rate of ESBL *E. coli* in food-producing animals in Guadeloupe, although moderate, is a concern. Further studies are in progress to better define the genetic background of the ESBL *E. coli* isolates.

Background

Currently, antimicrobial resistance (AMR) is one of the most urgent public health problem in the world [1], as it has dramatically increased morbidity and mortality in both humans and animals with serious repercussions for future treatment of infections in humans and for animal health and productivity [2]. The administration of antimicrobials to animals is regarded as a major contributor to the overall emergence of AMR worldwide. Several high-income countries report extensive use of antimicrobials and AMR in animals [3]. To reduce the use of antibiotics the risk factors for developing infectious diseases, such as the genetic background of breeds and the management of farms must be addressed [4].

Bacterial strain diversity has played a key role in the global emergence of AMR, and selection pressure by antibiotics imbalances diversity in favor of pathogens and greater resistance [5]. The emergence of extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, which hydrolyze key antimicrobials such as the expanded-spectrum cephalosporins, cefotaxime, ceftriaxone, ceftazidime and cefepime, is due mainly to the selective pressure of antibiotics given in both human and veterinary medicine [6], more antibiotics being used in animals than in humans [7]. The resistance is mediated mainly by acquired ESBL genes located on mobile genetic elements and frequently associated with resistance genes against several families of antimicrobial agents [8].

Guadeloupe, a French overseas department located in the Caribbean has been classified as a very high-resource country [9]. Less than one third of the surface of this small island is devoted to agriculture, and the livestock in 2018 comprised 44 900 cattle, 14 500 pigs, and 507 000 laying hens and broilers [10]. The latest of the few studies on AMR on Guadeloupe showed a low prevalence of ESBL-producing Enterobacteriaceae in human community-acquired urinary tract infections [11] and in waste water treatment plants [12]. As there is close contact between humans and domestic animals, it is important to see whether these animals are reservoirs of resistance genes; however, the only data on antibiotic resistance in local domestic animals is a study on horses, which showed the emergence of various ESBL-producing *E. coli* clones, some of which persisted for more than a month after antibiotic treatment [13].

The main objective of this work was to obtain information on antimicrobial use on farms on Guadeloupe and on AMR levels in the zootonic indicator bacteria *Escherichia coli* in poultry, pigs, and beef cattle.

Results

In the survey of use of medication containing antibiotics in pigs, beef cattle, and poultry on Guadeloupe, 64.4% (29/45) of all farmers reported use of antimicrobial agents (Table 1). Antimicrobials were usually administered (26/29, 57.7%) as curative treatment (20/29, 69.0%) and under veterinary prescription (22/29, 75.9%). The main causes for which antimicrobials were given were respiratory and digestive diseases in poultry (3/6, 50.0%), respiratory diseases in pigs (5/11, 45.5%) and skin diseases in cattle (5/12, 41.7%) (Table 1).

The most commonly used active substance was tetracycline (20/29, 69.0%); β-lactams and streptomycin were administered by 27.6% (8/29) and 24.1% (7/29) of farmers respectively. The proportion of farmers who used tetracycline was significantly higher in beef cattle production (100.0%) than in pig (54.5%) or poultry husbandry (33.3%), while β-lactams were administrated mainly by pig farmers (54.5%). Trimethoprim-sulfamethoxazole was used predominantly by poultry producers (83.3%, $P < 0.001$). Antimicrobials were used significantly less frequently in poultry (40.0%) than in pigs (78.6%) or

beef cattle (75.0%) farms ($P = 0.046$). The total annual costs for drugs and veterinary fees was estimated to be 2000 € by half of the pig farmers, and the median cost was estimated to be 500 €/year by beef cattle farmers and 50 €/year by poultry farmers. The median annual cost of veterinary treatment per 100 kg was estimated to be 7.2 € for pigs, 6.5 € for beef cattle, and 1.7 € for poultry.

Table 1 Antimicrobials used in poultry, pig and beef cattle production systems

An additional table file shows this in more detail [see Additional file 1].

A total of 216 samples were collected from food-producing animals living on 28 farms and the slaughterhouse. Fecal samples were collected from 124 pigs (90 collected on farms and 34 at the slaughterhouse), 75 beef cattle (51 on farms and 24 at the slaughterhouse), and 17 hen houses with about 53 000 broilers. ESBL *E. coli* were isolated mainly from broilers, (35.3% (6/17) sampled in four hen houses but 14.7% (11/75) of beef cattle on four farms, and 7.3% (9/124) of pigs on three farms. Two pigs were carriers of two distinct ESBL-*E. coli* and three distinct ESBL *E. coli* were isolated in two hen houses (Table 2).

Table 2
Distribution of ESBL E. *coli* positive animals and bla_{CTX-M} gene type

	Animals			**_E. coli_ isolates**					
	Total	ESBL E. *coli* positive		Total	*bla* carrier		$bla_{CTX-M-15}$		$bla_{CTX-M-1}$	
	(n = 216)	(n = 26)		(n = 265)	(n = 32)		(n = 10)		(n = 22)	
n, *(%)*										
Beef cattle	75	11	*(14.7)*	85	11	*(12.9)*	6	*(7.1)*	5	*(5.9)*
Pig	124	9	*(7.3)*	157	11	*(7.0)*	3	*(1.9)*	8	*(5.1)*
Broilers	17	6	*(35.3)*	23	10	*(43.5)*	1	*(4.3)*	9	*(39.1)*
ESBL, extendend spectrum β-lactamase producing										

bla, β-lactamase

The highest resistance levels were observed against ampicillin, cefotaxime, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. Significant differences in the frequencies of resistant *E. coli* isolates were found among the three production systems (Tables 1 and 3). The proportion of ampicillin-resistant *E. coli* was higher in broilers than in beef cattle or pigs (73.9% *vs* 25.9% and 20.4% respectively). A similar but less marked trend was observed for cefotaxime (43.5% of resistant *E. coli* in broilers, 12.9% in beef cattle and 7.0% in pigs). The proportion of streptomycin-resistant *E. coli* was higher in pigs than in beef cattle (47.8% *vs* 36.5% respectively). The lowest proportion of tetracycline-resistant *E. coli* was observed in beef cattle (18.8%) with 56.5% in broiler and 59.2% in pig isolates. Trimethoprim-sulfamethoxazole-resistant *E. coli* were found in a higher proportion in broilers (34.8%) than in pigs (18.5%) or beef cattle (12.9%).

Table 3
Comparison of antimicrobial use and resistant E. coli frequencies in broilers, pigs and beef cattle

	Farmers antimicrobial use								**Resistant _E. coli_ isolates**							
	Broilers (n = 6)		Pigs (n = 11)		Beef cattle (n = 16)		*P*		Broilers (n = 23)		Pigs (n = 157)		Beef cattle (n = 85)		*P*	
n, *(%)*								n, *(%)*								
TET	2	*(33.3)*	6	*(54.5)*	12	*(100.0)*	0.003	**TET**	13	*(56.5)*	93	*(59.2)*	16	*(18.8)*	< 0.001	
β-lact[a]	0	*(0.0)*	6	*(54.5)*	2	*(16.7)*	0.035	**AMP**	17	*(73.9)*	32	*(20.4)*	22	*(25.9)*	< 0.001	
SM	0	*(0.0)*	5	*(45.5)*	2	*(16.7)*	NS	**SM**	10	*(43.5)*	75	*(47.8)*	31	*(36.5)*	NS	
SXT	5	*(83.3)*	0	*(0.0)*	0	*(0.0)*	< 0.001	**SXT**	8	*(34.8)*	29	*(18.5)*	11	*(12.9)*	0.054	
QNR	0	*(0.0)*	0	*(0.0)*	0	*(0.0)*	NS	**NAL**	3	*(13.0)*	3	*(1.9)*	2	*(2.4)*	0.031	
[a]Penicillins such as ampicillin and amoxicillin																
TET, tetracyclines; SM, streptomycin; SXT, Trimethoprim-sulfamethoxazole; QNR, quinolones, AMP, ampicillin, NAL, nalidixic acid																

We compared the rates of resistance to tetracycline, ampicillin, streptomycin and trimethoprim-sulfamethoxazole according to the antibiotic or class of antibiotic stated in the survey, according to animal species. As shown in Table 1, tetracyclines were used more often in beef cattle than in broilers (100.0%

vs 33.3%, $P = 0.003$), but more tetracycline-resistant *E. coli* isolates were found in broilers (56.5%) and pigs (59.2%) than in beef cattle (18.8%, $P < 0.001$) (Table 3). Use of β-lactams also did not correspond to the level of *E. coli* β-lactams resistance in broilers (73.9%), as they did not receive these drugs. Congruent findings were observed between the use of sulfonamides in broilers (33.3% and none in pigs or beef cattle, $P < 0.001$) and the highest proportion of trimethoprim-sulfamethoxazole *E. coli*-resistant isolates from broilers (34.8%) as compared with 18.5% in pigs and 12.9% in beef cattle, $P = 0.054$). Congruent results were also observed between the absence of quinolone use and a low frequency of nalidixic acid-resistant *E. coli* in the three production systems.

The $bla_{CTX-M-1}$ gene was found predominantly in ESBL isolates (22/32, 68.8%), followed by $bla_{CTX-M-15}$ (10/32, 31.3%). The $bla_{CTX-M-1}$ gene was combined with the bla_{TEM-1C} gene in two pigs and with the bla_{TEM-1B} gene in one broiler isolate. The remaining ESBL *E. coli* carried a $bla_{CTX-M-15}$ gene, usually with combined cefotaximase-ceftazidimase activity. Comparison of phenotypic and genotypic profiles based on AMR/ARG combined patterns analysis is shown in Fig. 1. The bla_{CTX-M} genes were carried by ESBL *E. coli* isolates found in the three food producing-animals systems on four farms in distinct geographic areas. Genotypic comparative analysis generated 18 distinct patterns among the 32 ESBL *E. coli* (Fig. 1). A total of 20 (62.5%) isolates harboring patterns of close genetic relatedness were grouped into seven clusters of similar AMR/ARG patterns comprising two to five isolates, whereas 12 (37.5%) distinct patterns were not clustered. Three clusters included nine ESBL *E. coli* at the same farm, whereas the other clustered ESBL *E. coli* were not specific to a production system. Twelve clustered isolates with similar AMR/ARG profiles were found in different food-producing systems (12/32, 37.5%); e.g. one cluster of ESBL *E. coli* carriers consisted of two pigs and one hen house on three different farms.

Discussion

The study on use of antimicrobial on small-scale pig, broiler and beef cattle farms in Guadeloupe showed moderate ESBL-*E. coli* in pig and beef cattle production, probably due to rational use of antimicrobials. The island adheres to the French AMR reduction plan [14] on the use of veterinary antimicrobials and may reflect its effectiveness. The frequencies were nevertheless higher than those in the French national surveillance system for AMR on infected animals in 2018, in which where *E. coli* isolates resistant to third and fourth-generation cephalosporins (^{3rd}GC/4th GC) were detected in 2.3% of beef cattle and in < 2.0% of pigs, poultry, and turkeys [15]. The differences may be due to sampling in diseased rather than healthy animals for detection of ESBL-*E. coli.* The prevalence in our study is closer to that observed in Portugal, where 5.7% of fecal samples from 35 healthy pigs and 10% of those from healthy chicken were positive for ESBL-*E. coli* [16], but lower than that in Switzerland, where 15.3% of pigs and 13.7% of bovine fecal samples were positive [17]. Country- and production-specific factors influence the occurrence of resistance [18, 19]. In our study, most of the ESBL *E. coli* isolates were clustered and farm-specific or shared by distant farms and distinct animal species, suggesting that ESBL-producing *E. coli* were both transmitted among farms and evolved independently within farms.

None of the *E. coli* isolates was resistant to any of the tested quinolones (enrofloxacin, ciprofloxacin, nalidixic acid), probably reflecting the low use of these antibiotics in food-producing animals. Previous studies have shown that there was a significant positive correlation between antibiotic doses and the occurrence of antibiotic-resistant bacteria in animal feces [20]. In our study, the discrepancy between use of antimicrobials on the farms and the level of resistance is striking. Antimicrobial use was not always linked to AMR level, especially for β-lactams and tetracyclines. A potential bias might be underreported antimicrobials use, which would explain the inverse relation, but there may be other reasons. The low level of resistance to tetracycline in beef cattle in relation to the number of farms that used this drug might be due to targeted treatment in small beef cattle production rather than collective treatment as used in larger-scale broiler and pig husbandry. Antimicrobial treatments also reflect the veterinary cost, which is lower with collective administration, e.g. to poultry. Collective animal treatments could thus contribute to the emergence of resistance and could explain the high level observed in broilers. Such collective practice should therefore be more closely controlled.

The high frequency of ESBL resistance observed in broilers (35.3%) when the breeders declared that they did not use ^{3rd}GC and to a lesser extent, the ESBL resistance observed in pig and beef cattle production systems with no such use are difficult to explain. We tested imported 7-days old chicks 1 day after arrival from mainland France but found no resistance to ^{3rd}GC. It has been shown that a rapid increase in ESBL *E. coli* prevalence in the first week of life must be due to factors other than latent contamination of the majority of birds at arrival [21]. Therefore, as no ^{3rd}GC were administered in the production systems where ESBL *E. coli* resistance was detected, the resistance to these drugs observed in our study was probably due to co-selection of several resistance genes in the same genetic determinant through use of other antibiotics [22]. It has also been observed that tetracycline resistance in commensal *E. coli* is often linked to resistance to other antimicrobials such as ampicillin and trimethoprim-sulphonamides [19]. A recent study on a Danish pig production farm clearly indicated that commonly used antimicrobials such as tetracycline, which are not listed as critically important for human treatment can create resistance to critically important antimicrobials, limiting treatment possibilities [23]. A recent metagenomic study on bacterial communities showed that co-selection of resistance to tetracyclines among ESBL producers mobilized conjugative plasmids [24] or integrons [25]. The wide use of tetracyclines in our setting may explain some of the disproportion between the prevalence of resistance and the use of ^{3rd}GC. These results reinforce the importance of animal food-producing systems as a reservoir of mobile genetic elements carrying multiple resistance determinants.

Whatever the reasons for these discrepancies, further studies are warranted to better define the genetic background of ESBL *E. coli* isolates and to better clarify the AMR context on Guadeloupe, especially in food-producing animals that are not exposed to ^{3rd}GC.

Conclusion

Our study provides the first baseline information on levels of antimicrobial use and the dynamics of phenotypic and genotypic resistance to antimicrobials among *E. coli* in small-scale pig, beef cattle, and broiler production systems on Guadeloupe. Despite rational use of antimicrobials, ^{3rd}GC-resistant *E. coli* were found on the farms. Mechanisms other than ^{3rd}GC selective pressure involved in the emergence of AMR to these drugs remain to be elucidated.

Methods

Survey design

A prospective survey on the use of antimicrobial agents in veterinary medicine and food animal production was conducted between March and July 2018 on 15 broiler, 14 pig, and 16 beef cattle production farms. The farms were selected randomly in 16 of the 32 townships throughout the island to ensure representative production, covering small to large-scale production organized as cooperatives or independent. All the pig facilities were farrow-to-finishing farms with 30 − 3 120 head per farm for a total of 8 549 pigs, representing 59.0% of pig production on Guadeloupe. Broiler and laying hen breeder farms had 400 − 64 000 birds; for a total of 184 510, representing 36.4% of local chicken production. Beef cattle breeding was investigated on 13 small-scale grassland farms (≤ 90 head) and 3 large-scale farms, for a total 1 691 head, representing 4.3% of local production [10].

Antimicrobials use was documented in declarative face-to-face interview with farmers by an agronomist with a questionnaire specific for the study. Each participant provided information on farm characteristics (size, number of head) and routines for antimicrobial use, including frequency, reasons for treatment, name of the antibiotic drug used, route of administration, and estimated annual cost of treatment, including, laboratory analyses, veterinary services and drug purchase.

Sampling and collection

Between January 2018 and May 2019, poultry, pigs, and beef cattle on 28 farms and in the only slaughterhouse on Guadeloupe were screened for *E. coli*. Because of the predominance of small herds of beef cattle raised free in field, sampling of cattle was more difficult than that of pigs or chicken confined in blocks and is therefore less representative of the total of the production (4.3%) [10]. During the study period, 216 fecal samples (30 g) were collected randomly just after excretion (124 from pigs and 75 from beef cattle). Fecal material from broilers and laying hens (n = 17) was sampled by walking with boot socks (Sterisocks Tryptone SodiBox, Nevez, France) approximately 100 m around a flock. All the samples were stored and transported in sterile cups or bags on ice to the Institut Pasteur Laboratory within 4 h. Samples were stored at 4 °C and processed within 8 h of sampling.

Bacterial isolation and identification

A 10-µL loop of each fecal sample was mixed in Luria Bertani broth BD Difco™ (Humeau, La Chapelle-sur-Erdre, France) supplemented or not with 4 mg/L of ceftriaxone and incubated at 37 °C for 24 h. Cultures were streaked on chromogenic coliform agar plates (CHROMagar™, Paris, France), supplemented or not with 4 mg/L of ceftriaxone and incubated at 37 °C for 24 h. One susceptible and three resistant metallic blue colonies were picked up from selective and non-selective chromogenic coliform agar respectively. Presumptive *E. coli* were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on an Axima performance spectrometer (Shimadzu Corp, Osaka, Japan).

Antimicrobial susceptibility analysis

Susceptibility to 17 antimicrobials in six distinct classes was assessed in all *E. coli* isolates by the standard disk diffusion method on Mueller-Hinton agar, as recommended [26]. Strains were tested against: ampicillin (10 µg), amoxicillin–clavulanic acid (20 µg–10 µg), temocillin (30 µg), cefotaxime (5 µg), ceftazidime (10 µg), cefoxitin (30 µg), ertapenem (10 µg), gentamicin (10 µg), amikacin (30 µg), streptomycin (10 µg), enrofloxacin (5 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 IU), tigecycline (15 µg), trimethoprim–sulfamethoxazole (1.25 µg–23.75 µg), and fosfomycin (200 µg). ESBL producers were confirmed by the combined disk diffusion test with cefotaxime and ceftazidime, supplemented or not with clavulanic acid. Growth inhibition diameters were measured with the Adagio™ automated system (Bio-Rad, Marnes-La-Coquette, France). *E. coli* strains were classified as susceptible, intermediate, or resistant according to the epidemiological thresholds [26]. *E. coli* ATCC 25922 was used as a quality control. *E. coli* with a similar AMR profile and isolated from the same sample were considered duplicates of the same clone and were counted only once.

Resistance gene screening

For molecular characterization of AMR genes (ARGs), genomic bacterial DNA was extracted, from one colony with the InstaGene™ Matrix kit (Biorad, California, USA) according to the manufacturer's instructions. ESBL and tetracycline resistance coding genes were screened by PCR in all *E. coli* tetracycline-resistant isolates. bla_{CTX-M} multiplex PCR including phylogenetic groups 1, 2, and 9 was performed [27]. bla_{TEM} *gene* was screening by simplex PCR [28]. Amplified PCR products were sequenced (Eurofins, Ivry-sur-Seine, France) and compared with known resistance gene sequences in the GenBank database by multiple-sequence alignment with the BLAST program for further characterization. Tetracycline-resistant isolates were screened for the presence of *tet*A and *tet*B genes with specific *tet*A primers designed for this study (*tet*A-F 5'-TAGAAGCCGCATAGATCGCC-3' and *tet*A-R 5'-GCTTCATGAGCGCCTGTTTC-3') and published specific *tet*B primers [29]. The duplex PCR amplification conditions for *tet*A and *tet*B were: 5 min at 95 °C followed by 35 cycles of 95 °C for 30 s, 62 °C for 305 s, and 72 °C for 30 s. The amplicons were detected on gel electrophoresis. For quality control, a subsample of 10% was genotyped twice.

Combined numerical analysis

The combined numerical analysis was performed on ESBL-producing *E. coli* patterns with BioNumerics® v6.6 software (Applied Maths NV). Each file with experimental data from AMR and ARG screening was merged as a composite data set in the BioNumerics® database, with the similarity coefficient option taken from each experiment. The matrices from the individual experiments were averaged according to the same defined weight, and an individual similarity matrix was calculated in such a way that all characters had an equal influence on similarity. A dendrogram was drawn by using the unweighted pair group method with arithmetic averages with a tolerance of 1% to show the relations of the bacterial isolates. Under these conditions a “cluster” of ESBL *E. coli* isolates was considered clonal if the similarity index was 98% for the combined pattern of AMR and ARG.

Statistical analysis

Results are presented as means ± standard deviation, medians with the interquartile ranges for quantitative variables and numbers and percentages for qualitative variables. Intergroup differences between farms classified according to food-producing animal categories were assessed in the Kruskal–Wallis or chi-square test, as appropriate. The level of significance was defined as $P < 0.05$. Analyses were conducted with STATA® 11.2.

Declarations

Ethics approval and consent to participate

In this study fecal samples were taken from animals at the slaughterhouse and on the field after excretion. Therefore, there was no real "use of live animals for scientific purposes" (within the meaning of the Rural Code, Art R214-87 and following), involving the breeding and handling of animals, and there was no invasive procedure on live animals. Furthermore, the project has been considered, by the chairman of the CEMEAAG (Comité d’Ethique en Matière d’Expérimentation Animale des Antilles et de la Guyane) Ethics Committee (registered with the French Ministry of Higher Education, Research and Innovation under No. 69), outside the scope of the regulations on animal experimentation. Thus according to the French National law for the protection of animals No. 2013–118 that transcribes the European the EU directive 2010/63/UE on the protection of animals used for experimental and other scientific purposes, no ethics committee approval was deemed necessary based on the Article 7.1 (on the recommendations for animal welfare) and the Article 7.8 (on the use of animals in research and education) of the OIE Terrestrial Animal Health Code followed by France. The entity responsible for the animals was the slaughterhouse and authorization for sample collection was obtained by the director of the slaughterhouse. Also, the pig, beef cattle and chicken sampling was authorized verbally by the owners which are responsible for the animals.

Consent for publication

Not applicable

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interests.

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Authors’ contributions

GG, AS, HR, and MP performed sampling and bacteriological analysis. SB, SG, revised the manuscript, provided editing. AT supervised research and provided substantial revision of the draft. SF performed statistical analysis, designed the study, supervised research, drafted, and edited the manuscript. All authors approved the submitted version, have agreed to be accountable for the author's own contributions in ensuring that questions related to the accuracy or integrity of any part of the work, are appropriately investigated and resolved.

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Figures

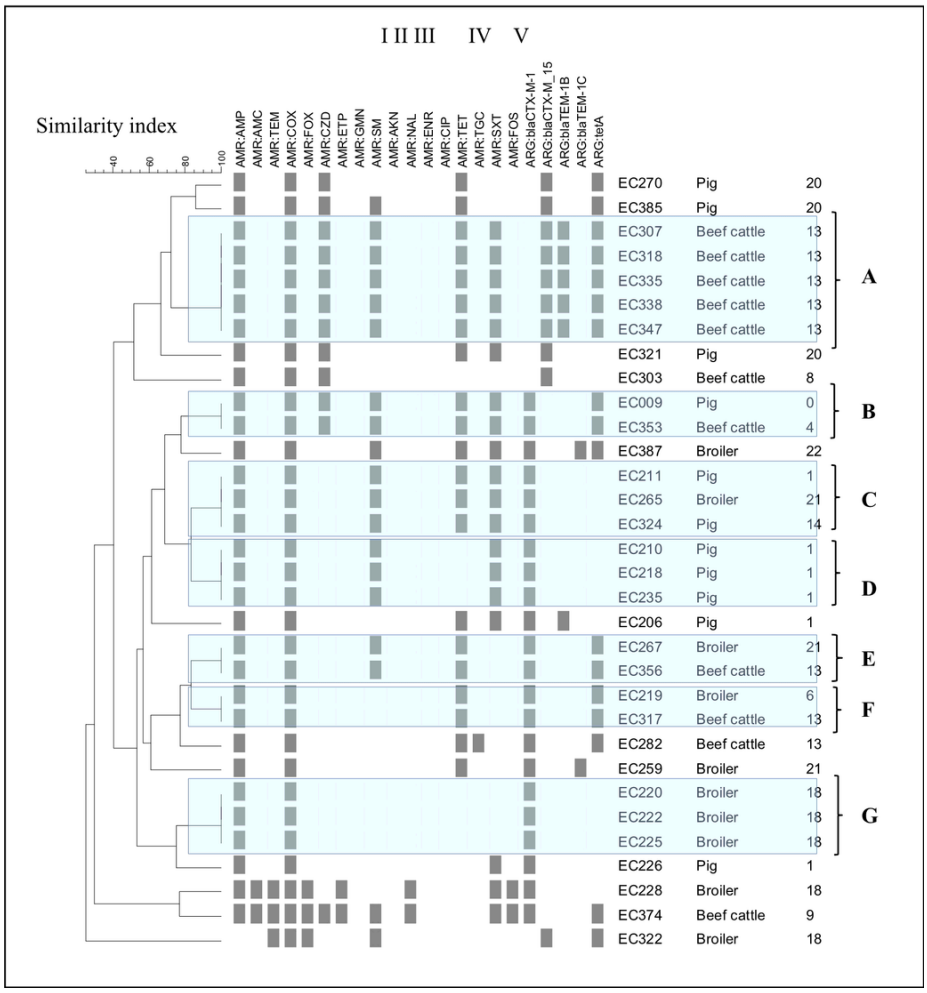

Figure 1

Combined numerical analysis of AMR and ARG patterns for 32 ESBL E. coli isolates from food-producing animals in Guadeloupe Patterns were based on antimicrobial resistance (AMR) profile and blaCTX-M and blaTEM (ARG) gene screening. Seven clusters containing isolates from two to five samples can be seen. The bar represents the similarity index obtained by the unweighted pair group method with arithmetic averages. Column I, combined pattern of AMR and ARG; column II, isolate number; column III, sample origin; column IV, farm identifying, column V, cluster designation. AMR, antimicrobial resistance phenotype; ARG, antimicrobial resistance gene AMP, ampicillin (10 µg); AMC, amoxicillin-clavulanic acid (20 µg-10 µg); COX, cefotaxime (5 µg); FOX, cefoxitin (30 µg); CZD, ceftazidime (10 µg); ETP, ertapenem (10 µg), GMN, gentamicin (10 µg); Sm, streptomycin (10 µg), AKN, amikacin (30 µg); NAL, nalidixic acid (30 µg); ENR, enrofloxacin (5 µg); CIP, ciprofloxacin (5 µg); TET, tetracycline (30 IU); TGC, tigecyclin (15 µg); SXT, trimethoprim-sulfamethoxazole (1.25 µg-23.75 µg); FOS fosfomycin (200 µg).

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