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Evaluation of mechanical transmission of *Enterococcus* spp., resistant to antibiotics, having flies as vectors of dissemination

Abstract

The objective of this work was to evaluate the mechanical transmission of *Enterococcus* spp., resistant to antibiotics, using flies as dissemination vectors. In this work, 50 flies were captured, 25 in "La Llanada" community, in Cumaná and 25 others in the surroundings of the "Juan Otaola Rogliani" outpatient clinic and the central cemetery of Carúpano. The main families of flies captured in the state of Sucre were Muscidae, Calliphoridae and Sarcophagidae. The flies captured in Cumaná had fewer strains of *Enterococcus* spp., on the surface than those from Carúpano, while those from Cumaná were more colonized in the intestine than those from Carúpano. A total of 14 strains of *Enterococcus* were isolated, among which were *E. faecium* (6%), *E. gallinarum* (36%) and *E. casseliflavus* (58%). The susceptibility profile of *E. casseliflavus* strains is resistance to linezolid, tetracycline, erythromycin, rifampicin and intermediate susceptibility to fluoroquinolones; *E. gallinarum* strains were sensitive to rifampicin and tetracycline, and resistant to the other antibiotics. The only strain of *E. faecium* isolated in Carúpano, presented low level of resistance to vancomycin. It did not amplify for the glycopeptide resistance ligase genes *vanA*, *vanB*, *vanD*, *vanE*, or *vanG*. Antibiotypes IA and IV of *E. caseliflavus* were detected in both Carúpano and Cumaná.

Keywords: flies, transmission, muscidae, calliphoridae, sarcophagidae, cumaná, carúpano, antibiotic resistance

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Introduction

Organizations that regulate health and public health have registered 21 species of flies as causative agents of gastrointestinal diseases, due to their predilection for contaminated environments and endophilia, that is, their tendency to enter buildings.[1] The fly is attracted to different substrates: food, waste, secretions and excreta to feed, making it an efficient mechanical vector of pathogens; This insect can transport microorganisms, externally, due to the morphology of its body covered by mushrooms, or internally, in its digestive tube (Moissant *et al*., 2004). The ways in which this common insect can transmit pathogens are: through its body surface; by regurgitation of contaminated food and by defecation of pathogens, this route being very important, due to the protective effect that the interior of their body gives to the pathogen present (Sasaki *et al*., 2000).

Isolating microorganisms from flies is a potential index of contamination.[2] The ease with which bacteria reproduce is shown with increasingly high incidences, becoming major health problems, which is why the Enterococcus genus also contributes to these health problems (Palomino and Gonzáles, 2014). Humans constitute the most important reservoir of *Enterococcus*, because this genus is part of the normal microbiota of the gastrointestinal tract. The most frequently isolated *Enterococcus* species are *E. faecalis*, 80 to 90%, followed by *E. faecium*, 5 to 10%, and other less frequent species: *E. casseliflavus*, *E. gallinarum*, *E. durans*, *E. flavescens*, *E. hirae*, *E. raffinasus* and *E. avium*, are rarely isolated from clinical samples and their role in infectious processes in humans is not coincidental. Bacteria belonging to the genus *Enterococcus* are characterized by being non-motile, with the exception of the species *E. casseliflavus*, *E. gallinarum* and *E. flavescens* (Prescott *et al*., 2002).

Enterococcus is responsible for a variety of infections such as those of the urinary tract, surgical wounds, bloodstream, central nervous system, endocarditis, intra-abdominal, hepatobiliary, pelvic and neonatal sepsis (Quiñones, 2010). The great capacity of bacteria to transfer resistance genes, essentially in plasmids and transposons, contributes to their dissemination, both among pathogenic bacteria and towards non-pathogenic bacteria.[3]

Material and methods

Sample

Flies were collected in different areas of the towns of Cumaná and Carúpano (in "La Llanada" community, in Cumaná and around the Juan Otaola Rogliani outpatient clinic, as well as the central cemetery of Carúpano). The captured flies were transferred to the Biological Control Laboratory for taxonomic identification and, subsequently, taken to the Bacterial Resistance Laboratory for the identification of the different species of Enterococcus; both laboratories located in the Institute of Research in Biomedicine and Applied Sciences of the Universidad de Oriente (IIBCA-UDO), in the Sucre Nucleus.

Catching flies

The flies were captured in plastic bottles, at the bottom of the container the decomposing baits (meat, fish entrails and fruits) were placed, previously protected with a mesh. Plastic funnels washed with water and disinfected with 70% isopropyl alcohol were adapted to the mouth of the containers, in the shape of inverted cones, to facilitate the entry of the flies, but not the exit.[2]

Those flies that explored various surfaces (tables, excrement, food and/or garbage) were collected for approximately one to two minutes. Observing this, traps were placed in strategic places in a way that facilitated their entry quickly. They were collected individually and one capture was carried out per environment, between 9:00 am and 2:00 pm. They were numbed with chloroform and separated into

0.5 ml Eppendorf tubes, then frozen at a temperature of -20°C and transported in a cellar with a cold pack compress to the Biological Control laboratory for identification, and then they were transferred to the Bacterial Resistance laboratory to be processed as soon as possible.[4]

Taxonomic identification flies

For the taxonomic identification of the collected flies, the illustrated dichotomous key was used.[5]

Obtaining whole and macerated flies

Whole flies

The test tubes were previously sterilized in the oven at 170°C for 30 minutes. In each tube, 1 ml of BHI (Sigma-Aldrich) was added for each whole fly, then the tubes were placed in a water bath at 35°C for 24 hours with shaking movements; after that time, the entire flies were removed with a gripper and placed in a mortar. Subsequently, inocula were taken from the supernatant of each tube.

Macerated flies

Once the bacteria were recovered from the surfaces of the flies, each fly was washed individually as follows: 70% ethanol was added for 30 seconds, then sterile distilled water was added for 20 seconds with triple repetitions. And the 0.05% NaCl solution was added, letting it act for 60 seconds and again, sterile distilled water was added for 20 seconds with triple repetitions. Once the flies had been washed separately and the mortar had previously been sterilized, 1 ml of BHI broth was added to the mortar and the flies were macerated in the porcelain mallet; This macerate was transferred to a sterile tube to incubate in a water bath at 35°C for 24 hours with shaking and the supernatant was taken.[2]

Isolation of *Enterococcus* spp strains

The inocula of the whole flies and the fly macerate were plated separately by the spread and streak method on agar plates selective for *Enterococcus* spp. Both plates were incubated at 35°C for 24 hours. Next, three colonies grown on *Enterococcus* confirmatory agar plates were taken, their morphological characteristics such as size, shape, appearance and color characteristics that oriented towards the genus under study were noted, and each developed colony was purified separately.[2]

For the purification of the colonies developed from the whole and macerated flies, 1 ml of BHI broth with 6.5 NaCl was added to each tube, the latter being a test that characterizes this genus, which consisted of the ability of *Enterococcus* to grow in a concentration of 6.5% NaCl. The salty broth was inoculated with the strain to be studied and incubated in a water bath at 45ºC for 24 hours to verify the presence of *Enterococcus* spp. They were then plated on nutrient agar plates and incubated for 24 hours. The morphological characteristics of the totally pure developed colonies were noted, and they were stored in vials of BHI broth plus 20% glycerol at – 20 ºC.[2]

Detection of the susceptibility profile of *Enterococcus* spp strains. isolated from flies

Antimicrobial susceptibility was performed using the disk diffusion method (Bauer *et al*., 1966), in MH (BD) agar. Antibiotic discs used in the test: vancomycin 30 µg, teicoplanin 30 µg, ciprofloxacin 5 µg, norfloxacin 10 µg, trimethoprim-sulfamethoxazole 25 µg, ampicillin 10 µg, ampicillin/sulbactam 10 µg, erythromycin 15 µg, rifampicin 5 µg, linezolid 30 µg, nitrofurantoin 300 µg, chloramphenicol 30 µg, high-load gentamicin 120 µg, high-load streptomycin 120 µg and tetracycline 30 µg. The results were reported with M100-S26 manual of CLSI,

Minimum inhibitory concentration detection (MIC)

The MIC was determined by the dilution method in MH agar following M100-S26 manual of CLSI. Liquid bacterial cultures including the negative control of *S. aureus* ATCC 25923 and positive control *E. feacalis* 77904 VanB, were prepared as well as the 0.5 McFarland inoculum and then 1:10 dilutions were made. The agar plates containing different concentrations of antibiotics ampicillin, ciprofloxacin and vancomycin (0.5, 1, 2, 4, 8, 16, 32, 128 µg/ml). The CMI corresponded where no growth was observed.[6]

Enterococcus species identification

DNA extraction

DNA extraction was performed from pure cultures on nutrient agar (BD) incubated at 37°C for 24 hours. The colonies obtained were placed in 100 µl of digestion buffer (10 Mm Tris-HCl [PH 7.4], 0.45% Triton in order to obtain the supernatant which contains the DNA, it was immediately used for PCR.

Polymerase chain reaction

The different species of *Enterococcus* were determined by means of the polymerase chain reaction (PCR), using the protocol for amplification of Depardieu *et al*.,.[7] The following bacterial strains were included as controls for molecular identification: *E. faecium* VanA 77903, *E. faecalis* VanB 77904 and *E. gallinarum* VanC 77905.

Determination of antibiotypes

Antibiotyping was carried out using the antimicrobial susceptibility tests described above, with the purpose of distinguishing through their susceptibility profile whether the colonies that were found in the flies were the same strain of *Enterococcus* or a different strain. In the first tables, the antibiotypes were grouped without Roman numerals or letters; only by species. In the summary table, the antibiotypes were designated with Roman numerals (I – VII). Two species with the same antibiotype were assigned a letter to differentiate the species with the same antibiotype.

Results

For a month, 50 flies were collected in houses in different locations between 9 in the morning and 2 in the afternoon. These were attracted by various substrates (bait) such as garbage waste, chicken remains, meat, fruits and fish viscera. With this last bait, more fly receptivity was observed, 10 in Cumaná and 11 in Carúpano, followed by chicken waste, meat, and garbage. It could be observed between the identified flies and the type of substrate that there was no similarity since not all of them were attracted to it. No flies were captured with the fruits (Data not shown).

The taxonomic identification of the flies was carried out in the IIBCA Biological Control Laboratory with dichotomous keys whose main characters used during recognition were: antennae, spiracle, legs, wing nerves and chaetotaxy (arrangement of the bristles, mainly on the head and in the thorax). During their study, the genera *Calliphora*, *Chrysomya*, *Lucilia*, *Musca* and *Sarcophaga* were identified (Table 1).

Of the 20 samples studied, a total of 14 of *Enterococcus* strains were obtained (Table 2), since 5 did not grow in 6.5% NaCl at 45°C and were catalase positive, which indicated not belonging to the *Enterococcus* genus. There was one sample that was discarded

because a fungus grew. In the present work, the study of the 14 bacterial cultures isolated from the flies revealed the presence of 8 strains of *Enterococcus* on the surface, achieving the greatest number of bacterial strains (57%), of which only one strain corresponded to the Cumaná flies and seven strains to Carúpano flies (Table 2).

Table 1 Frequency of the presence of flies in the towns of Cumaná and Carúpano in the month of April 2015 in the State of Sucre

Flies	Cumaná		Carúpano	
	Captured (N°)	Frequency (%)	Captured (N°)	Frequency (%)
Calliphora vicina	-	-	2	8
Chrysomya albiceps	5	20	2	8
Chrysomya rufifacies	-	-	5	20
Lucilia cuprina	7	28	2	8
Lucilia sericata	-	-	4	16
Musca domestica	7	28	3	12
Sarcophaga sp1	6	24	3	12
Sarcophaga sp2	-	-	4	16
Total	25	100	25	100

Table 2 Surface colonization with strains of *Enterococcus* spp., in flies captured in Cumaná and Carúpano, Sucre state

Moscas	Number of Enteroccocus strains	
	Cumaná	Carúpano
Calliphora vicina	-	2
Chrysomya albiceps	-	2
Chrysomya rufifacies	-	-
Lucilia cuprina	-	-
Lucilia sericata	-	-
Musca domestica	-	2
Sarcophaga sp1	1	-
Sarcophaga sp2	-	1

In the analysis of the macerate of the flies, isolation of six strains of *Enterococcus* (43%) was obtained, four strains belonging to the Cumaná flies and two in the Carúpano flies (Table 3). In the present work, the study of the 14 bacterial cultures isolated from the flies revealed the presence of 8 strains of *Enterococcus* on the surface, achieving the greatest number of bacterial strains (57%), of which only one strain corresponded to the Cumaná flies and seven strains to Carúpano flies (Table 3).

Table 3 Colonization of the gastrointestinal tract with strains of *Enteroccocus* spp., from flies captured in Cumaná and Carúpano, Sucre state

Moscas	Number of *Enteroccocus* strains	
	Cumaná	Carúpano
Calliphora vicina	-	1
Chrysomya albiceps	-	-
Chrysomya rufifacies	-	-
Lucilia cuprina	1	-
Lucilia sericata	-	-
Musca domestica	1	-
Sarcophaga sp1	2	-
Sarcophaga sp2	-	1

In the analysis of the macerate of the flies, isolation of six strains of *Enterococcus* (43%) was obtained, four strains belonging to the Cumaná flies and two in the Carúpano flies (Table 4).

In table 5, it was observed that the main *Enterococcus* species identified by PCR in this work were *E. casseliflavus* (58%), *E. gallinarum* (36%) and *E. faecium* (6%). In table 5 it can be seen that the predominant species in the flies in the state of Sucre is *E. casseliflavus* followed by *E. gallinarum*.

Table 4 Prevalence of the different glycopeptide resistance genotypes (*vanC1*, *vanC2*) in strains of *Enterococcus* spp., captured in Cumaná and Carúpano

City	Strains	Prevalence (%) *vanC1*	Strains	Prevalence (%) *vanC2*	Strains	Prevalence (%) *E. faecium*
Cumaná	1	7	4	29	-	-
Carúpano	4	29	4	29	1	6
Total	5	36	8	58	1	6

vanC1: *Enterococcus gallinarum* y *vanC2*: *Enterococcus casseliflavus*

Table 5 *Enterococcus* spp. species, isolated from different flies from Cumaná and Carúpano, Sucre state

Moscas	*Enteroccocus strains*	
	Cumaná	Carúpano
Calliphora vicina	-	*E. galllinarum, E. faecium*
Chrysomya albiceps	-	*E. casseliflavus, E. gallinarum*
Chrysomya rufifacies	-	-
Lucilia cuprina	*E. casseliflavus*	-
Lucilia sericata	-	-
Musca domestica	*E. casseliflavus*	*E. casseliflavus*
Sarcophaga sp1	*E. casseliflavus, E. gallinarum*	-
Sarcophaga sp2		*E. casseliflavus, E. gallinarum*

In Table 6 it can be seen that some strains of *E. casseliflavus* are resistant to linezolid, tetracycline, erythromycin, rifampin and intermediate susceptibility to fluoroquinolones. The last strain in Table 6 has several resistance mechanisms and is almost impossible to treat due to its susceptibility profile, being more resistant than the only *E. faecium* strain isolated in this work. In this work there were 25% of *E. casseliflavus* strains resistant to tetracycline and 50% resistant to erythromycin (Table 6).

Table 7 shows that all *E. gallinarum* strains were resistant to rifampin, some were resistant to linezolid, erythromycin and tetracycline. There is a decrease in the sensitivity of the strains to fluoroquinolones. In this work, no strains with a high level of glycopeptide resistance were found, but they could appear in the near future, since there is a strong selective pressure due to the abuse of vancomycin in the hospital environment, so epidemiological surveillance of these is recommended types of resistance.

Table 6 Antibiotypes of *Enterococcus casseliflavus* strains from flies from Cumaná and Carúpano, Sucre state

Strains	Antibiotics											
	AM	GM120	LZD	CIP	NOR	VA	TEC	E	C	TE	RA	F/M
I	S	S	S	I	S	S	S	I	S	S	S	S
3	S	S	I	I	I	S	S	R	I	S	R	S
2	S	S	R	I	I	S	S	I	S	S	R	S
I	S	S	I	I	I	S	S	I	S	R	R	S
I	S	S	R	I	I	I	S	R	R	R	R	S

S, Susceptible; I, Intermediate; R, Resistant; Am, Ampicillin; SAM, Ampicillin/sulbactam; GM120, high loading gentamicin; LZD, Linezolid, CIP, Ciprofloxacin; NOR, Norfloxacin; VA, Vancomycin; TEC, Teicoplanin; E, Erythromycin; C, Chloramphenicol; TE, Tetracyclin; RA, Rifampicin; F/M, Nitrofurantoin

Table 7 Antibiotypes of *Enterococcus gallinarum* strains from flies from Cumaná and Carúpano, Sucre state

Strains	Antibiotics											
	AM	GM120	LZD	CIP	NOR	VA	TEC	E	C	TE	RA	F/M
3	S	S	R	I	I	S	S	I	S	S	R	S
I	S	S	I	I	I	S	S	I	S	R	R	S
I	S	S	S	S	S	S	S	R	S	S	R	S

S, Susceptible; I, Intermediate; R, Resistant; Am, Ampicillin; SAM, Ampicillin/sulbactam; GM120, high loading gentamicin; LZD, Linezolid, CIP, Ciprofloxacin; NOR, Norfloxacin; VA, Vancomycin; TEC, Teicoplanin; E, Erythromycin; C, Chloramphenicol; TE, Tetracyclin; RA, Rifampicin; F/M, Nitrofurantoin

The only *E. faecium* strain isolated in this work presented resistance to ampicillin, a typical characteristic of this species, since resistance to beta-lactams is intrinsic due to the presence of PBP5' and its low affinity for these antibiotics. Additionally, the strain was resistant to fluoroquinolones (ciprofloxacin and norfloxacin) and rifampicin. Intermediate sensitivity to erythromycin and vancomycin. The MIC for ampicillin was 16 µg/ml and 8 µg/ml for both vancomycin and ciprofloxacin. This strain is intermediate to vancomycin, a PCR must be performed to determine the resistance operon it harbors, since from a phenotypic point of view it cannot be said.

In Table 8 it can be seen that the MICs for ampicillin of the strains isolated in the flies from the state of Sucre range from <0.5 to 16 µg/ml. It is worth remembering that when the ampicillin and ampicillin/sulbactam discs were placed on the MH agar plates, no variations were observed between the two discs, therefore, there were no beta-lactamase producing strains; The strain that obtained an MIC of 16 µg/ml was *E. faecium*. According to CLSI M100-S25, a strain with an MIC of 16 µg/ml or greater is resistant.

Table 8 Minimum inhibitory concentration to ampicillin of *Enterococcus* strains isolated from flies from Cumaná and Carúpano, Sucre state

	Concentration (µg/ml)								
Strains	<0.5	1	2	4	8	16	32	64	128
11	X	-	-	-	-	-	-	-	-
2	-	X	-	-	-	-	-	-	-
1	-	-	-	-	-	X	-	-	-

X: CMI (Concentración Mínima Inhibitoria)

Table 9 shows the distribution of the MICs to vancomycin of the strains isolated from the Carúpano and Cumaná flies. In this work, all strains had a vancomycin MIC below 4 µg/ml (except *E. faecium*), which indicates sensitivity according to table 2D of M100-S25 of the CLSI, however, it must be remembered that all Motile enterococci are intrinsically resistant to vancomycin only, due to the production of peptidoglycan precursors ending in D-Ala-D-Ser.

Table 9 Minimum inhibitory concentration to vancomycin of *Enterococcus* strains isolated from flies from Cumaná and Carúpano, Sucre state

	CMI (µg/mL)								
N° Cepas	<0.5	1	2	4	8	16	32	64	128
1	-	-	-	-	X	-	-	-	-
5	-	-	X	-	-	-	-	-	-
8	-	-	-	X	-	-	-	-	-

X: CMI (Concentración Mínima Inhibitoria)

In table 10 it can be seen that more than half of the strains are resistant to ciprofloxacin (57%).

Table 10 Minimum inhibitory concentration to ciprofloxacin of *Enterococcus* strains isolated from flies from Cumaná and Carúpano, Sucre state

	Concentration µ(g/ml)								
Strains	<0.5	1	2	4	8	16	32	64	128
6	-	-	X	-	-	-	-	-	-
7	-	-	-	X	-	-	-	-	-
1	-	-	-	-	X	-	-	-	-

X: CMI (Concentración Mínima Inhibitoria)

In table 11 you can see the 14 strains isolated from the flies from the state of Sucre; In the *Calliphora vicina* fly captured in Carúpano, two strains of *E. gallinarum* were isolated from the same fly, both from the intestine and from the surface, but with different antibiotypes (IB and III), therefore, it was not the same strain. Also from Carúpano, the *Sarcophaga* sp1 fly presented the *E. casseliflavus* strain, both on the surface and in the intestine, with the same antibiotic type (IV), therefore, there was clonality in these strains. and was located in both Carúpano and Cumaná in two different flies (*Sarcophaga* sp1 and *Chrysomya albiceps*). The *E. casseliflavus* strains isolated from *Musca domestica*, one in Cumaná and the other in Carúpano, presented the same antibiotype (AI).

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Table 11 Main species of *Enterococcus*, phenotypes and genotypes of resistance to glycopeptides isolated from flies captured in Cumaná and Carúpano, Sucre state

Fly	City	Ubication	Phenotype	Genotype	Specie	Antibiotype
M. domestica	Cumaná	Gut	VanC	*vanC2*	*E. casseliflavus*	IA
	Carúpano	Surface	VanC	*vanC2*	*E. casseliflavus*	II
	Carúpano	Surface	VanC	*vanC2*	*E. casseliflavus*	IA
Sarcophaga sp1	Cumaná	Gut	VanC	*vanC1*	*E. gallinarum*	V
	Cumaná	Surface	VanC	*vanC2*	*E. casseliflavus*	IV
	Cumaná	Gut	VanC	*vanC2*	*E. casselifflavus*	IV
C. albiceps	Carúpano	Surface	VanC	*vanC2*	*E. casseliflavus*	IV
	Carúpano	Surface	VanC	*vanC1*	*E. gallinarum*	IB
Sarcophaga sp2	Carúpano	Surface	VanC	*vanC2*	*E. casseliflavus*	IIIA
	Carúpano	Gut	VanC	*vanC1*	*E. gallinarum*	IB
C. vicina	Carúpano	Surface	VanC	*vanC1*	*E. gallinarum*	IB
	Carúpano	Gut	VanC	*vanC1*	*E. gallinarum*	IIIB
	Carúpano	Surface	–	–	*E. faecium*	VI
L. cuprina	Cumaná	Gut	VanC	*vanC2*	*E. casseliflavus*	VII

Discussion

This work is part of the mechanical evaluation of strains of *Enterococcus* spp., disseminated through flies in Cumaná (Sucre municipality) and Carúpano (Bermúdez municipality), Sucre State. The study arose due to the avidity and attraction of flies to inhabit urban or anthropized ecosystems, adapting to environmental conditions and their ability to transport pathogenic microorganisms with high levels of resistance to antibiotics for human clinical use.

Musca domestica belongs to the Muscidae family. The flies *Calliphora*, *Chrysomya* and *Lucilia* are part of the family Calliphoridae and the flies *Sarcophaga* sp1 and *Sarcophaga* sp2 are part of the family Sarcophagidae.[8] The main families of flies captured in Baltimore were Muscidae and Calliphoridae,[9] as in this work.

The results presented here coincide with the reports reviewed in a study that was carried out in the city of Coro, Falcón state, in the vicinity of markets, food outlets, homes, garbage deposits, including animal farms. The main families captured were Calliphoridae, Muscidae and Sarcophagidae, especially from Musca,[10] as in this study in the state of Sucre.

Enterococcus spp., represents 1% of the intestinal microbiota of humans, the most predominant species being *E. faecalis* and *E. faecium*. In insects, mainly diptera, the species *E. casseliflavus* (43.5%), *E. faecalis* (32%) and *E. faecium* (22.4%) have been detected.[11–14]

Of 262 flies captured in Baltimore, 36 *Enterococcus* strains (13.7%) were isolated,[9] much less than reported in this work. Flies carry bacteria in their digestive system and on their body surface, through their bristles. Due to their ability to fly, they can spread bacteria to any environment, this confirms the results obtained in the present study in which more bacteria were observed on the outside of the flies.[1]

A study carried out in India isolated 102 bacterial strains from the intestines of 65 flies; They used PCR to amplify the 16S rRNA genes and thus identify the bacterial species. Based on the sequences of the ribosomal genes, they obtained 22 different genera. Most of the bacteria identified were known potential pathogens of the genera *Klebsiella*, *Aeromonas*, *Shigella*, *Morganella*, *Providencia*, *Staphylococcus* and *Enterococcus*.[15]

Not all flies have species of the *Enterococcus* genus in their intestinal microbiota. In one study,[16] 50 flies were trapped and only 14 were colonized with *Enterococcus* (28%), leaving a large number of them uncolonized (72%).

The apparent non-colonization of the gastrointestinal tract of flies by *Enterococcus* spp. is due to the production of protective antimicrobial peptides or digestive compounds secreted by the salivary glands of diptera. Defensins (antimicrobial peptides) have properties against Gram-positive bacteria and have been found throughout the gastrointestinal tract except in the posterior region. It has been shown that the expression of defensins is inducible. On the other hand, the microbial load of the microbiota is also influenced by the insect diet.[17–19]

This study supports the possibility that flies may represent a danger to public health, since they can serve as vectors of opportunistic pathogens due to their ability to fly several kilometers, visit different substrates, and their adaptation to environmental conditions.[15] Another study demonstrates that house flies are fast in disseminating *Enterococcus* spp., and that it does not take so many flies or much time to achieve contamination with *Enterococcus* spp strains.[19]

E. faecalis is one of the most isolated bacteria in enterococcal infectious processes and house flies are involved in the dissemination of these strains.[20] In this work, *E. faecalis* was not isolated in any of the flies captured in the state of Sucre.

On swine farms in the United States, 162 specimens of *Musca domestica* were collected in North Carolina and Kansas. The flies were sterilized, as in this work, with sodium hypochlorite and ethanol. Almost all flies (98.1%) were colonized by *Enterococcus* and the main species in the gastrointestinal tract were *E. faecalis*, *E. faecium*, *E. hirae* and *E. casseliflavus*.[21] Unlike this study, strains of E. faecalis and *E. hirae* were isolated, most likely due to the presence of cattle feces colonized by these bacteria.

Molecular identification of the species is the best way to do this, as sometimes doubts can be left by some confusing biochemical tests. Two hundred and forty *Enterococcus* strains isolated from chicken fecal samples were characterized at the species level by PCR. They found that there were 40% strains of *E. faecalis*, 10.8% of *E. casseliflavus*, *E. faecium* and *E. mundtii*, 5.8% of *E. columbae*, 4.2% *E. gallinarum*.[22] In this work, no strains of E. mundtii or *E. columbae* were isolated.

The arrangement of these genes is similar in all three species, but each gene is species-specific. The VanC resistance phenotype is chromosomal, not transferable to other bacteria.[23] A study carried out in Italy in 2005 reported isolations of *E. gallinarum* strains from samples of cattle meat intended for human consumption (MIC 1-64 µg/ml) whose identification was carried out by multiplex PCR amplifying resistance ligases. (*vanC1*).

In recent decades, the isolation of *Enterococcus* strains in flies has increased, as well as various resistance mechanisms to most of the available antibiotics.[24] Although it is not considered virulent, it has become one of the most important intra-hospital pathogens due to its multi-resistance.[25]

The susceptibility profile of *E. casseliflavus* strains isolated from the intestine of *M. domestica* captured in pig farms shows a high level of resistance for tetracycline and erythromycin (> 60%), medium for quinolones (< 40%), low for aminoglycosides (< 20%) and phenicols (< 10%). The use of antibiotics as growth promoters in animals for human consumption, exerts selective pressure on the bacteria in the gastrointestinal tract of animals and those bacteria with resistance mechanisms to antibiotics for human clinical use are those that colonize the intestine of insects of farms.[21]

In this work there were 25% of *E. casseliflavus* strains resistant to tetracycline and 50% resistant to erythromycin (Table 6). Compared to other publications, it was low, since 43% of *E. casseliflavus* strains resistant to tetracycline and high for resistance to erythromycin (12%) have been reported, isolated from buffalo feces.[3] This is a problem, because it is a therapeutic option for patients allergic to beta-lactams,[22] and if there are already strains resistant to this antibiotic, it makes choosing the treatment in those cases difficult.

Table 7 shows that all *E. gallinarum* strains were resistant to rifampin, some were resistant to linezolid, erythromycin and tetracycline. There is a decrease in the sensitivity of the strains to fluoroquinolones. It has been described that the determinants of resistance to tetracycline (*tetM*) and erythromycin (*ermB*) are housed in conjugative transposons such as Tn*1545* of the Tn*916* family.[20]

Finding strains of *Enterococcus* spp. in the flies from the state of Sucre, resistant to erythromycin and tetracycline, would indicate the presence in these strains of this type of transposons, which also carry high-level resistance operons to glycopeptides.[26] In this work, no strains with a high level of resistance to glycopeptides were found, but they could appear in the near future, since there is a strong selective pressure due to the abuse of vancomycin in the hospital environment, so epidemiological surveillance of these is recommended types of resistance.

In two American swine farms, the families Muscidae and Calliphoridae were those with the highest isolation of resistant *Enterococcus* strains isolated from flies in American swine farms. The strains were resistant to at least two antibiotics.[9] In the flies from the state of Sucre, the same families were found colonized at the surface level and gastrointestinal tract with multidrug-resistant *Enterococcus*.

In a study conducted in Kansas, they found an ampicillin-resistant strain of *E. casseliflavus* with 16 µg/ml MIC isolated from Buffalo cattle feces.[3]

Neither the antimicrobial susceptibility testing nor the MIC are tools that allow making the decision to treat a strain isolated from an infectious process with vancomycin, since there are genotypes of resistance to glycopeptides that have low levels of resistance and are sensitive to the antimicrobial susceptibility testing and even the MIC. falls in susceptibility values, when they have resistance operons that will not allow therapeutic success [*vanB* (Arthur *et al*., 1996), *vanC*,[27,28] *vanE*,[29] *vanG*,[39] *vanL*,[31] *vanN*.[4] If a person develops an infection due to any of these strains, they should not be treated with vancomycin because there will be therapeutic failure.

Of 117 *E. faecalis* strains isolated from pigs in China, 64% resistance to ciprofloxacin was found.[32] This resistance mechanism is a problem in the treatment of strains that cause urinary infections, since fluoroquinolones are the antibiotics of choice due to their high concentration in the urinary tract and their broad spectrum in both Gram-positive and Gram-negative bacteria.[33] In a study conducted in Korea, of 81 strains of *E. faecalis* isolated from men, 47% were resistant to ciprofloxacin; If patients have uncomplicated urinary infections, they can be treated with fluoroquinolones, despite having strains resistant to ciprofloxacin, but if the infection is complicated, there will be therapeutic failure.[34]

Consuming food with fly droppings causes strains of *Enterococcus* spp., with antibiotic resistance genes, to reach the gut and become opportunistic pathogens depending on the patient's comorbidities. Cases of infections such as bacteremia, endocarditis, septicemia, endophthalmitis, or urinary tract infections due to strains of mobile enterococci have been described.[35,36]

The patients most affected by this type of infections are those who suffer from hepatobiliary and oncohematological disorders. Enterococcal infections are treated with vancomycin, linezolid and daptomycin, or combined treatments when strains have resistance mechanisms to the antibiotics mentioned above.[37]

In a study conducted on farms in Ha Nam Province, Vietnam, strains of *E. faecalis* resistant to linezolid were found in chickens, flies, and wastewater. This reaffirms the role of flies in the transmission of antibiotic-resistant pathogens.[38]

In a study, carried out solely with *Enterococcus* strains from different origins in Estonia, the mechanisms and genes of virulence and resistance of the isolated strains were studied; The clonal relationship was determined with molecular methods. They isolated closely related *E. faecalis* strains from different host species. This indicates a spread of strains between species and possible transfer of antibiotic resistance. Regardless of the fact that *E. faecalis* was not isolated in our study, it has a close relationship with the behavior of the strains of this study, because resistance genes are often present in the same genetic context in strains with diverse origins, which suggests the occurrence of transfer events.[39]

Flies are ubiquitous in the environment and coexist with both people and animals, making them ideal candidates to be important vectors of antibiotic resistance genes. It is not unreasonable to do research on bacteria and antibiotic resistance genes, because it is not thought about, but they play a fundamental role in the spread of resistance.[40]

Unfortunately, the strains isolated in this work harbor resistance determinants to linezolid, erythromycin, rifampicin, tetracycline and ciprofloxacin and flies are the main vectors of dissemination from one city to another. Flies carrying enterococci on their legs can be found 3 miles from the site where they were colonized, and even 10 miles due to the wind.[41]

Conclusion

The detection of the same antibiotypes of *E. caseliflavus* in both Carúpano and Cumaná indicates the high power of dissemination that flies have, of transporting these strains resistant to antibiotics for human clinical use, not only in the city but in a city to another (132.5 km), extending the problem of bacterial resistance to remote places.

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None

Conflicts of interest

The authors of the work declare that they have no conflict of interest.

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