Assessment of the prevalence of microbial antibiotic resistance in rabbit slaughterhouses

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INTRODUCTION

In order to investigate the prevalence of resistant bacteria to antibiotics in a rabbit slaughterhouse, animal and process area surfaces were analyzed in the present study. Rabbit´s carcass surfaces represent a suitable substrate for microbial growth of pathogenic bacteria due to improper animal handling from the slaughtering and the processing of carcass. Unhygienic slaughtering drives contamination of carcasses with microorganisms present in their skin, hair/wool, noses, urogenital and oral cavities, and besides, inside of intestinal tract.

In this sense, contamination with antibiotic resistant bacteria may increase the risk of resistance gene spread especially in the gut microbiome where horizontally transferrable resistance traits may take place between commensals and pathogens. This fact is enhanced by antimicrobials (antibiotics and biocides) used for different purposes in animal or the slaughterhouse environment.

The WHO Global Action Plan on Antimicrobial Resistance (2016) included surveillance and monitoring strategies of antibiotic resistance in farm animals and foodstuffs, in order to, develop new measures to combat this resistance.

OBJECTIVE

The aim of this study is to determine the prevalence of antibiotic resistant bacteria on carcass and environmental surfaces of a small production rabbit slaughterhouse.

MATERIAL AND METHODS

Two rounds of sampling were carried out (February and March 2018) with a total of nineteen samples collected in triplicate. In the first round, ten surfaces, not in contact with the carcasses, but close to the dressing and storage area, were sampled. The second round sampled two rabbit carcasses, three tooling and four installation surfaces (Table nº 1).

The non-destructive sampling method was performed by swabbing with a sterile 0.85% NaCl solution, in 5 cm2 areas, with the use of a template, and rubbing for 10 seconds in three directions. With the aim to isolate Antibiotic Resistant Bacteria (ABR), the resulting swabs were immersed in BHI (Brain Hearth Infusion) medium and TSB (Tryptone Soya Broth) containing Tetracycline, Amoxicillin or Sulfamethoxazole and stored at 37°C for 24h.

Samples were serially diluted, in sterile saline solution and plated in triplicate on TSA. Counts were obtained after 48 h of incubation at 37ºC for estimation of antibiotic resistant aerobic mesophilic bacteria.

The preliminary identification of resistant colonies was carried out by Gram staining and microscopic observation of colonies. Enterococci, Enterobacteriaceae, Gram+/- cocci and Gram+ Staphylococcus aureus were investigated.

Table 1. Sampling programme by slaughter areas

Code	Sample number	Sampling area	Sampling sub-area	Sampling point	Location
Z1CM	1	1	1	Carcass lower	Carcass 1
Z1CA	2	1	2	Carcass upper	Carcass 2
Z2G	3	2	3	Evisceration room	Hooks
Z2M	4	2	4	Cleaning room	Tables
Z2C	5	2	5	Packaging room	Boxes
1P	6	3	6	Cold room	Walls
2S	7	3	7	Cold room	Floors
3P	8	3	8	Slaughter room	Walls
4S	9	3	9	Slaughter room	Floors
5P	10	3	10	Evisceration room	Walls
6S	11	3	11	Evisceration room	Floors
7P	12	3	12	Packaging room	Walls
8S	13	3	13	Packaging room	Floors
9P	14	3	14	Box store room	Walls
10S	15	3	15	Box store room	Floors
Z4A	16	4	16	Toilets	Walls
Z4V	17	4	17	Locker room	Lockers
Z4AL	18	4	18	Cleaning area	Shelves
Z4OA	19	4	19	Office area	Tables

RESULTS AND DISCUSSION

The results obtained showed that 7.92% of the samples were resistant to Tetracyclines, in line with the 8.0% reported in Spain (2016).

The slaughterhouse area where the highest prevalence of resistant bacteria was detected (57.14%) within slaughtering, evisceration, chilling chamber, packing room and boxes store (Tables nº 2 and 3).

The area with the lowest prevalence of resistant bacteria (10.48%) was rabbit´s carcasses. The prevalence of bacteria resistant, in descendent order, was sulfamethoxazole (98.7%), amoxicillin (73.7%) and tetracycline (53.9%) was observed (Figures nº 1 and 2).

Table 2. Presence of microrganisms in selective broths

Samples	Kanamicyn Esculin	MacConkey	Tryptone Soy Broth	Giolitti-Cantoni Broth	Fraser Broth
1	-	+	+	+	+
2	-	+	+	+	+
3	+	-	+	+	+
4	+	-	+	+	+
5	+	-	+	+	+
6	+	-	+	+	+
7	+	+	+	+	+
8	+	-	+	+	+
9	+	+	+	+	+
10	+	-	+	+	+
11	-	-	+	+	+
12	+	-	+	+	+
13	+	+	+	+	+
14	+	-	+	+	+
15	-	-	+	+	+
16	-	-	+	+	+
17	+	-	+	+	+
18	+	-	+	+	+
19	+	-	+	+	+
Presence +	Absence -				

Table 3. Presence of microrganisms in selective broths

Kanamicyn/Esculin																		
Sample code	1								#	11	#	#	#	#	#	#	#	#
Growth	-	-	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	+
Sulfamethoxaz			+	+	+	+	+	+	+	0	+	+	+	0	0	+	+	+
Amoxicillin			-	-	-	-	-	-	-	0	-	-	-	0	0	-	-	-
Tetracyclines			-	-	+	-	-	-	-	0	-	-	-	0	0	+	-	-
MacConkey																		
Sample code	1								#	11	#	#	#	#	#	#	#	#
Growth	+	+	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-
Sulamethoxazo	+	+				+		+	0	0	0	+	0	0	0	0	0	0
Amoxicillin	+	-				+		-	0	0	0	-	0	0	0	0	0	0
Tetracyclines	-	-				-		-	0	0	0	-	0	0	0	0	0	0
Tryptone Soy Broth																		
Sample code	1								#	11	#	#	#	#	#	#	#	#
Growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulfamethoxaz	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amoxicillin	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-
Tetracyclines	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
Giolitti Cantoni Broth																		
Sample code	1								#	11	#	#	#	#	#	#	#	#
Growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulfamethoxaz	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amoxicillin	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	-	-	-
Tetracyclines	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-
Fraser Broth																		
Sample code	1								#	11	#	#	#	#	#	#	#	#
Growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sulfamethoxaz	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Amoxicillin	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-	-	-	-
Tetracyclines	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Presence +																		
Absence -																		

Figure nº 1. Post-antibiotic growth according to antimicrobials (172 from 228).

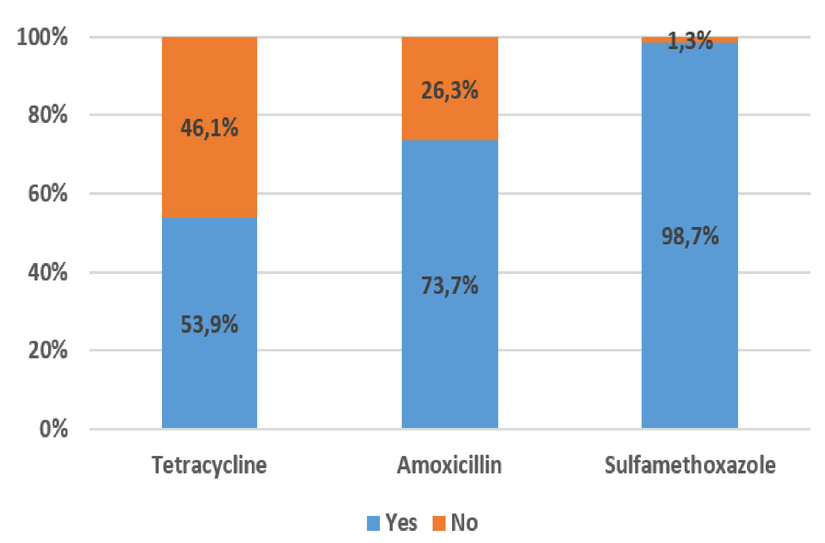
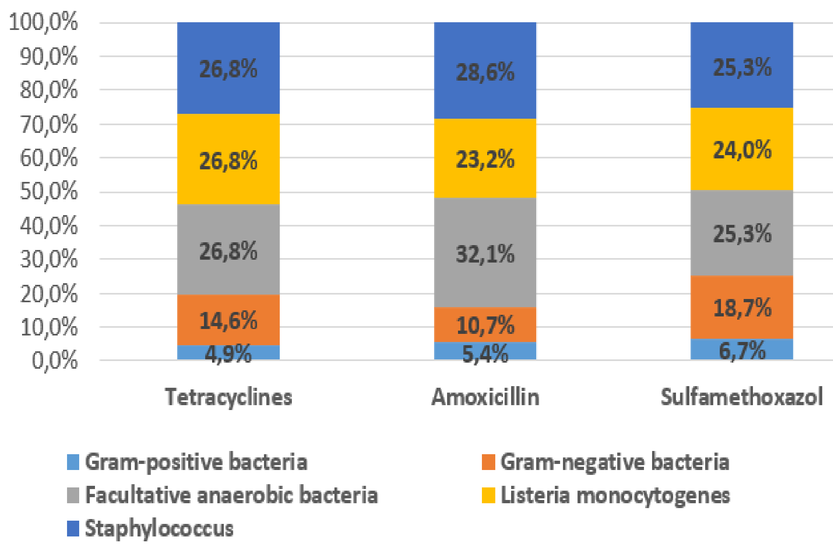


Figure nº 2. Samples of indicators microorganisms and pathogens bacteria identified as resistant to antimicrobials (172 from 228).



CONCLUSIONS

From this study, we can conclude that surfaces of equipment, installations and carcasses are the main reservoirs of Antibiotic Resistant Bacteria (ARB) leading to antibiotic resistance spread to humans and the environment and thus causing a great public health challenge.

The non-rational use of antimicrobials for veterinary or production purposes is a clear threat to animal and human health from a One Health perspective and highlights the problem of antibiotic effectiveness and bacterial resistance.