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BRIEF REPORT

Wild-type minimal inhibitory concentration distributions in bacteria of animal origin in Argentina

Florencia L. Pantozzi^{a,*}, Mariela P. Ibar^a, Victorio F. Nieves^a, Germán B. Vigo^b, Fabiana A. Moredo^b, and Gabriela I. Giacoboni^a

^a Laboratorio de Diagnóstico e Investigaciones Bacteriológicas, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

^b Cátedra de Microbiología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

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KEYWORDS

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Enterococcus spp;
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Abstract

The aim of this study was to determine the antimicrobial resistance profiles of indicator bacteria isolated from domestic animal feces. Minimal inhibitory concentration (MIC) was determined by agar dilution. Interpretative criteria on the basis of wild-type MIC distributions and epidemiological cutoff values (ECOFF or ECV) were used according to the 'European Committee on Antimicrobial Susceptibility Testing' (EUCAST) data. Results from 237 isolates of *Escherichia coli* showed reduced susceptibility for ampicillin, streptomycin and tetracycline, the antimicrobials commonly used in intensive breeding of pigs and hens. Regarding all the species of the genus *Enterococcus* spp., there are only ECOFF or ECV for vancomycin. Of the 173 *Enterococcus* spp. isolated, only one showed reduced susceptibility to vancomycin and was classified as 'non-wild-type' (NWT) population. This is the first report in Argentina showing data of epidemiological cutoff values in animal bacteria. © 2013 Asociación Argentina de Microbiología. Published by Elsevier España, S.L. All rights reserved.

PALABRAS CLAVE

Escherichia coli;
Enterococcus spp.;
Monitoreo antimicrobiano;
Punto de corte epidemiológico;
'Non-wild-type'

Distribución de la concentración inhibitoria mínima y puntos de corte "wild-type" en bacterias de origen animal en Argentina

Resumen

El objetivo de este estudio fue determinar los patrones de resistencia antimicrobiana en bacterias indicadoras aisladas de muestras fecales de animales domésticos. La concentración inhibitoria mínima (CIM) fue determinada por el método de dilución en agar. El criterio de interpretación usado se basó en la distribución de la CIM y el punto de corte epidemiológico (ECOFF o ECV) de acuerdo con los datos del *European Committee on Antimicrobial Susceptibility Testing* (EUCAST). Los resultados obtenidos de 237 aislamientos

* Corresponding author.

E-mail address: fpantozzi@fcv.unlp.edu.ar (F.L. Pantozzi).

de *Escherichia coli* mostraron sensibilidad reducida a ampicilina, estreptomina y tetraciclina, antimicrobianos comúnmente usados en porcinos y aves de explotación intensiva. Con respecto a todas las especies del género *Enterococcus* spp., solo existe ECOFF o ECV para la vancomicina. De los 173 *Enterococcus* spp. aislados, sólo uno presentó sensibilidad reducida a dicho agente y fue categorizado como población 'non-wild-type' (NWT). Este es el primer informe en Argentina que presenta datos de puntos de corte epidemiológico en bacterias animales.

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Antimicrobial resistance is a global public and animal health concern that is influenced by both human and non-human antimicrobial usage. The human, animal and plant sectors have a shared responsibility to prevent or minimise antimicrobial resistance selection pressures on both human and non-human pathogens¹⁰. Epidemiologists need to be aware of emerging changes in bacterial susceptibility, which may indicate emerging resistance, and allow for appropriate control measures to be considered³. In an attempt to overcome the problems of differences in interpretative criteria based on clinical or epidemiological data, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has decided to define separate dividing points for the detection of bacteria with resistance mechanisms and the monitoring of resistance development using wild-type cutoff values (WCV) or epidemiological cutoff values (ECOFF or ECV) and the guidance of therapy via clinical breakpoints⁹. ECOFF or ECV are determined on the basis of the distribution of minimal inhibitory concentrations (MICs) for an antimicrobial agent and a given bacterial species. EUCAST is in the process of collecting full range MIC data from as many sources as possible. Data are inserted into a database and each distribution is screened for acceptance, and then made freely available⁵. Although the use of ECOFF or ECV is important for the early detection of decreased susceptibility, this value is inappropriate to determine the percentage of clinical resistance¹⁴. This is because there are instances when a bacterial isolate will have a MIC value above the ECOFF or ECV but below the clinical susceptible breakpoint; in this case, such isolate will be clinically susceptible and should therefore not be categorized as resistant but as having decreased susceptibility⁴. In fact, when reporting data using ECOFF or ECV, the terms 'susceptible' or 'resistant' are inappropriate; instead, bacteria should be reported as 'wild-type' if the MIC or zone diameter falls below the epidemiological cutoff value and as 'non-wild-type' if the MIC is higher or the zone diameter is smaller than the epidemiological cutoff value¹³. Thus, the populations of microorganisms without an acquired phenotypically detectable resistance mechanism are defined as wild-type bacteria and the populations that clearly depart from the 'wild-type' populations are classified as 'non-wild-type' (NWT).

In Argentina, there is no current antimicrobial resistance surveillance system of public health importance in animals to describe the level of resistance to animal bacteria. In a previous study with the same bacterial isolates used in this work, we determined the antimicrobial susceptibility by the agar diffusion method and showed the results as resistance percentage¹².

The purposes of the present study were to determine the antimicrobial resistance profiles of indicator bacteria of

animal origin using the wild-type MIC distributions and ECOFF or ECV according to EUCAST data and to begin collecting data that could be used in a future monitoring program.

Escherichia coli and *Enterococcus* spp. were chosen and collected as indicator microorganisms for susceptibility testing. These bacteria are common commensals, which are considered to constitute a reservoir of antimicrobial resistance genes, which may be transferred to pathogenic bacteria causing disease in animals or humans¹¹. The isolates included in this study were collected from 2006 to 2007. A number of 237 *Escherichia coli* and 173 *Enterococcus* spp. were isolated from fifty fecal samples from healthy animals (cattle, horses, sheep, pigs, layer hens and dogs) without clinical signs. Samples were inoculated onto selective and differential media according to the bacterial genus¹¹. The *Enterococcus* species were identified on the basis of yellow pigment production, motility, deamination of arginine, utilization of pyruvate and carbohydrate fermentation of arabinose, sorbose, ribose, raffinose, sucrose and mannitol⁶. The agar dilution susceptibility test was used to determine the minimal inhibitory concentration (MIC)² of different antimicrobials. Interpretative criteria were used on the basis of wild-type cutoff values also called "epidemiological cutoff values" (ECOFF or ECV) according to EUCAST data⁵.

Escherichia coli ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as quality control. For *E. coli*, the following antimicrobial agents were tested according to the following ECOFF or ECV: ampicillin ≤ 8 $\mu\text{g/ml}$, cephalothin ≤ 32 $\mu\text{g/ml}$, gentamicin ≤ 2 $\mu\text{g/ml}$, amikacin ≤ 8 $\mu\text{g/ml}$, streptomycin ≤ 16 $\mu\text{g/ml}$, nalidixic acid ≤ 16 $\mu\text{g/ml}$, enrofloxacin ≤ 0.12 $\mu\text{g/ml}$, ciprofloxacin ≤ 0.06 $\mu\text{g/ml}$, chloramphenicol ≤ 16 $\mu\text{g/ml}$, florfenicol ≤ 16 $\mu\text{g/ml}$, tetracycline ≤ 8 $\mu\text{g/ml}$ and trimethoprim-sulfamethoxazole ≤ 1 $\mu\text{g/ml}$. For *Enterococcus* spp., ampicillin, vancomycin, tetracycline, erythromycin and gentamicin were tested.

According to EUCAST, there are no data of ECOFF or ECV in all *Enterococcus* species for some of the antimicrobials analysed. For *E. faecalis* and *E. faecium*, the ECOFF or ECV of ampicillin, vancomycin, tetracycline and erythromycin is ≤ 4 $\mu\text{g/ml}$ whereas that of gentamicin is ≤ 32 $\mu\text{g/ml}$. For *E. hirae*, the ECOFF or ECV of tetracycline is ≤ 4 $\mu\text{g/ml}$ whereas that of erythromycin is ≤ 2 $\mu\text{g/ml}$. For *E. avium*, *E. casseliflavus* and *E. gallinarum*, the ECOFF or ECV of ampicillin is ≤ 4 $\mu\text{g/ml}$, whereas for *Enterococcus* spp. the ECOFF or ECV of vancomycin is ≤ 4 $\mu\text{g/ml}$.

Table 1 shows the distribution of MICs and wild-type cutoff values according to EUCAST for *E. coli*. Table 2 shows the *Enterococcus* species and number of isolates among pigs, cattle, sheep, layer hens, horses and dogs. Table 3 shows the MIC distribution for *Enterococcus* spp.

Table 1 MIC distribution for *Escherichia coli* from pigs (n=43), cattle (n=43), sheep (n=18), hens (n=49), horses (n=35) and dogs (n=49)

ATM	Sample	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>128
AMP	Pigs										11	1	7			1	23
	Cattle										6	23	13				1
	Sheep										14	4					
	Hens										10	10	18	4		1	6
	Horses										7	20	7				1
	Dogs										4	10	25	3		1	6
CEP	Pigs											27	13	3			
	Cattle							1			1	23	18				
	Sheep											5	10	2		1	
	Hens											16	29	4			
	Horses											11	15	8	1		
	Dogs											20	27	2			
GEN	Pigs						7	22	10		1		1		1	1	
	Cattle							21	10	9	3						
	Sheep							9	7	2							
	Hens							22	8	16	2	1					
	Horses						1	15	8	9	1					1	
	Dogs						1	20	18	5	2	1			1	1	
AMK	Pigs								2	41							
	Cattle								4	19	20						
	Sheep								1	16		1					
	Hens								3	31	12	1	2				
	Horses								2	19	14						
	Dogs								1	24	24						
STR	Pigs										9	7	2	1	6	6	12
	Cattle										19	21	2		1		
	Sheep										1	8	7		2		
	Hens										22	17			2	3	5
	Horses										12	18	3		1		1
	Dogs										21	19		1	1	4	3
NAL	Pigs										5	10	13	3	3	4	5
	Cattle									1	24	14	4				
	Sheep											17	1				
	Hens										4	2	13	6	4		20
	Horses									4	16	12	3				
	Dogs									3	29	12		1			4
ENR	Pigs		12	10	11	3	3	2	1		1						
	Cattle		9	9	20	5											
	Sheep		1	5	12												
	Hens		2	9	1	3	4	10	11	7		1	1				
	Horses		6	13	10	6											
	Dogs		19	21	5			1	1	1			1				
CIP	Pigs	18	4	15		1	3	1	1								
	Cattle	12	10	16	4	1											
	Sheep		1	16	1												
	Hens	9	2	1	2	12	4	15	2			2					
	Horses	7	8	17	2	1											
	Dogs	35	6	4			1	2				1					

Table 1 (continuation) MIC distribution for <i>Escherichia coli</i> from pigs (n=43), cattle (n=43), sheep (n=18), hens (n=49), horses (n=35) and dogs (n=49)		≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>128
CMP	Pigs									1	9	6	6	1	4	2	14
	Cattle									11	20	11					1
	Sheep									1	1	11	5				
	Hens									1	2	34	10				2
	Horses									3	16	14	1				1
	Dogs									1	24	21	2	1			
	FLO	Pigs										1	12	11	1	18	
Cattle											10	12	21				
Sheep												9	9				
Hens											1	28	16	3	1		
Horses											5	14	15	1			
Dogs											9	15	25				
TET		Pigs									2	2	1			6	32
	Cattle								2	27	11					3	
	Sheep									15	1					2	
	Hens											16	1		4	28	
	Horses								1	22	9	1		1			1
	Dogs								3	27	5	3				11	
	TMS	Pigs							23	2	7	2		2	7		
Cattle								42	1								
Sheep								18									
Hens								45	1					3			
Horses								33					2				
Dogs								40	3	2		1	1	2			

ATM: antimicrobials, AMP: ampicillin, CEP: cephalotin, GEN: gentamicin, AMK: amikacin, STR: streptomycin, NAL: nalidixic acid, ENR:: enrofloxacin, CIP: ciprofloxacin, CMP: chloramphenicol, FLO: florfenicol, TET: tetracycline, TMS: trimethoprim-sulphametoxazole.
 The vertical lines indicate wild-type cutoff value.
 The grey zone indicates the number of bacteria with decreased susceptibility above ECOFF or ECV denominated as 'non-wild-type'.

Table 2 <i>Enterococcus</i> species and number of isolates among pigs, cattle, sheep, hens, horses and dogs		Pigs	Cattle	Sheep	Hens	Horses	Dogs
<i>E. casseliflavus</i> (n = 75)		9	2	17	3	29	15
<i>E. mundtii</i> (n = 39)		7		3	18	4	7
<i>E. gallinarum</i> (n = 5)		3				1	1
<i>E. dispar</i> (n = 9)		5		1			3
<i>E. faecalis</i> (n = 35)		1	1	2	19	2	10
<i>E. hirae</i> (n = 2)				2			
<i>E. faecium</i> (n = 3)			1		1		1
<i>E. raffinosus</i> (n = 5)		1	1		1	1	1

Table 3 MIC distribution for *Enterococcus* species from pigs (n=26), cattle (n=5), sheep (n=25), hens (n=43), horses (n=37) and dogs (n=37)

	<i>Enterococcus</i> species	≤ 0.25	0.5	1	2	4	8	16	32	64	> 128
AMP	<i>E. casseliflavus</i>		9	21	34	8	2				1
	<i>E. mundtii</i>	6	5	2	19	3	3				1
	<i>E. gallinarum</i>				1	2	2				
	<i>E. dispar</i>			2	2	2	3				
	<i>E. faecalis</i>	3	3	4	15	5	5				
	<i>E. hirae</i>	1	1								
	<i>E. faecium</i>				1	1	1				
	<i>E. raffinosus</i>	2		1		2					
VAN	<i>E. casseliflavus</i>				16	44	14	1			
	<i>E. mundtii</i>	7	6	16	8	2					
	<i>E. gallinarum</i>				4	1					
	<i>E. dispar</i>		5	4							
	<i>E. faecalis</i>	9	1	24			1				
	<i>E. hirae</i>	1		1							
	<i>E. faecium</i>	2		1							
	<i>E. raffinosus</i>		2	3							
TET	<i>E. casseliflavus</i>			49	1		1	6	6	3	9
	<i>E. mundtii</i>			13	1	1	3	2	6	4	9
	<i>E. gallinarum</i>			1				1			3
	<i>E. dispar</i>			2		1	1			1	4
	<i>E. faecalis</i>			13			2	1	1	4	14
	<i>E. hirae</i>			2							
	<i>E. faecium</i>			2							1
	<i>E. raffinosus</i>			3							2
ERY	<i>E. casseliflavus</i>	28	12	17	1	5				12	
	<i>E. mundtii</i>	16	1	4		4				14	
	<i>E. gallinarum</i>		1	1						3	
	<i>E. dispar</i>	4		1						4	
	<i>E. faecalis</i>	13		3	2					17	
	<i>E. hirae</i>	2									
	<i>E. faecium</i>	2								1	
	<i>E. raffinosus</i>	3								2	
GEN	<i>E. casseliflavus</i>						72	2			1 ^a
	<i>E. mundtii</i>						36	1			2 ^a
	<i>E. gallinarum</i>						5				
	<i>E. dispar</i>						9				
	<i>E. faecalis</i>						34				1 ^a
	<i>E. hirae</i>						2				
	<i>E. faecium</i>						3				
	<i>E. raffinosus</i>						5				

AMP: ampicillin, VAN: vancomycin, TET: tetracycline, ERY: erythromycin, GEN: gentamicin

The vertical lines indicate wild-type cutoff value.

The grey zone indicates the number of bacteria with decreased susceptibility above ECOFF or ECV denominated as 'non-wild-type'.

^a = 2000 µg/ml; *E. casseliflavus* and *E. faecalis* isolated from canines and *E. mundtii* from hens.

Indicator bacteria constitute a natural part of the intestinal flora of many different animal species that easily acquire resistance, and allow to compare levels of antimicrobial resistance among animal populations. In addition, indicator bacteria allow the direct comparison of resistance among different animal species and the analysis of resistance trends over time.

The results obtained showed decreased susceptibility in intensive breeding of pigs and hens. In pigs, 24 NWT of the 43 *Escherichia coli* strains isolated showed reduced susceptibility to ampicillin and streptomycin, 20 to chloramphenicol, 18 to florfenicol and 38 to tetracycline, coinciding with the antimicrobials most used in pig farms. In hens, 24 NWT of the 49 *Escherichia coli* strains isolated showed reduced susceptibility to quinolones, 30 to enrofloxacin, 2 to ciprofloxacin and 32 to tetracycline, the drugs most commonly used in this animal species.

In intensive pig and poultry production, animals are kept confined in overcrowded conditions and they are bred and managed for maximum yield. These conditions compromise their health and their immune responses and encourage infectious disease to develop and spread easily^{7,8}. Up to now, without the aid of drugs for disease prevention, it would not be possible to keep the animals productive under these intensive conditions.

In *Enterococcus* species, there are ECOFF or ECV only for vancomycin ($\leq 4 \mu\text{g/ml}$), and in this study only one *E. faecalis* isolate was classified as NWT. For tetracycline, a bimodal distribution was observed in some species. Based on the results, 26 *E. casseliflavus* of the wild-type population were isolated from horses and 8 from dogs, where the selective pressure of antimicrobials is minimal. For *E. mundtii* and *E. faecalis* most NWT strains came from layer hens, in which the same behaviour was observed for erythromycin. On the other hand, 75 strains of *E. casseliflavus* and 5 *E. gallinarum* were found to have MICs between 2 and 32 $\mu\text{g/ml}$ for vancomycin, phenotypically corresponding to the gene VanC, which is characterized by chromosomally mediated, non-transferable, intrinsic low-level resistance to vancomycin¹.

Some monitoring programs show the resistance percentage as well as the MIC distribution in their result tables¹⁵. In the present study, we decided not to determine this percentage, and, instead, to follow Schwarz's criterion¹³, which considers that when comparing resistance percentages among published studies, authors must make sure that the same methodologies and the same interpretative criteria have been used.

This study represents the first published data of wild-type MIC distributions of bacteria isolated from different animals in Argentina and we consider that the values herein obtained might serve as a starting point for a future monitoring program.

Ethical responsibilities

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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