



BRIEF REPORT

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

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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

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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 humans⁸. 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 mannitol. The agar dilution susceptibility test was used to determine the minimal inhibitory concentration (MIC)¹² 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.

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