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Brief communication

Prevalence of zoonotic *Arcobacter* species in pigs at slaughterhouse level in Ecuador

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ABSTRACT

Objective. To establish the prevalence, diversity and antimicrobial resistance of the zoonotic species of *Arcobacter* in healthy pigs at slaughterhouse level. **Material and methods**. Fifty fecal samples were taken by rectal swabs from healthy pigs, before the beginning of the slaughter at the slaughterhouse of Loja city, Southern Ecuador. Sampling was done by means of a non-probabilistic method for convenience. Isolation of *Arcobacter* strains was done by microbiological methods and species identification using biochemical and molecular (multiplex PCR) tests. Antimicrobial behavior was performed using the disk diffusion method. **Results**. The four zoonotic species of *Arcobacter* were found. The isolation rates were *A. thereius* (18.0%), *A. skirrowii* (18.0%), *A. cryaerophilus* (6.0%) and *A. butzleri* (2.0%). High resistance to ciprofloxacin was found and multi-resistant strains were isolated from these four species. **Conclusions**. The fecal carriage of the zoonotic species of *Arcobacter* was demonstrated in pigs at slaughterhouse level. These species showed high resistance to ciprofloxacin being isolated muti-resistant strains among these four species.

Keywords: Antimicrobial drug resistance; epidemiology; reservoir; zoonoses (*Source*: *DeCS*).

RESUMEN

Objetivo. Se pretende determinar la prevalencia, la diversidad y la resistencia antimicrobiana de las cuatro especies zoonóticas de *Arcobacter* en cerdos sanos, a nivel de matadero. **Materiales y métodos**. Fueron recolectadas, mediante muestreo no probabilístico por conveniencia, 50 muestras fecales obtenidas por hisopado rectal de cerdos sanos a nivel de matadero, antes de su faenamiento. El aislamiento de las cepas de *Arcobacter* fue realizado por métodos microbiológicos, utilizando enriquecimiento selectivo en caldo y filtración pasiva, mientras que para la identificación de especie fueron utilizadas pruebas bioquímicas y moleculares (multiplex PCR). El comportamiento frente a los antimicrobianos fue determinado por el método de disco difusión. **Resultados**. Fueron aisladas las cuatro especies zoonóticas, las cuales presentaron las siguientes frecuencias de aislamiento: *A. thereius* (18.0%), *A. skirrowii* (18.0%), *A. cryaerophilus* (6.0%) y *A. butzleri* (2.0%). Se encontró alta frecuencia de resistencia a ciprofloxacina y en las cuatro especies fueron aisladas cepas multirresistentes (resistentes a más de tres antibióticos).

Palabras clave: Antibiótico resistencia; epidemiología; reservorio; zoonosis (*Fuente*: *DeCS*).

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INTRODUCTION

The first isolations of *Arcobacter* species were made from bovine and porcine fetuses in 1997 and 1978, being initially described as *Vibrio*/*Spirillum*. Later, they were known as aerotolerant *Campylobacter*-like microorganisms and finally classified into the genus *Arcobacter* in 1991 (1,2).

Through the years, genus *Arcobacter* has experimented a great expansion being, at present, composed of 27 species. Of these, 11 species have been isolated from environmental samples, seven from shellfish or mollusks and nine from animals or animal food (4). Among the latter nine, only four -*A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius*- are considered emerging, zoonotic food-borne bacteria producing diarrhea and other infectious processes in human beings (2,3,4).

These four *Arcobacter* species were reported producing abortions, mastitis and diarrhea in domestic mammals. However, they can be recovered frequently from feces of healthy animals which may act as reservoirs of these bacteria and contamination sources to humans and the environment (1,5,6). Among domestic animals used to produce food for human consumption, swine are important reservoir of different *Arcobacter* species as well as source of pork meat contamination (1,5,6,7,8).

Considering that in Latin America, particularly in Ecuador, the information available about *Arcobacter* in swine is limited and that a significant proportion of the Ecuadorian population frequently consumes pork meat (9), the objective of this work was to determine the prevalence, species diversity and antimicrobial behavior of *Arcobacter* spp. isolated from fecal material obtained of healthy pigs at slaughterhouse level.

MATERIALS AND METHODS

Ethical aspects. The Ethics Committee of the Universidad Técnica Particular de Loja approved the execution of this study and fecal sampling was done respecting animal welfare and with veterinary assistance.

Samples collection. Fifty fecal samples were randomly taken (non-probabilistic for convenience method) by rectal swabs from healthy pigs, before the beginning of the slaughter at the slaughterhouse of Loja city, Southern Ecuador.

Laboratory methods. To isolate *Arcobacter* spp., each sample was seeded into CAT enrichment broth (cefaperazone, amphotericin B and teicoplanin) being incubated at 30 °C during 72 h in aerobiosis (10). After them, 200 µL aliquots of every enriched broth were passive filtered through 0.45 µm filter membrane on blood agar plates. Filtration lasted for 30 min, the filters were then aseptically removed, and the plates incubated using the same conditions described above (6,10). The isolates were preliminary identified by their phenotypic characteristics [motile curved Gram-negative rods with aerobic growth, and oxidase and catalase positive] (6). Definitive species identification was done employing the multiplex PCR (mPCR) test described by Douidah *et al* that characterizes the five most common *Arcobacter* species associated to human and mammals [*A. butzleri*, *A. cibarius*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius*] (11).

Since specific breakpoints for defining resistance in *Arcobacter* are not available, the resistance to the five antibiotics studied (ciprofloxacin, erythromycin, gentamycin, amoxicillin and tetracycline) was assessed using the disk diffusion method recommended for *Campylobacter* by EUCAST (12).

RESULTS

Of the 50 swine fecal samples studied, 20 (40%) were positive being identified the four zoonotic species of *Arcobacter*. In two of the positive samples, two *Arcobacter* species were simultaneously isolated (*A. butzleri* + *A. thereius* and *A. skirrowii* + *A. thereius*). In the remaining 18 samples, only one single species was isolated each time. The isolated species were *A. butzleri* (2.0%), *A. cryaerophilus* (6.0%), *A. skirrowii* (18.0%) and *A. thereius* (18.0%).

As shown in Table1, resistance to ciprofloxacin and tetracycline was found in all the four species isolated. The highest resistance frequency was found for ciprofloxacin. Multi-resistance was found in one strain of *A. butzleri*, *A. cryaerophilus*, *A. skirrowii* and *A. thereius*. No resistance was observed for i) erythromycin in *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*; ii) gentamycin in *A. skirrowii* and *A. thereius* and iii) to ampicillin in *A. cryaerophilus*.

Table 1. Occurrence, diversity and antimicrobial resistance of *Arcobacter* species in swine fecal samples (50) at slaughterhouse level.

Species	No. of Isolates	%	Antimicrobial resistance				
			Cipro	Erythro	Genta	Ampi	Tetra
A. butzleri	3*	6.0	1/3[a]	0/3	1/3	3/3	1/3
A. cryaeophilus	1	2.0	1/1	0/1	1/1	0/1	1/1
A. skirrowii	9*	18.0	3/9	0/9	0/9	1/9	1/9
A. thereius	9	18.0	6/9	1/9	0/11	2/9	2/9

*A strain of *A. butzleri* and *A. skirrowii* were isolated simultaneously with *A. thereius*
[a] No. of resistant strains/No. of strains tested
Cipro= ciprofloxacin; Erythro= erythromycin; Genta= gentamycin; Ampi= ampicillin; Tetra= tetracycline

DISCUSSION

The *Arcobacter* species prevalence reported in swine in different countries ranged from 4.0% in Brazil (8) to 59.3% in Chile (6), but intermediate prevalence data have been reported in Belgium (11.3-50.0%), Peru (29.2%) and India (23.3%) (13,14,15). The isolation frequency found in this work seems to be relatively high and falls within the ranges described in the literature. These data suggest that swine should be considered a significant reservoir of *Arcobacter* spp. in different latitudes.

In agreement with the reports of de Smet *et al.* in four Belgian pig farms (13), we also isolate the four zoonotic species of *Arcobacter*. However, other authors isolated mostly *A. butzleri, A skirrowii* and *A. cryaerophilus*. This is because molecular methods did not cover the identification of all zoonotic species (6,8,15). Even, in some works, only phenotypic methods have been used as it was in the Peruvian experience (14). These differences highlight the lack of standardized methods, universally accepted, both to isolate and to identify accurately de different species of *Arcobacter*.

A. butzleri has been reported as the species more frequently found in clinical, environmental and animal samples (1). However, in this study *A. thereius* and *A. skirrowii* were the most frequent (18.0% each), followed by *A. cryaerophilus* (6.0%) and *A. butzleri* (2.0%). Lower isolation rates of *A. skirrowii* have been reported (1,14) but, Ho *et al.* and de Smet *et al.* informed higher frequencies of this bacterium (7,13). In addition, Ho *et al.* demonstrated that transmission of *Arcobacter* spp. from sows to their offspring could be vertical as well as horizontal; the latter through environmental sources (7). *A. thereius* was originally isolated from pigs and ducks (16), having been isolated relatively frequently from healthy pigs' fecal samples and also from a couple of hospitalized patients with enteritis (4,16). At present, these four species are considered as emerging zoonotic and food-borne agents having pigs as one of their main reservoirs (4,5). Other species of the genus such as *A. throphiarum*, *A. suis* and *A. lanthieri* have also been isolated from pigs (17). Recently, Figueras *et al*. described the new species *A. porcinus,* closely related to *A. thereius* (18). However, until now, none of these species, except *A. thereius*, have been shown to be zoonotic. Considering that pigs are reservoirs of *Arcobacter* species not yet described as zoonotic, clinical microbiologists should be aware about the possibility that some of them could infect humans. Therefore, they should be prepared to be able to identify them correctly.

Regarding their antimicrobial behavior, resistance to ciprofloxacin and tetracycline was found in all the four species isolated, drawing attention to the high resistance to ciprofloxacin in *A. thereius* (6/9) and A. *skirrowii* (3/9). No resistance was observed for erythromycin in *A. butzleri*, *A. cryaerophilus* and *A. skirrowii*; gentamycin in *A. skirrowii* and *A. thereius* and to ampicillin in *A. cryaerophilus*. Multi-resistant strains were found, one strain in each of the four species. Resistance to ciprofloxacin and to other antibiotics as well as multi-resistance have been reported previously (19).

The small number of isolates studied does not let us to propose sound conclusions but points out the need to conduct long-range studies to define both the epidemiological situation of *Arcobacter* and its antimicrobial behavior in Ecuador.

The obtained results demonstrate that pigs at slaughterhouse level in Ecuador could be reservoir of the four zoonotic species of *Arcobacter*, which can display resistance to

several antibiotics, highlighting the resistance to ciprofloxacin and the isolation of multi-resistant strains. Additional long-range studies are needed to provide information on both the epidemiological situation of *Arcobacter* and its antimicrobial behavior in Ecuador.

Conflict of interest

The authors state that they have no conflicts of interest.

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