Science of the Total Environment 647 (2019) 362–368

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Escherichia coli used as a biomarker of antimicrobial resistance in pig farms of Southern Brazil

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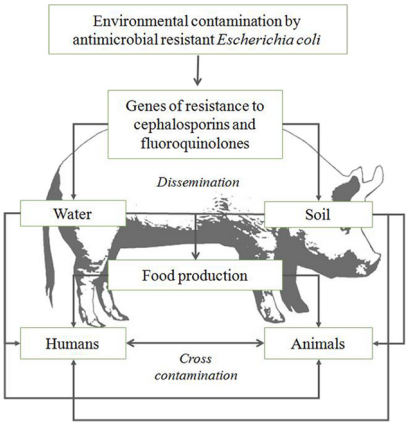
HIGHLIGHTS

- Prevalence of *E. coli* in fecal, water and soil samples of swine farms from Southern Brazil
- Phenotypic profile of resistance and multiresistance of strains of *E. coli* to five classes of antimicrobials
- Detection of Extended Spectrum Beta-lactamases (ESBLs) in *E. coli* strains
- Coexistence of ESBLs and *qnr* genes in phenotypically ESBL-producing isolates

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 March 2018
Received in revised form 29 May 2018
Accepted 30 July 2018
Available online 31 July 2018

Editor: Henner Hollert

Keywords:
Escherichia coli
ESBLs
Fecal contamination
Fluoroquinolones
Resistance

ABSTRACT

The objective of this study was to verify the presence of antimicrobial resistant strains of *Escherichia coli* in pig farms and to use it as a biomarker to evaluate phenotypic and genotypic profiles of antimicrobial susceptibility, as well as the presence of Extended Spectrum Beta-lactamases (ESBLs) and fluoroquinolone resistance genes. Several samples (n = 306) collected from swine farms (n = 100) of Southern Brazil were used for *E. coli* isolation: 103 of swine feces, 105 of water, and 98 of soil. *E. coli* isolates were submitted to the disk-diffusion test to verify their antimicrobial susceptibility, to disk-approximation test to detect ESBL-producers, and to PCR analysis to search for ESBLs genes (*bla*CTY-M2, *bla*SHV-1, *bla*TEM-1, *bla*CTX-M2, *bla*OXA-1, *bla*PSE-1) and quinolone resistance genes (*qnr*A, *qnr*B and *qnr*S). The percentage of *E. coli* isolates found in feces, water and soil samples was 66.02%, 30.48% and 35.71%, respectively. The highest percentages of resistance were obtained for sulfamethoxazole associated with trimethoprim (63.70%), colistin (45.19%) and enrofloxacin (39.26%). Regarding the levels of multidrug resistance, 37.04% of the isolates were resistant to three or more classes of antimicrobials. The most common profile (16%) of multirresistance was GEM-SUT-ENO-COL. The index of multiple resistance to antimicrobials (IRMA) was above 0.2 in 78% of the multiresistant isolates. Out of 135 *E. coli* isolates, 7.41% was ESBL-producers, of which 50% showed the *bla*CMY-M2 gene, 40% the *bla*TEM-1 and 70% the *qnr*S gene. Of non-ESBL-producing strains resistant to enrofloxacin, 13.04% were positives for *qnr*S gene. These results demonstrated

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https://doi.org/10.1016/j.scitotenv.2018.07.438

the presence of fecal contamination in the environment, in addition to high resistance indexes for several antimicrobials, including beta-lactams and fluoroquinolones, which was confirmed by the genetic detection of ESBLs and *qnr* genes.

1. Introduction

Brazil is one of the largest animal protein producer, with 3.3 million tons of pork meat produced in 2017, where 600 thousand tons were exported to more than 70 different countries (ABPA, 2017). In 2016, the state of Santa Catarina (Southern Brazil) accounted for 38% of all exported Brazilian pork meat (EPAGRI, 2017). However, high concentration of animal production can lead to environmental problems, such as soil saturation caused by animal waste and also soil and water contamination by pathogenic microorganisms (Silva et al., 2015).

Contaminated water and soil may spread pathogenic microorganisms, and the bacterium *Escherichia coli* can be used as a biomarker of environmental contamination. *E. coli* is a commensal microorganism of the gastrointestinal tract of humans and many animals that can cause diseases and the spread antimicrobial resistance genes (Caldorin et al., 2013). It is indicated as a biomarker due to its abundance and direct contact with the gastrointestinal tract of animals, being susceptible to all environmental conditions of this organ, such as feed, additives and performance enhancers (Rochelle-Newall et al., 2015).

Recently, bacteria isolated from many environments have been identified as important reservoirs of resistance genes that can be transferred to other pathogens through mobile elements, such as genes that encode the production of Extended Spectrum Beta-lactamases (ESBLs), confering resistance to beta-lactam antibiotics (Jiang et al., 2011). These same mobile elements confer resistance to quinolones and fluoroquinolones through genes encoding the production of proteins capable of protecting the bacterium from drug action (Yeh et al., 2017).

Cephalosporins and fluoroquinolones are widely used in human and animal medicine and are classified as "critically important antimicrobials" by the World Health Organization (WHO, 2014). Although Santa Catarina State has been a state with a large pork meat production for many decades, little is known regarding its antimicrobial resistance status. In this sense, the objective of this work was to isolate *E. coli* from feces, water and soil of farms with swine production in order to evaluate its phenotypic profile of antimicrobial susceptibility, as well as to correlate the presence of ESBLs and *qnr* genes in beta-lactam and fluoroquinolone resistant strains of *E. coli*.

2. Materials and methods

2.1. Sampling and E. coli isolation

A total of 306 samples were collected from March 2016 to May 2017 (103 samples of swine feces, 105 of water and 98 samples of soil) from rural farms (n = 100) with pork production in many cities (n = 20) of Santa Catarina state, mainly in the municipalities of Seara and Xavantina, where the highest volume of meat production takes place (EPAGRI, 2017). The identification and origin of the samples are described in Tables 3, 4 and 5. The samples were conditioned in sterilized bottles and transported under refrigeration to the laboratory. *E.coli* isolation was performed according to the technique of Quinn et al. (2005) with some adaptations, where the samples were incubated in lactosate broth (1:10) for 24 h at 36 ± 1 °C. They were then seeded in petri dishes containing Eosin Methylene Blue Agar (EMB) and MacConkey Agar and incubated at 36 ± 1 °C for 24 h. The colonies with green metallic characteristics on EMB Agar and pink on MacConkey Agar were submitted to biochemical tests (Urea Base Agar, TSI Agar, Sim Medium Agar and Agar Simmons Citrate) and subsequently incubated at 36 ± 1 °C for 24 h. After biochemical confirmation of the colony as *E. coli*, it was incubated in microtubes containing Tryptone Soya Agar medium at 36 ± 1 °C for 24 h and stored −20 °C after the addition of steril glycerol.

2.2. Antimicrobial susceptibility test

For the antimicrobial susceptibility test, the methodology approved by the Clinical and Laboratory Standards Institute (CLSI, 2018) and the National Agency for Sanitary Surveillance (ANVISA, 2003) was used, which is included in the Normative Instruction M-2 A-8 Antimicrobial Susceptibility by Disk Diffusion, where five classes of antimicrobials (ATBs) with different mechanisms of action were tested, with the selected antimicrobials commonly used in swine production in Brazil: beta-lactams (amoxicillin associated with clavulanic acid 30 μg - AMC) and third-generation cephalosporin (ceftiofur 30 μg - CTF), fluoroquinolones (enrofloxacin 5 μg - ENO), aminoglycosides (gentamicin 10 μg - GEM), sulfonamides (trimethoprim associated with sulfamethoxazole 25 μg - SUT) and polymyxins (colistin 10 μg - COL) by

Table 1
Genes of resistance related to extended-spectrum beta-lactamases (ESBLs), primers, conditions for their amplification and sizes of each expected fragment (bp).

Gene	Nucleotide sequence	PCR conditions	Cycles	bp
*bla*CMY-2	(F) TGG CCG TTG CCG TTA TCT AC (R) CCC GTT TTA TGC ACC CAT GA	95 °C - 10 min; 95 °C - 30 s; 55 °C - 1 min; 72 °C - 1 min; 72 °C - 7 min; 4 °C - ∞		870
*bla*SHV-1	(F) GGC CGC GTA GGC ATG ATA GA (R) CCC GGC GAT TTG CTG ATT TC			714
*bla*TEM-1	(F) CAG CGG TAA GAT CCT TGA GA (R) ACT CCC CGT CGT GTA GAT AA			643
*bla*CTX-M2	(F) GGC GTT GCG CTG ATT AAC AC (R) TTG CCC TTA AGC CAC GTC AC		30	486
*bla*OXA-1	(F) AAT GGC ACC AGA TTC AAC TT (R) CTT GGC TTT TAT GCT TGA TG			595
*bla*PSE-1	(F) TGC TTC GCA ACT ATG ACT AC (R) AGC CTG TGT TTG AGC TAG AT			438
AmpC	(F) AAC ACA CTG ATT GCG TCT GAC (R) CTG GGC CTC ATC GTC AGT TA	95 °C – 9.5 min; 95 °C – 45 s; 59 °C - 45 s; 72 °C - 1 min; 72 °C - 7 min; 4 °C - ∞	40	1226