Antibiotic resistant Enterobacteriaceae from Portuguese piggeries

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ABSTRACT

Background: Antibiotic (AR) overuse in animals, food chain, and human medicine has contributed to the spread of antibiotic-resistant (AR) bacteria outside hospitals. Data about AR occurrence among food animals in Portugal is limited to specific countries. Objectives: To evaluate the occurrence of AR Enterobacteriaceae in Portuguese piggeries. Methods: We analyzed 57 samples (30/37 piggery; nasal/rectal; drinking/water; food, air, predatory; surface) from 5 Portuguese piggeries located in different regions (2006-07). After enrichment, samples were plated on MacConkey agar. Antibiotics for resistance to quinolones among ESBL-producing Enterobacteriaceae were searched by double-disc synergy test. Results: We found several combinations of AR Enterobacteriaceae and AR Enterobacteriaceae isolates being similar among piggeries. Isolates were commonly resistant to AmpC (96%), aminoglycosides (80%), sulfonamides (72%), and tetracyclines (70%), and also to chloramphenicol (25%), nalidixic acid (16%), other aminoglycosides (<12%) and ciprofloxacin (2%). Among Enterobacteriaceae, resistance to chloramphenicol was frequent (76%). ESBLs were detected in 14% of isolates (23 E. coli, 2 Proteus vulgaris, 1 Salmonella marcescens, 1 Citrobacter freundii), being consistently recovered from two piggeries, qnrB was found in one ESBL-producing E. coli, qnrB, and qepB were absent among ESBL producers. Conclusions: Enterobacteriaceae resistant to AmpC used in clinical practice are frequently recovered from Portuguese piggeries. The potential dissemination to human highlights the need for public health efforts to implement surveillance, epidemiological, environmental health, and policy-making components.

INTRODUCTION

• Antibiotic overuse in animals could create an important reservoir of antibiotic resistant bacteria and genes that can spread to humans through the food supply (1, 2).

• Some antimicrobial agents used in veterinary and human medicine belong to the same antibiotic family. Intensive selective pressures exercised in food producing animals environments might contribute for the selection and dissemination of similar resistance genes (3, 4).

• To ensure future effectiveness of antimicrobials in human medicine, WHO is developing international consensus guidelines for reducing the use of critically important antimicrobials in food animals (1). Other control measures to reduce the exposure are also being proposed by EFSA (5).

• In a previous survey performed in Enterobacteriaceae isolates between 1998 and 2006, prevalence of ESBL was 15% (6). There is a need for continuous surveillance in a bigger scale, comprising food-producing farms from diverse geographic areas, is imperative for better control of antibiotic resistance.

OBJECTIVES

• To evaluate the occurrence of antibiotic resistant Enterobacteriaceae in Portuguese piggeries.

• To investigate the presence of extended-spectrum beta-lactamase (ESBL) enzymes among Enterobacteriaceae recovered from Portuguese piggeries.

• To evaluate the occurrence of genes coding for resistance to quinolones among ESBL-producing Enterobacteriaceae.

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MATERIAL AND METHODS

Bacterial isolates. We analyzed 57 samples from 5 intensive-production piggeries located in the North (n=18, Pg A), Centre (n=25, Pg C and Pg E) and South (n=14, Pg B) regions of Portugal, recovered between April 2006 and December 2007. Different types of samples were analyzed: fresh/dry feces, nasal/rectal, drinking/water, food, air, predatory, surfaces. All Enterobacteriaceae isolates presumptively identified as Enterobacteriaceae by standard biochemical profiles were selected for further analysis. All susceptibility (22 AMs) was performed by standard CLSI methods. Presence of extended spectrum-lactamases (ESBL, conferring resistance to third-generation cephalosporins) was searched by double-disc synergy test. Genes encoding resistance to quinolones (qnr, qep) were searched by PCR. Results: We Table 1. Enterobacteriaceae antibiogram showing resistance (R) and susceptibility (S) in Portuguese piggeries. Enzymes activities were determined using discs with the corresponding antibiotic. All enzyme activities were confirmed by E-test method following CLSI guidelines (7).

RESULTS

Bacterial isolates. Antibiotic resistant Enterobacteriaceae were found in 57 samples from 5 intensive-production piggeries located in the North (n=18, Pg A), Centre (n=25, Pg C and Pg E) and South (n=14, Pg B) regions of Portugal, recovered between April 2006 and December 2007. Different types of samples were analyzed: fresh/dry feces, nasal/rectal, drinking/water, food, air, predatory, surfaces. After pre-enrichment in buffered peptone water for 18 h at 37°C, samples were plated (0.2 mL) on MacConkey agar with and without ceftazidime (1 μg/mL), cefotaxime (1 μg/mL), tetracycline (0.5 μg/mL) or sulfonamides (256 mg/mL). Presumptive Enterobacteriaceae were selected for further studies and identified by API ID32GN (bioMérieux, Marcy l’Etoile, France).

Antimicrobial susceptibility testing to beta-lactam and non-beta-lactam antibiotics was determined by the standard disk diffusion method following CLSI guidelines (7). The antibiotics tested were the following: amoxicillin-clavulanic acid, cefazolin, cefotaxime, ceftazidime, cefoxitin, aztreonam, imipenem, ciprofloxacin, nalidixic acid, tobramycin, spectinomycin, kanamycin, gentamicin, amikacin, tobramycin, apramycin, neomycin, and netilmicin. All intermediate- to resistant isolates were selected as non-susceptible enterobacteriaceae.

Expression of ESBL was screened by the standard double disk synergy test using Mueller-Hinton agar plates with and without cefoxitin (250 μg/mL) (8, 9).

Genes encoding resistance to quinolones, namely qnrA, qnrB, qnrS and the recently discovered qep gene, were searched by PCR using primers and amplification conditions previously described (10, 11) (Table 1).

Fig. 1. Resistance patterns to beta-lactam antibiotics among Enterobacteriaceae from different piggeries. The unique Enterobacteriaceae recovered from Pg A was susceptible to all beta-lactam antibiotics. Among Pg B and Pg C isolates, resistance to amoxicillin-clavulanic acid and cefotaxim (22% and 22% versus 21% and 18%, respectively), indicating a possible high occurrence of AmpC-producing Enterobacteriaceae. Resistance to cefotaxime was frequently observed in Enterobacteriaceae recovered from Pg E and Pg F (22%, 10/45 and 24%, 16/66, respectively).

Overall, resistance to cefotaxime was the most frequently observed (16%, 30/191) when analyzing resistance rates obtained for beta-lactam tested. Resistance to cefopime was very low (0.5%) and all isolates were susceptible to imipenem.

Occurrence of ESBL phenotypes: ESBL expression was observed in 14% (27/191) of the isolates included in this study and identified as E. coli (n=23), P. vulgaris (n=2), S. marcescens (n=1), and C. freundii (n=1).

ESBL-producing Enterobacteriaceae were consistently recovered from two piggeries, Pg E and Pg F, localized in the Centre and North regions of Portugal, respectively, and showed a clear spread in these piggeries environment, being detected in different samples: feces (n=5), hide (n=2), feed (n=9), drinking water (n=4) and wastewater (n=4) samples.

The majority of the isolates presenting an ESBL phenotype were susceptible to ceftazidime and resistant to cefotaxime, suggesting the dissemination of CTX-M enzymes in Portuguese piggeries.

Distribution of multidrug resistant Enterobacteriaceae among different piggeries samples analysed: Multidrug resistant Enterobacteriaceae (resistance to 2 or more antibiotics tested) were frequently recovered from xenure (100%, 4/4), drinking/water (71%, 48/68), nasal/rectal (100%, 10/10), powder (100%, 3/3), and fresh feces (97%, 33/34) samples.

Occurrence of genes coding for resistance to quinolones among ESBL-producing Enterobacteriaceae: qnrS was found in one ESBL-producing E. coli recovered from a feed sample from Piggery E.

qnrA, qnrB, and qepA were absent among all ESBL-producing Enterobacteriaceae.

CONCLUSIONS

Enterobacteriaceae resistant to antibiotics used in clinical practice are frequently recovered from pigs and piggeries environment in Portugal.

We describe a high occurrence of ESBL-producing Enterobacteriaceae in Portuguese piggeries. This finding is worrisome as these enzymes confer resistance to cephaplosporins of third- and fourth-generation, relevant for human infections treatment.

Presence of acquired genes conferring resistance to fluoroquinolones suggests the emergence of plasmid-mediated quinolone resistance in Enterobacteriaceae from Portuguese piggeries and a possible animal reservoir for the qnr genes.

Our findings are worrisome as transmission of antibiotic resistance to humans via pork products and throughout contamination of environment is likely to occur, compromising several relevant antibiotics for treatment of human infections.