Preliminary Study on Phenotypic Detection of ESBL and Amp-C β -Lactamase-Producing *Escherichia Coli* Isolates from Slaughtered Pigs

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SHORT COMMUNICATION

Abstract

The presence of extended-spectrum beta-lactamase-producing *Escherichia coli* in food animals is a public health concern. The aim of this study was to phenotypically detect the extended β -lactamase and AmpC β -lactamase-producing *E. coli* isolated from pig caecum from three slaughterhouses in the North-East of Romania. After collecting the cecal samples, they were phenotypically processed. ESBL and AmpC screening was carried out by cultivation on MacConkey medium with addition of cefotaxime (MC+CTX). The isolates were confirmed as being *E. coli* based on the biochemical properties using MIU, TSI and API 20E and as ESBL based on synergy tests between clavulanic acid and ceftazidime and/or clavulanic acid and cefotaxime. Phenotypical detection of AmpC beta-lactamase-producing strains was carried out through a stable AmpC cephalosporin (cefepime). Following ESBL/AmpC screening, out of the 128 analysed samples, 51 (39.84%) grew on MC+CTX and they were identified as *E. coli*. After performing microdilution in broth using EUVSEC2 plates, 78.43% isolates were identified with ESBL phenotype, 9.8% isolates with ESBL/AmpC phenotype, and 11.77% isolates with AmpC phenotype. In this study, although a limited number of strains were analysed, the obtained results phenotypically confirm the presence of ESBL and AmpC enzymes in slaughtered pig populations from the North-East of Romania.

Keywords: AmpC; *E. coli*; ESBL; slaughtered pigs.

INTRODUCTION

Extended-spectrum beta-lactamase (ESBL) enzyme and AmpC beta-lactamase enzyme-producing *Enterobacteriaceae* strains represent a major public health concern as a result of the resistance to third-generation cephalosporins, antibiotics that are commonly used both in human and in veterinary medicine (Salviati et al., 2014). The carriage of ESBL and AmpC enzymes and the risk of their transmission from production animals to humans through various routes (direct contact, contaminated food) is known and signalled in the scientific literature (Kola et al., 2012; Dahms et al., 2015). Several studies from Europe revealed the association of consumption of cephalosporins in pig farms with the high prevalence of ESBL-producing *E. coli* isolates (Hammerum et al., 2014; Dahms et al., 2015; Schmithausen et al., 2015). The aim of the study was to assess the current situation regarding the faecal carriage with positive ESBL/AmpC *E. coli* strains isolated from the caecal content of slaughtered pigs.

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MATERIALS AND METHODS

Isolation of ESBL/AmpC-producing *Escherichia coli* strains from the samples of swine caecal content was carried out according to the European Reference Laboratory for Antimicrobial Resistance (EURL-AR) (Hasman et al., 2018). After sterile collection of caecal content, a pre-enrichment was carried out first using peptone water. The ESBL/AmpC screening was performed using the selective MacConkey medium (Oxoid, Basingstoke, UK), with the addition of cefotaxime. All colonies with morphology typical for *Escherichia coli* were confirmed based on the biochemical properties using the triple-sugar-iron (TSI) agar test, the motility-indole-urea (MIU) medium or Api 20E for atypical *E. coli* strains. For phenotypical confirmation of ESBL isolates, the microdilution method was carried out in broth using EUVSEC2 plates that contain cefoxitine, cefepime, cefotaxime, ceftazidime and clavulanate combined with cefotaxime and ceftazidime. The results were interpreted based on the synergy tests between clavulanic acid and ceftazidime and/or clavulanic acid and cefotaxime. Phenotypical detection of AmpC beta-lactamase-producing strains was carried out through a stable AmpC cephalosporin: cefepime.

RESULTS AND DISCUSSIONS

A total of 128 samples of caecal content were analysed of which, following ESBL/AmpC screening, 51 (39.84%) presumptive extended-spectrum beta-lactamase enzyme-producing and/or AmpC enzyme-producing *E. coli* strains have been isolated and identified. The synergy test between the potentiated cephalosporin and the simple cephalosporin phenotypically confirmed that 100% of the *E. coli* isolates identified following screening are presumptive ESBL and/or AmpC-producing isolates. Also, following centralization of the results obtained from the 51 *E. coli* isolates of animal origin, attained following ESBL/AmpC screening, most of them showed ESBL phenotype (40/51; 78.43%), 5/51 (9.80%) showed ESBL+AmpC phenotype, and 6/51 (11.77%) showed AmpC phenotype (Table 1).

Table 1. Phenotypes identified in *E. coli* isolates

Source of <i>E. coli</i> strains isolated on MC+CTX [†]	No. of strains isolated on MC+CTX [*]	Type of phenotype		
		No. of <i>E. coli</i> strains phenotype ESBL (2016+2017)	No. of <i>E. coli</i> strains phenotype ESBL+AmpC (2016+2017)	No. of <i>E. coli</i> strains phenotype AmpC (2016+2017)
IS [†] slaughterhouse	6	5	1	0
BT [†] slaughterhouse	32	26	3	3
SV [†] slaughterhouse	13	9	1	3
Total isolates of animal origin	51	40	5	6

MC+CTX[†] - MacConkey medium with the addition of cefotaxime; IS[†] - Iași; BT[†] - Botoșani; SV[†] - Suceava

For swine, the faecal carriage with ESBL enzyme-producing *E. coli* strains has big geographic differences, with proportions that vary between 15.2% in Switzerland (faecal samples) and 23.4% in the United Kingdom (caecal samples) or 49% in Portugal (faecal samples) (Geser et al., 2011; Ramos et al., 2013; Randall et al., 2014). Furthermore, in Belgium, the study carried out by Van Damme et al. (Van Damme et al., 2017) highlighted the presence of positive ESBL/AmpC *E. coli* strains in 75% of the samples of faecal batches and 47% of the samples of tonsils collected upon slaughtering of pigs. The preliminary results from this study fall into the range listed in other similar studies carried out in Europe. Moreover, the big differences between various areas of Europe can be justified by the policy of each country on the administration of antibiotics in production animals. In some countries, such as the Netherlands, following a high prevalence obtained, starting January 2013, veterinarians have been legally forced to limit the use of third/fourth-generation cephalosporins and fluoroquinolones only in infections confirmed by bacteriological examinations and susceptibility tests (SDa, 2013).

Considering that pigs and, subsequently, pork add to the zoonotic spread of ESBL/AmpC *E. coli* strains (Carmo et al., 2014; Evers et al., 2017; Lazarus et al., 2015) and that ESBL/AmpC strains can persist in the human gastrointestinal track for up to 5 years (Brolund, 2014), intervention strategies are necessary to reduce the presence of these isolates on pig carcasses and to reduce the exposure of humans to ESBL/AmpC-producing enterobacteria.

CONCLUSIONS

In conclusion, the high percentage (39.84%) of presumptive extended-spectrum beta-lactamase enzyme-producing and/or AmpC enzyme-producing *E. coli* strains isolated from the caecum of slaughtered pigs can represent an important source of contamination of carcasses and pork, respectively, and there is a risk of their transmission from production animals to humans through various routes.

Author Contributions: M.T. - collected the samples and performed the phenotypic analysis of clinical isolates; A.P.C. - analyzed the data and wrote the manuscript; D.A. E.I., L.O., A.A - analyzed the data; G.S - planned and coordinated the study, analyzed the data and wrote the manuscript.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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