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DETECTION OF RESISTANT *ESCHERICHIA COLI* DURING MEAT PROCESSING IN FOOD ESTABLISHMENTS

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ABSTRACT

Meat is an important food source, the nutritional composition of which, in conjunction with its desirable taste, positively affects human development and health. From a veterinary point of view, meat obtained from food-producing animals is also a potential reservoir for foodborne pathogens, including *Escherichia coli*. The prevalence and spread of antibiotic-resistant *E. coli* represent a threat to public health. For these reasons, the aim of this work was to identify the isolates obtained from food processing plants and to confirm the presence of *E. coli* species based on phenotypic and genotypic expression. The susceptibility of the investigated strains to antibiotics and their combinations was determined using the modified microdilution method. Dominant resistance was detected against ampilicin, tetracycline, cotrimoxazole, ampilicin + sulbactam, ciprofloxacin, and cefuroxime. Resistance to ertapenem, cefotaxime, tigecycline, and ceftazidime was also detected sporadically. Frequent mechanism responsible for resistance was generated an incomplete fluoroquinolone resistance induced by mutation and penicillinase with low enzyme expression. The occurrence of multidrug-resistant strains was also detected. Selected *E. coli* isolates were subjected to detection of genes encoding resistance to tetracyclines (*tet*A and *tet*B) and quinolones (*qnr*A, *qnr*B, and *qnr*S) by PCR. The *tet*A gene was confirmed in 68 % of the isolates, and the *tet*B gene in one isolate. The prevalence of the *qnr*A gene was found to be 29 % in the examined isolates. Two isolates showed the presence of the *qnr*S gene. The *qnr*B gene, as well as a combination of the *qnr*A and *qnr*B genes, was detected in one isolate.

Key words: antibiotics; *E. coli*; food establishment; meat; resistance

INTRODUCTION

Currently, in addition to microbial food safety, transmission, and emergence of foodborne diseases, antibiotic resistance of foodborne pathogens, including *E. coli*, has become another global public health concern [13].

E. coli bacteria inhabit the gastrointestinal tracts of humans and animals. They also enter and persist in the environment after excretion from the body. Over the past decades, the overuse and misuse of antibiotics has greatly increased the trend of antibiotic resistance, with *E. coli*

representing a significant source of genes encoding resistance to the action of antibiotics [16]. The close association and interactions between humans, animals, and the environment allow the transport of this pathogen between them [10].

It has been shown that the way animals are handled before, during, and after slaughter makes the resulting meat a potential reservoir of foodborne pathogenic bacteria [1]. Transfer of pathogenic bacterial species can occur at any level of the food chain [18]. During carcass processing, meat can become contaminated in several steps, such as skinning or deboning [21]. During boning, the internal organs are separated from the carcass. It needs to be done correctly; otherwise, the stomach or intestines may be damaged, and their contents spilled, resulting in contamination of the meat surface. Ingesta and excreta contain a variety of bacterial species, of which pathogenic species such as *E. coli*, *Salmonella* spp., *Campylobacter jejuni*, and others may be present [9]. Contamination of meat with *E. coli* also occurs during handling by workers during packaging, improper storage, transportation, and failure to adhere to hygienic principles during sale [16].

In particular, consumers represent an important but also often the most endangered component of the food chain [3]. Pathogens can spread to consumers in two ways. The direct way is through contact with infected people, animals, or their biological components (such as faeces, urine, blood, or others). Consumption of contaminated food or water constitutes an indirect route of entry for microbes [22]. Residues of antibiotics or their toxic metabolites are often present in various types of meat, milk, eggs, and their products, as well as in foods of plant origin. The consumption of such foods poses a huge health risk for consumers in several ways. In the host's organism, the healthy intestinal microflora is disturbed, antibiotic resistance arises and spreads, as well as other harmful effects, which include allergies and other immunological hardships, hepatotoxicity, mutagenicity, teratogenicity, carcinogenicity, and reproductive disorders [15].

Our study was based on a microbiological investigation, namely the detection of antibiotic-resistant *Escherichia coli* bacteria from litter samples and thigh muscles of slaughtered broiler chickens and pigs, as well as from swab samples of butcher's utensils.

MATERIALS AND METHODS

Sampling and isolation of *E. coli* bacteria

E. coli isolates were recovered from 28 samples of poultry litter and from 13 swab samples obtained from tools used in meat processing at two abattoirs where pigs were slaughtered. At the same time, 13 samples of thigh muscle meat from slaughtered chickens and 10 samples of thigh muscle meat from pigs were obtained. Litter samples were collected in sterile plastic samplers. The stock and 10-fold dilutions were prepared according to EN ISO 6887-1 [24], which were spread on the surface of Endo agar (HiMedia Laboratories, India), and the plates were incubated in a thermostat at 37 °C and 43 °C for 24 hours, respectively. Smears from the investigated tools and muscle samples were examined according to EN ISO 6887-2 [25] and EN ISO 16649-2 [26] using agar supplemented with 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (TBX agar; Oxoid, UK).

Species identification of *E. coli* isolates

Typical colonies were transferred from the surface of arbitration medium to the surface of Nutrient agar (HiMedia Laboratories, India) and HiChrome agar (HiMedia Laboratories, India) and incubated at 37 °C for 24 hours. After incubation, the typical colonies were inoculated on the surface of nutrient agar, and after subsequent incubation at 37 °C for 24 hours, the obtained bacterial culture was used for species identification based on phenotypic expression using commercial biochemical test kits: INDOLtest, OXItest, OFtest, VPtest, and ENTEROtest 24 N. For confirmation, phenotypically identified *E. coli* isolates were further subjected to more precise identification by the PCR method, according to W a n g et al. [27]. Subsequently, the obtained PCR products were subjected to sequencing by a commercial company (SEQme s.r.o., Czech Republic). The obtained isolates were compared with the sequences available in the nucleotide database GenBank - EMBL (NCBI), which confirmed the species identification of *E. coli* strains available at http://www.ncbi.nlm.nih.gov/BLAST (accessed August 19, 2023).

Antibiotic susceptibility testing

The susceptibility of *E. coli* to the selected 20 antibiotics was determined by minimum inhibitory concentration (MIC) testing using a modified microdilution method ac-

cording to CLSI VET01-S2 [2] and EUCAST [4] using a commercial diagnostic kit (Bel-Miditech, SR). The reading of the result was performed using a scanner and interpretation software (Bel-Miditech, SR).

Identification of genes encoding antibiotic resistance by PCR

Based on the MIC results, the presence of genes encoding tetracycline resistance (*tet*A and *tet*B) in tetracycline-resistant *E. coli* isolates was investigated by multiplex PCR reactions according to Guillaume et al. [7], and in ciprofloxacin-resistant *E. coli*, the presence of genes encoding quinolone resistance (*qnr*A, *qnr*B and *qnr*S) was detected according to Robicsek et al. [20], under optimized conditions. For both groups of ATBs, an increasing trend of resistance in *E. coli* isolates derived from both clinical and non-clinical samples has recently been confirmed.

RESULTS

Based on the results of biochemical tests, it can be concluded that *E. coli* has the ability to cleave the amino acid tryptophan to form indole (INDOLtest), does not have the enzyme cytochrome oxidase present (OXItest), has an enzymatic type of metabolism (OFtest), and does not produce acetoin (VPtest). We identified *E. coli* at the species level at 97.98 % by means of the ENTEROtest 24N. To confirm the species identification, we proceeded to the identification of the isolates by PCR, which identified all isolates examined as *E. coli* species.

As also shown in Fig. 1, in general, *E. coli* isolates showed dominant resistance to ampicillin (AMP; 61 %), followed by tetracycline (TET; 34 %), cotrimoxazole (COT; 31 %), ampicillin + sulbactam (SAM; 19 %), ciprofloxacin (CIP; 16 %), and cefuroxime (CXM; 11 %). Resistance to ertapenem (ETP), cefotaxime (CTX), tigecycline (TGC), and ceftazidime (CAZ) was detected in less than 10 % of the bacterial isolates. With ciprofloxacin, the increased intermediate susceptibility of *E. coli* isolates was also confirmed (26 %). Overall, however, the strains appeared to be the most sensitive (100 % sensitivity) to piperacillin + tazobactam, cefoperazone + sulbactam, meropenem, gentamicin, tobramycin, amikacin, and colistin, and therefore these ATBs are not included in the Figure.

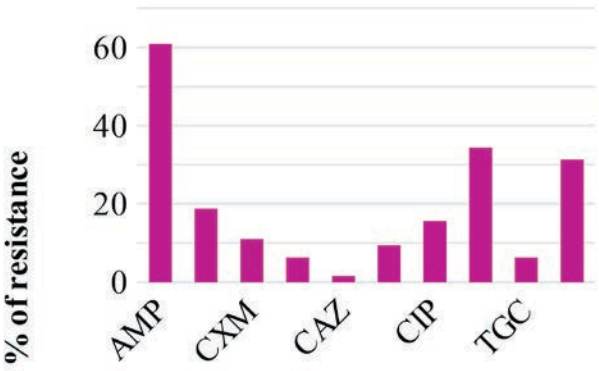

Fig. 1. Rates of resistance to selected antibiotics in the isolates investigated

In general, mutation-induced incomplete fluoroquinolone resistance was the most reported occurrence, followed by penicillinase with low enzyme expression, penicillinase with high enzyme expression, and suspected carbapenemase/metallo-β-lactamase (= β-lactam resistance) and multidrug resistance. Phenotypic identification also revealed multidrug resistance in the *E. coli* isolates studied, most frequently to two and three ATBs simultaneously. Resistance to seven ATBs (AMP-SAM-CXM-ETP-TGC-TET-COT or AMPSAM-CXM-CTX-ETP-CIP-TET) was detected in four strains, and resistance to up to eight ATBs simultaneously (AMP-SAM-CXM-CTX-ETP-TGC-TET-COT) was confirmed in two strains investigated.

Based on the PCR results, the presence of the *tet*A gene was confirmed in a total of 68 % of the 22 isolates examined (11 isolates from litter, 4 isolates of poultry meat and tool swabs, and 3 isolates of pork). The *tet*A gene was present in all *E. coli* from broiler chicken litter, as well as in three isolates from poultry meat and one isolate from a butcher's tool swab. The *tet*B gene was identified in only one strain of *E. coli* isolated from the thigh muscles of carcass chickens. The presence of both genes was not confirmed simultaneously. Neither gene was confirmed in the six isolates examined.

Genetic determinants encoding quinolone resistance were detected in almost half of the 24 isolates tested (7 isolates from litter, 11 isolates from poultry, 5 isolates from tool swabs, and 1 isolate from pork). In general, the *qnr*A gene was demonstrated in 29 %, i.e., one strain from broiler chicken litter, four from poultry carcass meat, and two from used butcher's tool swabs. The *qnr*S gene was detected in two *E. coli* strains isolated from broiler chicken litter. The presence of the *qnr*B determinant was

also confirmed in one isolate. In an *E. coli* isolate derived from poultry carcass meat, the *qnr*A and *qnr*B genes were present simultaneously. In the remaining 13 *E. coli* isolates examined, no *qnr* gene prevalence was observed.

The resulting products of both PCR reactions are shown in Figures 1 and 2.

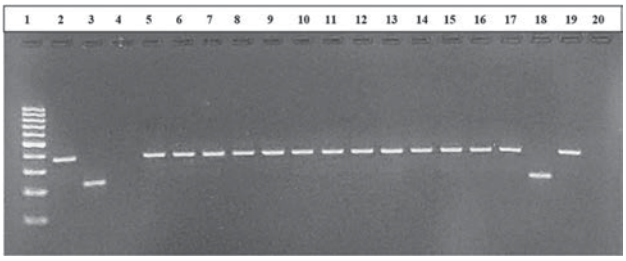

Fig. 1. Detection of tetA and tetB genes by PCR
1 – GeneRuler 100 bp ladder standard; 2 – positive control tetA (372 bp); 3 – positive control tetB (228 bp); 4 – negative control; 5–17 and 19 – positive isolates for tetA; 18 – isolate positive for tetB; 20 – isolate negative for tet genes

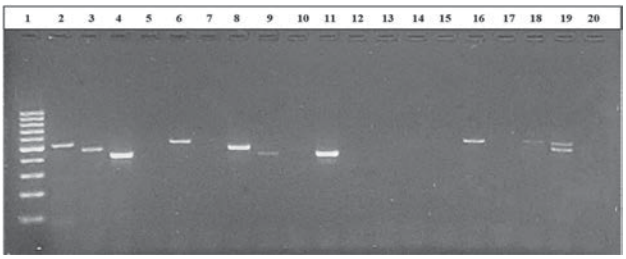

Fig. 2. Detection of qnrA, qnrB and qnrS genes by PCR
1 – GeneRuler 100 bp ladder standard; 2 – qnrA positive control (516 bp); 3 – positive control qnrB (469 bp); 4 – positive control qnrS (417 bp); 5 – negative control; 6, 16 and 18 – isolates positive for qnrA; 8 – isolate positive for qnrB; 9 and 11 – isolates positive for qnrS; 19 – isolate positive for qnrA and qnrB simultaneously; 7, 10, 12–15, 17 and 20 – isolates negative for qnr genes

DISCUSSION

From a veterinary perspective, food-producing animals are still considered potential reservoirs of pathogenic bacterial species such as *E. coli*, *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, *Enterococcus* spp., etc., and vectors of resistance determinants, which can enter the human population through direct contact with infected animals, contact with agricultural waste, or through the food chain [17]. For this reason, the use of ATBs in livestock accounts for around 50–80 % of the global production of both human and veterinary ATBs [23]. Subsequently, this leads to the agricultural and food sectors being a significant contributor to the emergence and spread of ATB resistance [12].

The bacterium *E. coli* belongs to the group of so-called coliform bacteria, whose presence in drinking water, food, or the environment indicates faecal contamination, non-compliance with hygiene conditions, and the presence of other faecal microorganisms, including pathogens [8]. In meat processing plants, contamination with *E. coli* strains can occur during slaughter when digestive tract breaches spill contents onto the carcass. Similarly, in meat processing and meat product manufacturing, when recommended conditions are not followed, e.g., contaminated employee hands, knives and other tools, tables, etc., which do not represent primary sources of *E. coli* and thus must be cross-contaminated [1].

Antibiotic-resistant strains of *E. coli* are now widely prevalent in the European Union. High levels of resistance to ampicillin, ciprofloxacin, cotrimoxazole, and tetracycline have been identified in the EU, as reported in the EFSA and ECDC [6] synthesis reports. Low levels of resistance have been identified against cefotaxime, ceftazidime, colistin, tigecycline, and azithromycin. No *E. coli* isolate showed resistance to meropenem. Our results correlate with these data. *E. coli* isolates from litter samples, butcher's tool swabs, and thigh muscle from slaughtered chickens and pigs showed predominant resistance to ampicillin. Higher levels of resistance in all groups investigated were observed to cotrimoxazole and ciprofloxacin, but also to ampicillin, sulbactam, and cefuroxime. Resistance of *E. coli* to cefotaxime, ceftazidime, tigecycline as well as ertapenem was also found which was generally below 10 %. In our study, none of the isolates studied showed resistance to meropenem, piperacillin + tazobactam, cefoperazone + sulbactam, gentamicin, tobramycin, amikacin and colistin. Additionally, the results of several other experiments conducted reported the most frequent occurrence of resistance of *E. coli* isolated from food animals to ampicillin, tetracycline, ciprofloxacin, nalidixic acid, and cotrimoxazole [19].

Also, the results for the detection of multidrug-resistant isolates are similar to the ECDC and WHO reports [5]. *E. coli* strains resistant to two and three ATBs simultaneously were the most prevalent. The isolates examined, obtained from pork thigh muscle and from tool swabs, showed multidrug resistance to seven to eight ATBs simultaneously. A high prevalence of multi-drug resistant *E. coli* strains isolated from food-producing animals was also found by Martínez-Vázquez et al. [14] in Mexico.

Tetracyclines are among the most commonly used veterinary ATBs on livestock farms. Both *tet*A and *tet*B genes, encoding tetracycline resistance, are most commonly identified in *Enterobacteriaceae* [11]. Of the 22 *E. coli* isolates examined, the presence of the *tet*A gene was confirmed in 68 % of the isolates. The *tet*B gene was identified in only one *E. coli* isolate. The simultaneous presence of both genes was not detected in either strain. At the same time, no gene encoding tetracycline resistance was detected in the six isolates studied. Corresponding results were found in a similar study by K u r n i a et al. [11].

Quinolone resistance is also of interest, with the involvement of the *qnr*A and *qnr*S genes being particularly important [19]. Also, in our work, an increased frequency of these two genes was observed in the studied isolates. Of the 24 isolates, the prevalence of the *qnr*A and *qnr*S genes was 29 % and two isolates, respectively. The presence of the *qnr*B gene was confirmed in one isolate, as was the presence of a combination of *qnr*A and *qnr*B genes at the same time. The presence of *qnr* genes was not detected in 13 *E. coli* isolates examined. Similar results were also found by K u r n i a et al. [11] in their experiment.

The increased prevalence of the *tet*A, *qnrS,* and *qnr*A genes, together with confirmed resistance to tetracycline and quinolones in the studied *E. coli* isolates obtained from food companies during meat processing, may lead to the transfer of these genes into the food chain and to a reduction in the efficacy of the respective ATBs administered for the treatment of various infectious diseases in the population.

CONCLUSIONS

The aim of this work was to detect the occurrence of antibiotic-resistant *Escherichia coli* strains from samples collected during meat processing in food processing plants. Subsequently, the aim was to determine the susceptibility of the obtained isolates to the selected antibiotics using the microdilution method and to identify genetic determinants of resistance to tetracyclines and quinolones using the PCR method. Based on the results, it can be concluded that poultry and pigs represent a suitable reservoir of antibiotic-resistant *E. coli*. The increased prevalence of *tet*A, *qnr*S, and *qnr*A genes, together with confirmed tetracycline and quinolone resistance in the isolates studied, may lead to the transfer of this pathogen or genes into the food chain, threatening the health of the consumer and reducing the efficacy of the respective ATBs. Therefore, it is also necessary in the future to reduce the consumption of ATBs in food-producing animal farms to protect the effects of ATBs for future generations, to improve the safety measures and monitoring of the occurrence of resistance on a global scale, and to comply with the hygiene and recommended measures associated with the killing of animals, from handling to the consumption of prepared foodstuffs.

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