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Extraintestinal pathogenic *E. coli* infections: The spread of antibiotic resistance through the food products

Zakażenia wywołane przez pozajelitowe patogenne szczepy *E. coli* – rozprzestrzenianie się oporności na antybiotyki poprzez produkty spożywcze

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
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G Funds Collection

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Summary

With the increasing demand for poultry meat and poultry products and the growing poultry industry around the world, food safety is an important challenge for public health. To assess the dissemination of extraintestinal pathogenic *E. coli* (ExPEC) strains, one should examine the level of genetic similarity between isolates from different hosts. In the proposed review paper, multiple levels of genotyping are proposed, in which typing of strains, plasmids, and genes are compared in order to obtain the more complete picture of this complex issue. The ExPEC group includes uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), and sepsis-associated *E. coli* (SEPEC). ExPEC presents an elaborated phylogenetic structure, a wide range of virulence factors (VF), and considerable plasticity of the genome. These strains cause not only uncomplicated UTIs, but also other dangerous illnesses such as bacteremia or sepsis. Mechanisms underlying ExPEC transmission dynamics and the selection of resistant to drugs clones are still poorly understood and require further investigations. Overuse and inappropriate use of antibiotics and chemotherapeutics has led to a global threat, which is the emergence and spread of microbial resistance. Food, depending on certain products and processing technology, provides an excellent substrate for the growth of microorganisms. Intensive trade and wide use of antibiotics in contemporary food production favor the emergence and spread of resistant bacteria. Currently, antibiotic use in vegetable and animal food production is significantly higher compared to the number of antibiotics used in medicine to treat infections, which is a huge threat. We need new strategies to prevent, quickly diagnose, and treat ExPEC infections, especially in the context of the recently observed clonal expansion of strains with increased antibiotic resistance.

Keywords: **multi-drug resistance strains • vegetable • meat • β-lactamase genes • ESBL**

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Abbreviations: **APEC** – Avian Pathogenic *Escherichia coli*, **ESBL** – extended spectrum β-lactamase; **ExPEC** – Extra--intestinal pathogenic *Escherichia coli*; **MDR** – Multi drug resistance; **EPEC** – Enteropathogenic *Escherichia coli*; **ST** – sequences type; **UTI** – Urinary Tract Infection.

BACKGROUND

Excessive and inappropriate use of antibiotics and chemotherapeutics has led to the emergence and spread of microbial resistance, which is a global threat. The first step to reduce resistance is to identify the epidemiological situation by monitoring antimicrobial resistance and the sources of resistant strains. Food, depending on certain products and processing technology, provides an excellent substrate for the growth of microorganisms. Intensive trade and wide use of antibiotics in contemporary food production favor the emergence and spread of resistant bacteria [14, 50].

For many years, antibiotic resistance was linked only to the hospital environment. However, the increasing knowledge about bacterial resistance and transmission has captured the interest of researchers. The selection of resistant strains may results from their improper use. In agriculture, the source of resistant strains may come from meat of farm animals, fruits, vegetables or water [18]. The presence of antibiotic-resistant bacteria in food suggests that they may play a role in the transfer of genes encoding resistance to antibiotics [44, 48]. The main place of resistance transmission from the environment to humans is the food chain [12, 54].

Many studies on the pathogenesis of extraintestinal diseases show that when human ExPEC (Extraintestinal pathogenic *E.coli*) is introduced into models of avian diseases, it behaves like APEC (Avian pathogenic *Escherichia coli*), whereas APEC, after it is introduced into the mammalian model of extraintestinal infections in humans, behaves like human ExPEC [1, 23, 25]. The most common diseases caused by APEC both in living poultry and wild birds are respiratory tract infections that may lead to systemic infections and even to sepsis. It has been observed that serotypes identified in APEC also cause diseases in humans, including UTI (urinary tract infection), and neonatal meningitis (NMEC – Neonatal meningitis *E. coli* or sepsis (SEPEC – Human sepsis-associated *E. coli*) [13, 52]. *E. coli* sequence types (ST), i.e., ST10, ST23, ST69, ST95, ST117 and ST131, which are associated with human ExPEC infections, are found in poultry or in retail poultry meat. It was reported that human ExPEC reservoirs may also exist in water, sewage, and in other environmental sources, although there are hypotheses that one of the most important reservoirs for ExPEC is food, with poultry being particularly hazardous [2, 23, 25].

The aim of this study was to characterize *E. coli* strains isolated from humans, animals and food for the presence of bacterial genes encoding antibiotic resistance determinants in the context of an increasing spread of ExPEC infections.

Antibiotic resistance is the ability of bacteria to "resist" its bacteriostatic or bactericidal activity. Bacteria display innate and acquired resistance. Natural resistance most often results from ineffective antibiotic penetration through the cell wall and membrane structures due to the presence of elements inhibiting the possibilities of its reaching the destination. Such resistance is usually chromosomally encoded and thus non-transmissible [22, 33]. While innate resistance is a characteristic feature of a given species, associated with the information encoded in the bacterial chromosome, acquired resistance appears as a result of changes in the genome [51]. Such changes may occur as a result of point (random) mutations, which are most often errors in the nucleotide sequence of genetic material, or may result from acquiring genes or sets of genes that determine antibiotic resistance from other resistant bacteria. This second phenomenon occurs much more frequently, but they both consequently lead to permanent resistance inheritance and to its spread by horizontal transfer, which leads to the appearance of new resistance genes in cells, carried on mobile genetic elements. This phenomenon often leads to the formation of the so-called multi-drug resistance strains (MDR), i.e., showing insensitivity to at least one antibiotic from three or more classes active toward a given microorganism species. The appearance of MDR is favored by the simultaneous encoding of several different resistance genes on the same genetic element [56].

MECHANISMS OF ANIBIOTICS RESISTANCE IN EXPEC STRAINS

Bacteria in the Enterobacteriaceae family, including important food-borne pathogens such as *E.coli* causing a variety of infections (from wound infections to meningitis), play a significant role in hospital-acquired infections [26, 28]. The production of extended-spectrum β-lactamases (ESBL) is an important resistance mechanism [36]. These enzymes hydrolyze penicillins, II, III and IV generation cephalosporins (with the exception of cephamycins), and monobactams (aztreonam). Plasmid-determined β-lactamases with a broad substrate profile are divided into classic TEM and SHV and so-called extended spectrum β-lactamases, which are either TEM- or SHV-derived mutants or natural enzymes. Point mutations in the genes involved in the synthesis of classical

enzymes led to the formation of the ESBL phenotype [39]. Despite in vitro susceptibility to ESBL substrates, especially to some cephalosporins, it was recommended to treat ESBL-producers as resistant to all penicillins (without inhibitors), cephalosporins (with the exception of cefamycin), and aztreonam [47]. Until recently, ESBL+ strains have been treated as typical hospital-acquired pathogens, but recently they have also been routinely identified as etiologic agents of out-of-hospital infections (especially UTI) [31, 35, 38]. They are also found in healthy people, pets, farm animals, and even in animal food products [11]. Cephalosporins and penicillins belong to β-lactam antibiotics, used most frequently in medicine and veterinary. The mechanism of antibiotic resistance in gram-negative bacilli involves enzymatic hydrolysis of the ring in the structure of β-lactam antibiotics, which causes its inactivation. Genes coding for β-lactamases (bla) are often located on plasmids. TEM (bla_{TEM}) and SHV (bla_{SHV}) β-lactamase, which emerged evolutionary earlier, were able to inactivate penicillins and first-generation cephalosporins (penicillinase). Currently, *E. coli* strains are dominant ESBL-producing Enterobacteriaceae [40]. In turn, CTX-M enzymes, which can be divided into five clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25), belong to most frequently detected ESBL phenotypes, with the most widespread CTX-M 1 and CTX-M-9, although their incidence varies greatly among different geographical areas [7]. In Europe, in the case of *E. coli* strains, different types of CTX-M have been isolated from farm animals, pets and healthy people, and CTX-M-1 is the most frequently detected phenotype [10, 41, 59]. The CMY-2 is the most common ampC β-lactamase gene among Enterobacteriaceae. bla_{CMY-2} gene was detected in *E. coli* isolated from domestic and farm animals in Tunisia [6]. IncA/C, IncFIA-IncFIB, IncL/M and IncI1 plasmids are carriers of bla_{CMY-2}. Plasmids harboring bla_{CMY-2} or bla_{CTX-M} genes often carry multi-resistance determinants, such as genes resistant to amino-glycoside, sulphonamides, tetracyclines and fluoroquinolones [5, 9, 20]. AmpC β-lactamases produced by Gram-negative bacteria can hydrolyse several β-lactam antibiotics, including cephamycins and cephalosporins [21]. Recently, bla_{CMY-2} has been found in cephalosporin-resistant *E. coli* from retail chicken meat in Canada and Taiwan [58]. Many studies have shown that antibiotic resistance genes in plasmids, transposon area or in the chromosomal DNA of bacteria, are integrated into integrons, which play a significant role in propagating antibiotic resistance genes in Gram-negative bacteria. Integrons are commonly found among hospital Gram-negative bacilli, but recently such genetic structures have been reported to occur in strains from meat intended for sale [15, 45]. Class 1 and 2 integrons were first detected in *E. coli* strains from meat, meat products, and poultry carcasses in Spain, the United States and Norway [24, 37, 49]. Multi-drug resistance genes, in the case of bacteria isolated from animals and animal origin products, are considered a significant risk related to the possibility of rapid spread of multi-drug resistant strains in the human environment [16, 55].

OCCURRENCE OF RESISTANCE GENES IN EXPEC STRAINS ISOLATED FROM FOOD

Recently, numerous studies have shown similarities between human and poultry ExPEC strains, mainly in their virulence genes. This fact suggests that poultry products could be a source of ExPEC. Poultry meat exhibits the highest overall level of *Escherichia coli* contamination and *E. coli* strains isolated from poultry tend to be resistant to more drugs (MDR) than *E. coli* strains derived from meat products of other origin (e.g. pork) [26].

Bacteria in the poultry environment are a very important reservoir of antibiotic resistance genes. The presence of antibiotic resistant bacteria in poultry can lead to the contamination of poultry products, which increases the risk of these bacteria or their genes being transmitted to humans. Studies have shown that women infected with multi-resistant *E. coli* strains have reported more frequent chicken consumption [27].

Ahmed et al. [1] conducted a study of 69 *E. coli* strains from raw retail poultry meat products from various supermarkets in Hiroshima. Multi-drug resistance was reported in 28 *E. coli* strains (40.6%). The most commonly detected resistance phenotypes were against ampicillin, streptomycin, spectinomycin, kanamycin, tetracycline, trimetho-prim/sulfamethoxazole, nalidixic acid, cefoperazone, cephalotine, cefoxitin, and ciprofloxacin. Using the PCR method, class 1 and 2 integrons were detected in eight strains (11.6%) and in one (1.4%) strain, respectively. Detailed DNA sequence analysis revealed the presence of four class 1 integrons, which carry: dihydrofolate reductase genes (dfrA1, dfrA7), which confer resistance to trimethoprim, and aminoglycoside adenyltransferase gene A (aadA1), known to confer resistance to streptomycin and spectinomycin. Analysis of the class 2 integron DNA sequence showed 3 gene cassettes: dihydrofolate reductase (dfrA1), streptothricin acetyltransferase (sat2), aminoglycoside adenyltransferase (aadA1) that confer resistance to trimethoprim, streptomycin and streptomycin/spectinomycin, respectively. Genes coding for β-lactamase were detected in the examined *E. coli* strains. The bla_{TEM-1}gene was detected in 12 (17.3%) strains, and the bla_{CMY-2} gene, encoding AmpC beta-lactamase, was detected in 16 (23.2%) strains. It is important for human health to isolate cephalosporin-resistant *E. coli* carrying the bla_{CMY-2} gene from chicken meat, because the spread of the bla_{CMY-2} gene from food and animals to humans has been documented before.

Seiffert et al. [43] examined the $bla_{CMY-2/4}$ carrying INcB/O/K-like plasmids of seven *E. coli* strains derived from poultry, poultry meat and human urine samples. One *E.coli* from poultry meat and one from human urine contained the same plasmid. The presence of the same IncK2 plasmid in *E. coli* strains from poultry meat and human urine indicated that the IncK2 plasmids originated from the same source and had the ability to spread to genetically diverse *E. coli* in different reservoirs. This is an

alarming discovery and it stresses the need for rapid introduction of strict hygiene measures throughout the food chain, which can limit the spread of such plasmids in the human settings.

Other reports available in the literature indicated that INcI1 family of plasmids is associated with the spread of ESBL genes. Martinez-Martinez L. et al. [29], in a study conducted in France, detected $bla_{CTX-M-1}$ IncI1 plasmid in ExPEC strains isolated from sick people and healthy poultry, which suggests the potential of strains and/or plasmids to be transferred between humans and animals.

Hoek et al. [53] compared *E. coli* strains isolated from broilers (n = 149), people working in contact with these broilers (n = 44), healthy people (n = 63), and patients with UTI (n = 10). The most frequently detected genes encoding for ESBL and AmpC in strains from broilers and people working with broilers were the following: $bla_{CTX-M-1}$, bla_{CMY-2}, bla_{SHV-12}. In healthy people, the most frequently detected genes were the following: $bla_{CTX-M-1}$, $bla_{CTX-M-14,}$ $bla_{CTX-M-15}$, and in patients with UTI $bla_{CTX-M-15}$. These results support the claim that antibiotic resistance genes can be spread from broilers, which are a reservoir of resistant *E. coli*.

Antibiotic resistance genes can spread through different food products, including dairy. In Iran, pathogenic *E. coli* strains resistant to ciprofloxacin, trimethoprim, oxytetracycline, gentamycin, nalidixic acid, ampicillin, and streptomycin were detected in raw milk and unpasteurized cheeses [8]. In the Czech Republic, *E. coli* strains were detected in 243 out of 263 (92.4%) samples of raw milk. Genetic analysis identified that the majority of strains had bla_{CTX-M} and another resistance genes (tet(B) and qnrB) determining resistance to β-lactam antibiotics, tetracycline and quinolones [46].

In turn, Singh et al. [42] examined 19 samples of fresh seafood (14 fish, 3 shrimps, 1 mussel, 1 squid) from several supermarkets in India. Of the 19 examined samples, the authors isolated 215 strains of different bacteria, including 66 *E. coli* strains, 53 of which were ESBL+. Genes encoding the ESBL phenotypes were detected using the PCR method. In the case of *E. coli*, the bla_{CTX} gene was detected in 41 strains, bla_{SHV} in 29 strains, bla_{TEM} in 24 strains, and bla_{NDM} in two strains. The study for the first time showed the simultaneous occurrence of more than one ESBL genes in the case of *E. coli* isolated from seafood samples. The New Delhi metallo-β-lactamase encoding gene (bla_{NDM}) had not been previously found in seafood samples anywhere in the world. The fact that ESBL+ isolates were detected in the majority of genes suggests health risks, but also implicates seafood as a potential reservoir of such bacteria in food. It is important to identify the critical points of contamination of seafood with antibiotic-resistant bacteria. To guarantee the quality and safety of seafood, strategies like scientific management of domestic sewage, regulation of human settlement, reduction of pollution along the coast, and the development of hygienic conditions in market facilities for seafood are need to be implemented.

Araújo et al. [3] characterized *E. coli* strains present in irrigation water and vegetables (cucumber, lettuce, tomato, spinach, kale) from 16 household farms in northern Portugal. Authors isolated 210 *E. coli* strains from irrigation water and 239 from vegetables. A total of 449 *E. coli* strains were isolated from eight samples of well water (n = 210) and from seven samples of vegetables (n = 239). After antibiotic susceptibility testing, 126 *E. coli* strains exhibited resistance to drugs from more than one class. Streptomycin-resistance was reported in 89% of cases (86.7% – water, and 93% – vegetables), tetracycline-resistance was much less frequent – in 25% of strains (20.5% – water and 34% – vegetables), and resistance to amoxicillin/clavulanic acid was reported in water and vegetable strains (25.3% and 16.1%, respectively). The most frequently detected gene was tet(B) and it occurred with similar frequency in strains from water and vegetables, respectively 15% and 13%. Another genetic determinant of resistance in strains isolated from water and vegetables were the following: bla_{TEM}, tet(A), strA/strB and sul2. In the examined strains, the authors reported a lack of quinolone resistance determinants, except gyrA gene mutations, lack of resistance genes to carbapenems and third-generation cephalosporins, whereas sul1, dfrA1, aadA and aadA2 genes were detected only in isolates from water. Class 1 integron was detected in five strains, including two strains with resistance genes to trimethoprim and β-lactam antibiotics (dfrA1-aadA1 i dfrA16-bla_{P1b}-aadA2-ereA, respectively). Class 2 integron was detected in one *E. coli* isolate from water and it had dfrA1-sat2-aadA1 genes. Genetic analysis of integrons confirmed the presence of gene cassettes encoding resistance to popular antibiotics. These results suggest that irrigation water may be the source of *E. coli* that enter the food chain through vegetables ingestion.

Ojer-Usoz et al. [34] analyzed 448 *E. coli* ESBL+ strains isolated from environmental (waste water treatment plant, rivers, farm beds), human (e.g. nasal swabs, urethral swabs, bronchial aspirates, sputum, urine, blood) and food samples (e.g. vegetables, fish, cheese, poultry, pork) in Spain. Multidrug-resistant strains were present in 50% of the analyzed material. The most common β-lactamase genes were $bla_{CTXM-14}$ (26%), bla_{CTXM-1}(21.4%), followed by bla_{SHV-12}, $bla_{CTX-M-15}$, and bla_{TFM-42}. The wide dissemination of ESBL-type drug resistance among bacteria from different environments encourages the introduction of measures to prevent and control dissemination between the environment and consumers of genes conditioning such resistance.

Mazurek et al. [30] aimed to determine the prevalence of type 1 integrons in *E. coli* isolated from treated wastewater (n = 89) and coastal waters, which are wastewater collectors (n = 70). From the epidemiological point of view, studies on the occurrence of *E. coli* in sewage water and in aquatic environmental sources provide information about the reservoir of bacterial genes, which may be

useful in assessing potential risks resulting from the presence of bacteria with drug resistance genes. In Poland, data assessing contamination with drug-resistant *E. coli* are still scarce. In the examined strains, class 1 integron was found in seven strains from treated wastewater (8%) and in seven strains from sewage collector water (10%), and six types of various sizes gene cassettes were identified. *E. coli* strains resistant to penicillin, first-generation cephalosporins, or tetracyclines were reported in coastal waters and in sewage collectors. Water contaminated with sewage containing antibiotic-resistant bacteria not only poses a threat to human health, but it also, through horizontal gene transfer in complex microbial populations, may contribute to the dissemination of these traits in the environment.

Currently, antibiotic use in vegetable and animal food production is significantly more prevalent compared to the amount of antibiotics used in medicine to treat infections, which is a considerable threat. In 2009, approximately 80% of all antibiotics sold in the United States were used on farms in meat and poultry production [32]. Due to the widespread use of antibiotics, migration of people and animals, and, as a result, the mobility of mobile genetic elements, drug-resistant strains isolated from different food products are reported all over the world. The release of resistant strains to the environment, in which food is an important reservoir of antibiotic-resistance genes, is particularly dangerous. Drug resistance has become a global problem. Resistance determinants spread through the environment and we can only control the emergence, selection and propagation of genes in bacterial strains in contact with humans, animals and plants [19, 57]. More and more scientists are debating whether to treat patients or the environment [4]. New strategies of combating antibiotic resistance have been developed, e.g., eco-evo - integrating ecology and developmental biology, or eco-evo-devo - integrating ecology, evolution and developmental biology. Proponents of these theories argue that antibiotic-resistant bacteria live in the environment that requires healing. Looking for compounds that will inhibit the spread of antibiotic resistance and block processes leading to the emergence of new determinants conditioning insusceptibility to antibiotics is one of the concepts. This hypothesis assumes that we should treat food contaminated with antibiotic-resistant bacteria [18]. The occurrence of resistance genes in ExPEC strains isolated from different sources is presented in Table 1.

CONCLUSIONS

Comparative analysis showed that avian and human *E. coli* isolates contain similar sets of genes encoding virulence factors, and that they belong to the same phylogenetic groups, which is suggested by a zoonotic origin of ExPEC [17]. Many authors confirm the presence of genetically closely related strains isolated from infections of epidemic character, which usually presented unusual virulence profile or antibiotics susceptibility. The researchers are particularly interested in the problem of food contamination by ExPEC/UPEC strains in correlation with their virulence factors. With the increasing demand for poultry meat and poultry products and the growing poultry industry around the world, food safety is an important challenge for public health. In order to assess the dissemination of ExPEC strains, we should examine the level of genetic similarity between isolates from different hosts. Multiple levels of genotyping are proposed, in which typing of strains, plasmids and genes is compared in order to obtain a more complete picture of this complex problem [53].

We also need new strategies to prevent, quickly diagnose, and treat ExPEC infections, especially in the context of the recently observed clonal expansion of strains with increased antibiotic resistance. To establish the transmission dynamics and clone selection, large-scale epidemiological and clinical studies should be carried out and database, which would help develop new preventive or therapeutic strategies. Potential strategies may include implementation of targeted infection control measures or development of targeted national and international antimicrobial rules.

Table 1. The occurence of resistance genes in ExPEC strains isolated from different sources.

Authors	Year of isolate collection	Source of resistance genes	Locale
Ahmed et al. [1]	2009	raw retail poultry meat	Japan
Mazurek et. Al. [30]	2013	treated wastewater, coastal waters	Poland
Skočková et al. [46]	2015	raw cow's milk	Czech Republic
van Hoek et al. [53]	2016	broilers, human on broilers farm, UTI patients	The Netherlands
Seiffert et al. [43]	2017	poultry, poultry meat, human urine	Switzerland
Sanjit Singh et al. [42]	2017	fresh seafood	India
Araújo et al. [3]	2017	irrigation water, vegetables	Portugal
Ojer-Usoz et al. [34]	2017	human samples, food products (vegetables, cheese, fish, fresh meat, cooked meat), environment (WWTP, rivers, farm beds)	Spain

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