Resistance and virulence factors in *Escherichia coli* from meat and milk

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Abstract

This study focused on the comparison and characterisation of *Escherichia coli* (*E. coli*) isolates obtained from the meat of slaughtered animals (n = 34) and from raw cow's milk (n = 33). Resistance to important antimicrobial substances and the presence of virulence factors were determined. Resistance to antimicrobials was tested using a disk diffusion method, while a polymerase chain reaction was used for the detection of selected genes encoding virulence – *eae*A, *hly*, stx_1, stx_2. More than half the isolates of meat origin were resistant to at least one antimicrobial substance (67.7%). Resistance to ampicillin (50%) and tetracycline (41%) was found most frequently. Of 34 isolates from meat, 13 (38.2%) were multi-resistant. No stx_1, *hly* or *eae*A genes were detected in any of the *E. coli* isolates from meat. The stx_2 gene was detected in one isolate. Only 4 (12.1%) isolates of milk origin were resistant to at least one antimicrobial substance – resistance to tetracycline was found in all cases. No virulence genes were detected in milk isolates. The results of our study indicate that resistance to antimicrobial substances is more common in isolates from meat than in isolates from milk. The only virulence gene stx_2 detected in our study was found in an *E. coli* isolate of meat origin.

Escherichia coli, resistance, virulence genes, meat, milk

Introduction

Determination of *E. coli* is used in the microbiological analysis of foodstuffs as an indicator of possible faecal contamination. Its presence may signal the occurrence of pathogenic bacteria. *E. coli* is a common part of the intestinal microflora of the majority of mammals, including humans and livestock, though if certain virulence factors are present it may be the cause of serious alimentary diseases (Altalhi and Hassan 2009). Strains producing Shiga toxin, known as STEC, are the most important in this regard. Uncooked food of animal origin, including meat and milk, is a frequent reservoir of *E. coli* bacteria. Contamination may occur directly through animal faeces or indirectly through employees or contaminated working aids (Desmarchelier and Fegan 2003).

The degree of antibiotic resistance in *E. coli* is considered a good indicator of selective pressure caused by the use of antibiotics (Lei et al. 2010). In recent years, the occurrence of bacteria resistant to antimicrobials has been recorded increasingly frequently (Scaria et al. 2010). Foodstuffs contaminated by resistant bacteria may pose a health risk to humans as the genes encoding resistance to antimicrobials are carried by mobile genetic elements that may be transferred to sensitive bacteria, including the pathogenic ones (Van et al. 2008). *E. coli* is a suitable vehicle for the transfer of genes of resistance in view of both its great diversity and the fact that it makes up part of the general microflora of the gastrointestinal tract of humans and animals. The increase in antimicrobial resistance in bacteria limits the possibilities available for the treatment of bacterial infections (Scaria et al. 2010).

The aim of this work was to characterise and compare isolates of *E. coli* obtained from meat and milk, focusing on the assessment of resistance to antimicrobials and the detection of genes encoding selected virulence factors.

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Materials and Methods

Thirty-four isolates of *E. coli* obtained from raw meat (beef, pork, turkey) from the retail market in the Czech Republic and 33 isolates from untreated cow's milk from six various farms in the Czech Republic were tested and compared. All the isolates tested were indole positive and β-D-glucuronidase positive.

A disk diffusion method was used to test resistance to 13 medically important antimicrobials, a list of which is given in Table 1. Determination was performed in accordance with the CLSI methodology (2006a). Antibiotic disks were obtained from the company Oxoid (GB). The isolates were assessed as sensitive (S), intermediate resistant (I) or resistant (R) according to the criteria given by the CLSI (2006b) on the basis of the size of inhibition zones.

A polymerase chain reaction (PCR) was used to detect genes encoding selected virulence factors – *eae*A, *hly*, stx_1 and stx_2. Bacterial DNA was isolated from a 24-hour culture on blood agar (BioRad, France). Isolation was performed by the lysis of a suspension of bacterial cells at 95.5 °C for 10 minutes with the addition of 20% Chelex 100 (BioRad, France) with subsequent centrifuging. The supernatant was used as template DNA. The detection of virulence factors was performed by means of multiplex PCR according to Fagan et al. (1999). A QIAGEN PCR Master mix (Qiagen, Germany) was used in 25 µl of reaction mixture. PCR products were analysed by gel electrophoresis in 1.5% agarose (Serva, Germany) with subsequent visualisation in a transilluminator following staining with ethidium bromide.

The isolate positive to stx_2 gene was serotyped using a U-type microplate agglutination assay (Salajka et al. 1992). Agglutination was performed with a set of 70 types of O-antisera, including the most common O-serogroups.

Subtyping of stx_2 gene was performed by method according to Alexa et al. (2000).

Results and Discussion

Table 1. The occurrence of resistance in isolates of *Escherichia coli* from meat and milk

Antimicrobial substance		Content of disk [µg]	Meat (n = 34)		Milk (n = 33)	
			n*	%	n*	%
Ampicillin (AMP)	β-lactams	10	17	50	0	0
Amoxicillin/clavulanic acid (AMC)		30	6	17.7	0	0
Cefotaxime (CTX)		30	2	5.9	0	0
Chloramphenicol (CMP)	Chloramphenicol	30	3	8.8	0	0
Streptomycin (STR)	Aminoglycosides	10	10	29.4	2	6.1
Kanamycin (KAN)		30	1	2.9	0	0
Gentamicin (GEN)		10	3	8.8	0	0
Sulfamethoxazole/trimethoprim (SXT)	Antimetabolites	25	6	17.7	0	0
Trimethoprim (TMP)		5	9	26.5	2	6.1
Tetracycline (TET)	Tetracyclines	30	14	41.2	4	12.1
Nalidixic acid (NA)	Quinolones, fluoroquinolones	30	7	20.6	0	0
Ciprofloxacin (CIP)		5	5	14.7	0	0
Colistin (COL)	Polymyxins	10	0	0	0	0

n = number of isolates tested, n* = number of positive isolates, % = percentage of positive isolates

The majority of isolates from meat were resistant to at least one tested antimicrobial substance (67.7%). Resistance was found most frequently to ampicillin (50%), tetracycline (41.2%), streptomycin (29.4%) and trimethoprim (26.5%). In contrast to isolates from meat, resistance to at least one antimicrobial agent was found in isolates from milk in only 4 cases (12.1%). All these isolates were found to be resistant to tetracycline (12.1%), with sporadic cases of resistance to streptomycin (6.1%) and trimethoprim (6.1%). The isolates from milk were sensitive to all the other antimicrobial substances. A comparison of the occurrence of resistance to the individual antimicrobials in isolates of *E. coli* from meat and milk is given in Fig. 1.

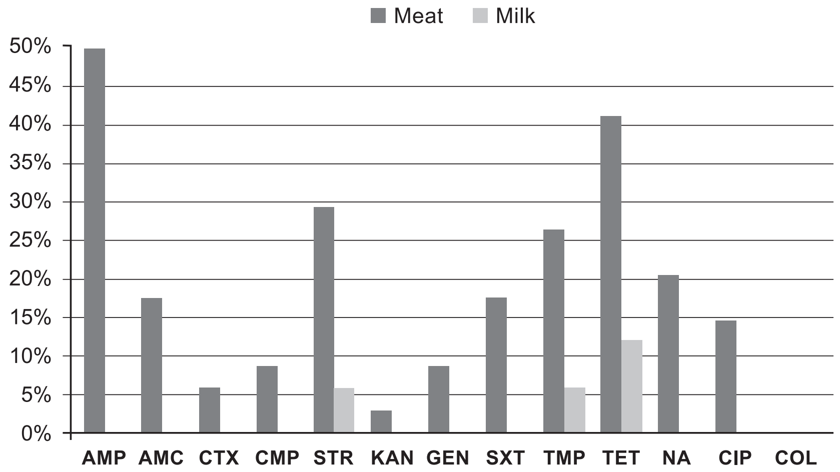

Fig. 1. A comparison of resistant isolates of *Escherichia coli* from meat and milk

E. coli resistant to three or more groups of antimicrobials (multi-resistant *E. coli*) was strongly represented (38.2%) among isolates from meat. The phenotypes of multi-resistance differed from one another; only the phenotype AMP-SXT-TMP-TET was discovered in two isolates. The other phenotypes of resistance found are given in Table 2.

Van et al. (2008) also recorded the extremely frequent occurrence of resistant *E. coli* (84.0%) in raw meat, with the majority of the isolates they obtained being multi-resistant. Similarly to our study, the most frequently determined resistance was to tetracycline, ampicillin, amoxicillin, trimethoprim and streptomycin, followed by resistance to chloramphenicol, nalidixic acid and gentamicin. Lee (2009) also refers to the frequent occurrence of resistance to ampicillin, tetracycline and streptomycin in *E. coli* isolated from cattle. The German scientists Schwaiger et al. (2012) note the frequent occurrence of multi-resistant strains of *E. coli*, particularly in chicken meat. They recorded the dominant occurrence of resistance to ampicillin and amoxicillin/clavulanic acid in *E. coli* isolated from chicken meat, and resistance to streptomycin in particular in pork.

A high percentage of resistance to streptomycin (100%), tetracycline (95%) and ampicillin (47%) was recorded in strains of *E. coli* isolated on dairy farms by Scaria et al. (2010). The Finnish authors Suojala et al. (2011) assessed the resistance of *E. coli* bacteria

Table 2. Phenotypes of multi-resistance in *E. coli* isolates from meat

Phenotype of resistance	No of isolates	Phenotype of resistance	No of isolates
AMP-STR-SXT-TMP-TET	1	AMP-AMC-STR-SXT-TMP-TET	1
AMP-AMC-CMP-KAN-TMP-TET-NAL-CIP	1	AMP-AMC-GEN-TET-NAL-CIP	1
AMP-AMC-TET-NAL	1	AMP-AMC-CTX-STR-GEN-TET	1
AMP-AMC-CMP-STR-GEN-SXT-TMP-TET-NAL-CIP	1	CTX-STR-TET-NAL	1
AMP-STR-TMP-NAL-CIP	1	AMP-STR-SXT-TMP-TET	1
AMP-STR-NAL-CIP	1	AMP-SXT-TMP-TET	2

isolated from cattle suffering from mastitis, in which they refer to the dominant occurrence of resistance to ampicillin, streptomycin, tetracycline and sulfamethoxazole. The fact that we recorded a low proportion of resistance to antimicrobials in our study in isolates from untreated cow's milk testifies, first and foremost, to the good health of the dairy cows in question and the appropriate antibiotic policy on the given farms. In view of the size of the set of isolates tested in this study, however, no universal conclusions may be deduced. The presence of selected virulence factors (*eae*A, *hly*, stx_1, stx_2) was performed by the PCR method in all *E. coli* isolates from milk and meat. The presence of neither the gene *eae*A encoding the production of intimin, nor the gene *hly* responsible for the production of haemolysin was detected in any of the isolates from milk. The presence of the gene stx_1 was also not detected, though the gene stx_2 was detected in one isolate from pork meat. The gene stx_2 encodes the production of Shiga toxin 2 and is typical of the group of Shiga toxigenic *E. coli* (STEC). STEC strains that cause haemorrhagic colitis and haemolytic-uraemic syndrome (HUS) are known as enterohaemorrhagic *E. coli* (EHEC). The production of haemolysin and (usually) intimin is typical of these strains, in addition to the production of Shiga toxin (Fagan et al. 1999). In our study, the strain carrying the stx_2 gene was further typed. It was found that this isolate did not belong to any of the 70 serogroups tested. Subtyping of stx_2 gene revealed a subtype stx_{2e}, which is commonly associated with pig edema disease but is only seldomly found in human STEC infections and has not been implicated in diarrhea or severe illnesses (Beutin et al. 2008).

Conclusions

The results of our study indicate that meat is a more significant source of resistant *E. coli* than milk. The frequent presence of multi-resistant isolates is particularly important and makes raw meat a significant reservoir of genes of resistance that can be transferred to sensitive strains. The stx_{2e} gene was detected in one isolate from meat. This subtype is commonly associated with disease of pigs but only seldomly found in human STEC infections.

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