Phenotypic and genotypic characterization of *Escherichia coli* O157 strains isolated from humans, cattle and pigs

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ABSTRACT: A total of 90 *Escherichia coli* O157 isolates recovered from humans, cattle, and pigs, were examined for the presence of the H7 antigen, ability to produce Shiga toxins and enterohemolysin as well as for antimicrobial resistance and biochemical properties. Fourteen of the human strains (n = 23) and 21 of the bovine isolates (n = 29) were of the O157:H7 serotype as determined by agglutination and PCR methods. All *E. coli* O157 of porcine origin (n = 38) were H-negative. Based on the ability to produce Shiga toxins (Stxs), all human and cattle isolates and 11 strains recovered from swine were identified as Shiga toxin-producing *E. coli* (STEC). Among STEC, most human strains (18 isolates) were Stx1- and Stx2-positive whereas cattle strains were mostly Stx2-positive. Eleven porcine STEC produced either Stx1 (7 isolates) or Stx2 (4 strains) toxins; an additional 20 isolates recovered from these animals had the Stx2e toxin gene as previously determined by PCR. All human and cattle *E. coli* O157 produced enterohemolysin whereas only 4 strains recovered from pigs were *ehly*-positive. Moreover, the PCR identification of the lpf_{O113} gene performed earlier revealed that this putative virulence marker was present in all porcine isolates, only in 5 strains of bovine origin but in none of *E. coli* O157 recovered from humans. All 90 *E. coli* O157 strains tested displayed 10 biochemical profiles that were different at least in one of the reaction tested. The most common atypical reaction observed among porcine O157 isolates was ability to ferment sorbitol (all strains) and production of β-glucuronidase (25 isolates). Moreover, none of the sorbitol-positive strains was able to produce indol. Four antimicrobial resistance profiles among 90 *E. coli* O157 strains tested were observed. Most of the isolates recovered from humans and all strains from cattle were resistant only to rifampicin whereas the porcine strains showed resistance to either 3 antimicrobials (4 isolates) or to 4 drugs tested (34 isolates). The phenotypic data shown in the present study, together with the previously published genotypic analyses of these strains, confirm earlier suggestions that the porcine *E. coli* O157 strains are mostly different from those of bovine and human O157 isolates and could therefore play less important role in human STEC O157 infections.

Keywords: *E. coli* O157; phenotypic properties; virulence marker genes; antibiotic resistance

Shiga toxin-producing *Escherichia coli* (STEC) is a major serologically diverse group of foodborne, zoonotic pathogens, of which two serovars – O157:H7 and O157:H$^-$ have been epidemiologically significant in North America, Europe, and increasingly in other areas of the world (Armstrong et al., 1996; Paton and Paton, 1998; Tarr and Neill, 2001). These bacteria can cause a range of human diseases, including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Griffin and Tauxe, 1991; Tarr and Neill, 2001). STEC possess several factors implicated in their pathogenesis, including Shiga toxins (Stx1, Stx2, or Stx2 variants). There are other putative virulence markers found in *E. coli* O157 and among them there is enterohemolysin, which is connected with nearly all O157 STEC and many non-O157 isolates, seems to play a role in the pathogenicity of STEC infections (Armstrong et al., 1996; Paton and Paton, 1998).

Cattle are the major reservoir of *E. coli* O157:H7 and O157:H$^-$ but STEC of these serovars have been also isolated from pigs, sheep, goats, horses, and poultry (Armstrong et al., 1996; Mainil, 1999). Most outbreaks caused by these organisms have been

food or water related. Moreover, transmission by person-to-person contact or drinking of unpasteurised milk or water has been described (Armstrong et al., 1996; Karch et al., 1999; Tarr and Neill, 2001). There is little information concerning the prevalence and characteristics of *E. coli* O157 originating from pigs and pork products are not regarded as a risk factor for human STEC infection (Beutin et al., 1993; Chapman et al., 1997; Heuvelink et al., 1999). However, studies from Chile and Japan have documented a carriage rate of *E. coli* O157 in pigs comparable to the carriage rate in cattle (Nakazawa et al., 1999; Rios et al., 1999).

The detection of *E. coli* O157 is based on the isolation of the bacteria from samples and determination of its virulence-associated factors or their genes. Selective media for the identification of O157 strains are based on the fact that most of these bacteria possess typical biochemical characteristics: delayed (or lack) of fermentation of D-sorbitol and lack of β-D-glucuronidase activity (Vernozy-Rozand, 1997; Coia, 1998). These two phenotypic markers help to differentiate *E. coli* O157 from non-O157 *E. coli* strains. However, it has been shown that some *E. coli* O157, especially those of serotype O157:H$^-$, may ferment sorbitol within 24 h (Karch et al., 1993; Bielaszewska et al., 1998). Moreover, in 1995 the first isolation of a β-D-glucuronidase-producing strain of O157:H7 associated with HC was reported (Hayes et al., 1995). Furthermore, Ware et al. (2000) described several *E. coli* O157 possessing other aberrant biochemical properties. These observations suggest that, despite a high clonality of *E. coli* O157:H7, an increasing phenotypic variation among these isolates may potentially lead to some failures in detection *E. coli* O157 bacteria.

Several antimicrobial agents are used therapeutically in human and veterinary medicine. In addition, some antibiotics are routinely used for disease prevention and growth promotion in animal production. This practice leads to selection of drug resistance among bacteria, including *E. coli* O157, in food animals. Antimicrobial-resistant strains may then enter the human population and may create a public health problem (Witte, 1998; Tollefson et al., 1999; Van den Bogaard and Sobberingh, 1999).

The major objectives of this study were to characterize *E. coli* O157 strains isolated from humans, cattle, and pigs, by the determination of their phenotypic traits: biochemical properties, antimicrobial resistance, and production of the main virulence markers – Shiga toxins and enterohemolysin. An additional objective was to define, on the basis of the above characteristics, if *E. coli* strains of different origin display similarities or differences.

MATERIAL AND METHODS

Bacterial strains. A total of 90 *E. coli* O157 isolates, collected from humans, cattle, and pigs during the years 1998–2000, was used in this study. The human isolates (n = 23) were obtained from the National Institute of Hygiene, Warsaw, the University of Lodz, and the Federal Institute of Protection of Consumers and Veterinary Medicine, Dessau, Germany. The strains recovered from cattle (n = 29) were isolated from 3–5 months old animals as described previously (Osek et al., 2000). One strain was obtained from one animal that represented one cattle farm. The strains of porcine origin (n = 38) were isolated from weaned pigs (four to six weeks old) with diarrhea from eight geographically separated pig farms in the west part of Poland as described previously (Osek, 2002).

After isolation and identification, the *E. coli* bacteria were stored in agar stabs at room temperature without any subculturing.

Biochemical tests. The strains were characterized biochemically using the ID 32E system (bioMerieux, Marcy-l'Etoile, France) according to the manufacturer's instruction. Additionally, sorbitol utilization was analyzed by growing the bacteria at 37°C for 48 h in peptone water supplemented with 0.5% of D-sorbitol.

Antimicrobial susceptibility determination. Antimicrobial susceptibility profiles were performed using the ABT VET system (bioMerieux) according to the producer's recommendation. The following Gram-negative-targeting antimicrobial agents were included in the test: Amoxicillin (AMO; 4 mg/l), Amoxicillin-Clavulanic Acid (AMC; 4 mg/l, 2 mg/l, respectively), Cephalothin (CFT; 8 mg/l), Colistin (COL; 4 mg/l); Cefoperazone (CFP; 4 mg/l); Cotrimoxazole (TSU; trimetoprime 2 mg/l and sulfamethoxazole 378 mg/l), Stereptomycin (STR; 8 mg/l), Sulfamethizole (SUL; 100 mg/l), Spectinomycin (SPE; 64 mg/l), Flumequine (FLU; 4 mg/l), Kanamycin (KAN; 8 mg/l), Oxolinic Acid (OXO; 2 mg/l), Gentamicin (GEN; 4 mg/l), Enrofloxacin (ENR; 0.5 mg/l), Apramycin (APR; 16 mg/l), Nitrofurantoin (FUR; 25 mg/l), Chloramphenicol (CMP; 8 mg/l), Tetracycline (TET; 4 mg/l), Rifampicin (RFA; 4 mg/l), and Doxycycline

(DOT; 4 mg/l). The *E. coli* strains resistant to one antimicrobial were classified as profile A, to two as profile B, to three as proifile C whereas those resistant to four antimicrobials tested were designated as profile D.

Enterohemolysin production. Detection of the enterohemolytic phenotype was performed on blood agar plates containing 5% of washed sheep erythrocytes and 10 mM $CaCl_2$ (Beutin et al., 1989). The narrow turbid zone of hemolysis occurring after 18 h incubation at 37°C was regarded as a positive result. A blood agar with unwashed sheep blood was used as a comparison plate.

Production of Shiga toxins. Toxin production in the isolated bacteria was detected by the reverse passive latex agglutination test (VTEC-RPLA; Oxoid, Basingstoke, UK) with polymyxin B sulfate (Sigma, St. Louis, Mo) extraction as described (Karmali et al., 1985).

Serological examinations. The O157 and H7 antigens were tested using slide agglutination tests (Denka Seiken, Tokyo, Japan). All *E. coli* isolates were analyzed for O157 after boiling the bacteria at 100°C for 1 hour. Strains which were nonmotile after three passages through semi-solid agar were defined as nonmotile (H^-).

PCR analyses. All PCR tests were performed before and their results had been published previously (Osek, 1999, 2002; Osek et al., 2000, 2003). Briefly, *E. coli* strains were grown overnight at 37°C on LB agar. A small amount of the culture was resuspended in 50 µl of distilled water, heated to 99°C for 10 min and centrifuged for 1 min at 12 000xg. The resulting supernatant was used as a template for PCR. The following genes were amplified: *rfb*O157 (*E. coli* O157), *fliC* (H7), *stx*1 and *stx*2 (Shiga toxin 1 and 2, respectively), *stx2e* (Shiga toxin 2e variant), *ehly* (enterohemolysin), *eaeA* (intimin), and lpf_{O113} (long polar fimbriae) using the PCR primers and amplification conditions as described previously (Osek, 1999, 2002; Osek et al., 2000, 2003).

RESULTS

Characteristics of *E. coli* strains tested

It was shown (Table 1) that the H7 flagellar structure was present only on isolates recovered from humans (14; 60.9% strains) and from cattle (21; 72.4% isolates). None of the 38 porcine *E. coli* O157 was H7-positive as determined by the slide agglutination test.

The results of enterohemolysin production are shown in Table 1. All *E. coli* O157 isolates of human and bovine origin regardless of the presence of the H7 antigen, were hemolytic as shown by the presence of the hemolysis zone after 18 h incubation at 37°C. Among 38 porcine strains, only 4 isolates were positive in this assay; these *ehly*-positive isolates were able to produce Shiga toxin 2 as determined by the VTEC-RPLA and PCR analyses.

Table 1. Characteristics of the 90 *E. coli* O157 strains isolated from humans, cattle, and pigs, used in this study (number and percent of strains)

E. coli phenotype and genotype[a]	Humans $n = 23$	Cattle $n = 29$	Pigs $n = 38$
O157:$H7^+$ (*rfbO157* and *fliC* genes)	14 (60.9)	21 (72.4)	0
O157:H^- (*rfbO157* gene)	9 (39.1)	8 (27.6)	38 (100)
Enterohemolysin (*ehly* gene)	23 (100)	29 (100)	4 (10.5)
Stx1 (*stx1* gene)	2 (8.7)	2 (6.9)	7 (18.4)
Stx2 (*stx2* gene)	3 (13.0)	22 (75.9)	4 (10.5)[b]
Stx1/2 (*stx1* and *stx2* genes)	18 (78.3)	5 (17.2)	0
Intimin (*eaeA* gene)	23 (100)	26 (89.6)	7 (18.4)
LPF fimbriae (*lpfAO113* gene)	0	5 (17.2)	38 (100)

[a]Phenotypic properties were tested as described in Material and Methods. The respective genes were analysed previously by PCR (Osek, 2002; Osek et al., 2003)

[b]Additional 20 strains possessed genes encoding Stx2e toxin variant as determined by PCR (Osek, 1999)

It was demonstrated that Shiga toxins 1 and 2 were produced by *E. coli* O157 strains isolated from all 3 sources (humans, cattle, pigs) (Table 1). It was found that all human and bovine isolates could be classified to the STEC group and were able to produce either one toxin (Stx1 or Stx2) alone or both. On the other hand, only 11 out of 38 *E. coli* O157 strains of swine origin were STEC although additional 20 isolates possessed the *stx2e* gene of Shiga toxin 2e variant as determined previously by PCR (Osek, 1999). The most prevalent toxin profile among human strains was Stx1/Stx2 (18; 78.3% isolates) whereas the strains recovered from cattle mainly produced Shiga toxin 2 (22; 75.9% isolates).

The *E. coli* O157 phenotypic properties listed in Table 1 were confirmed earlier by the identification of the respective genes using PCR (Osek, 1999, 2002; Osek et al., 2000, 2003). There was a 100% correlation between the results of the phenotypic analyses performed in this study and the results of the previous genotypic tests. Moreover, in the present results, two additional genetypic markers (*eaeA* and lpf_{O113}) responsible for the expression of *E. coli* O157 attaching properties were included (Osek et al., 2000, 2003). It was found that all 23 isolates of human origin and the majority of strains (26; 89.6%) recovered from cattle were intimin gene positive. Only 7 strains (18.4%) isolated from pigs had the *eaeA* marker (Table 1). On the other hand, all porcine O157 *E. coli* bacteria possessed the lpf_{O113} gene cluster that was either absent in human strains or found only in a few (5; 17.2%) isolates of cattle origin.

Biochemical profiles of the *E. coli* O157

Table 2 lists the biochemical reactions recorded in *E. coli* O157 strains tested. All 90 isolates produced ten biochemical profiles (designated 1 to 10) that were different at least in one of the reactions tested. Human strains were classified into 3 biochemical profiles (groups 1, 2, and 3) with the majority of the isolates belonging to the profile 1 (14 strains). The only difference between this group and the biochemical profile of group 2 (5 isolates) was the ability to ferment sucrose by strains of profile 1. It has also been noted that this sugar was not utilized by the isolates classified to profile 3. However, strains of this group were able to decarboxylate lysine which was not utilized by the *E. coli* with biochemical properties of groups 1 and 2 (Table 2). Typical *E. coli* O157 biochemical pattern (lack of D-sorbitol fermentation after 24 h and lack of β-glucuronidase) was found among all 23 isolates recovered from humans. However, four of these strains were able to ferment sorbitol after 48 h as well as decarboxylate ornithin and hydrolyze arginine; therefore, they were classified into a separate biochemical group 3.

Strains of bovine origin (29 isolates) could be classified into 3 groups (designated as profiles 4, 5, and 6) (Table 2). *E. coli* belonging to the profile 4 were identical with the isolates of group 3 (recovered from humans) except for the ability to ferment sorbitol which was still negative after 48 h at 37°C. Moreover, the strains with profile 4 were able to hydrolyze arginine and utilize rhamnose, which both were negative among the isolates of group 5. Additionally, the strains of bovine origin, which were classified to biochemical profile 6, were not able to decarboxylate ornithin. This reaction was always positive with the isolates of groups 1–5, recovered either from humans or from cattle (Table 2).

E. coli O157 strains of porcine origin ($n = 38$) were classified into four biochemical profiles (designated as 7, 8, 9, and 10). The most characteristic atypical reaction of all these isolates was the lack of lysine decarboxylation and indol production, a negative reaction with maltose and fermentation of sorbitol within 24 hours. Moreover, most of the strains (25 out of 38 isolates) produced β-glucuronidase, the marker which was absent in all *E. coli* O157 isolates of human and cattle origin (Table 2).

Antimicrobial susceptibility

Antimicrobial resistance of the *E. coli* O157 strains was tested to 20 drugs belonging to different antimicrobial classes (Table 3). In general, four drug resistance patterns, designated A, B, C, and D, were observed. The most common resistance profile A was found among 16 (69.6%) strains of human origin and in all 29 isolates recovered from cattle. An additional 7 human strains exhibited the resistance pattern B. Strains of this group differed from the isolates of profile A in the resistance to cephalothin only.

The porcine O157 strains showed two antimicrobial resistance properties (groups C and D) that were different in resistance to sulfamethizole, tetracycline and doxycycline and in sensitivity to rifampicin, the antibiotic which was only efficient to the isolates of porcine origin (Table 3).

Table 2. Biochemical profiles among *E. coli* O157 strains isolated from humans, cattle, and pigs

	Biochemical profile									
	1	2	3	4	5	6	7	8	9	10
Ornithin decarboxylase	+	+	+	+	+	–	–	+	+	+
Arginine dihydrolase	–	–	+	+	–	–	–	–	–	–
Lysine decarboxylase	–	–	+	+	+	+	–	–	–	–
Urease	–	–	–	–	–	–	–	–	–	–
L-Arabitol (acidification)	–	–	–	–	–	–	–	–	–	–
Galacturonate (acidification)	+	+	+	+	+	+	–	+	+	+
5-Ketogluconate (acidification)	–	–	–	–	–	–	–	–	–	–
Lipase	–	–	–	–	–	–	–	–	–	–
Phenol red (acidification)	+	+	+	+	+	+	+	+	+	+
β-Glucosidase	–	–	–	–	–	–	–	–	–	–
Mannitol (acidification)	+	+	+	+	+	+	+	+	+	+
Maltose (acidification)	+	+	+	+	+	+	–	–	–	–
Indol (production)	+	+	+	+	+	+	–	–	–	–
N-Acetyl-β-Glucosaminidase	–	–	–	–	–	–	–	–	–	–
β-Galactosidase	+	+	+	+	+	+	+	+	+	+
Glucose (acidification)	+	+	+	+	+	+	+	+	+	+
Sucrose (acidification)	+	–	–	–	–	+	+	–	+	+
L-Arabinose (acidification)	+	+	+	+	+	+	+	+	+	+
D-Arabitol (acidification)	–	–	–	–	–	–	–	–	–	–
α-Glucosidase	–	–	–	–	–	–	–	–	–	–
α-Galactosidase	+	+	+	+	+	+	+	+	+	+
Trehalose (acidification)	+	+	+	+	+	+	+	+	+	+
Rhamnose (acidification)	+	+	+	+	–	+	+	+	–	+
Inositol (acidification)	–	–	–	–	–	–	–	–	–	–
Adonitol (acidification)	–	–	–	–	–	–	–	–	–	–
Palatinose (acidification)	–	–	–	–	–	–	–	–	–	–
β-Glucuronidase	–	–	–	–	–	–	–	+	+	+
Celiobiose (acidification)	–	–	–	–	–	–	–	–	–	–
Sorbitol (acidification)	–	–	+[a]	–	–	–	+	+	+	+
α-Maltosidase	–	–	–	–	–	–	–	–	–	–
Malonate	–	–	–	–	–	–	–	–	–	–
L-Aspartic acid acrylamidase	–	–	–	–	–	–	–	–	–	–
No. (%) of isolates	14 (60.9)	5 (21.7)	4 (17.4)	12 (41.4)	9 (30.0)	8 (27.6)	13 (34.2)	11 (28.9)	9 (23.7)	5 (13.2)
Source of isolation	Humans (n = 23)			Cattle (n = 29)			Pigs (n = 38)			

[a]Positive after 48 h

The highest prevalence of antimicrobial resistance was observed among *E. coli* O157 recovered from swine: 34 (89.5%) isolates were resistant to 4 rugs used and additional 4 (10.5%) strains were resistant to 3 antimicrobials assayed. On the other hand, all 29 bovine isolates and the majority (16 out

of 23) of human strains were not sensitive to only one drug (rifampicin) tested. It has to be noted that 7 strains of human origin were resistant to cephalothin (Table 3).

Multiple antimicrobial resistance

Most of the *E. coli* O157 isolates analyzed during this study (45; 50.0%) were resistant to one antimicrobial (rifampicin) and 34 strains (37.8%) to 4 drugs (streptomycin, sulfamethizole, tetracycline, doxycycline) tested (Table 4). Among STEC isolates (n = 63), the majority (45 strains; 71.4%) was resistant to rifampicin only and the remaining 18 *stx*-positive strains were resistant to either 2 (cephalothin, rifampicin), 3 (sulfamethizole, tetracycline, doxycycline) or 4 (sulfamethizole, tetracycline, doxycycline, streptomycin) antimicrobials assayed. Among non-STEC *E. coli* O157 bacteria (n = 27, all recovered from pigs), all except one displayed the highest multi-resistance pattern (4 antimicrobials). One non-STEC strain (recovered from pigs) was resistant to 3 drugs tested.

DISCUSSION

E. coli strains of the O157 serogroup, isolated from humans, cattle, and pigs, were characterized

Table 3. Antimicrobial resistance profiles among *E. coli* O157 strains recovered from humans, cattle, and pigs

Antimicrobials	Drug resistance profile[a]				
	A	B	A	C	D
Amoxicillin (AMO)	S[b]	S	S	S	S
Amoxicillin-Clavulanic Acid (AMC)	S	S	S	S	S
Cephalothin (CFT)	S	R	S	S	S
Colistin (COL)	S	S	S	S	S
Cefoperazone (CFP)	S	S	S	S	S
Cotrimoxazole (TSU)	S	S	S	S	S
Streptomycin (STR)	S	S	S	S	R
Sulfamethizole (SUL)	S	S	S	R	R
Spectinomycin (SPE)	S	S	S	S	S
Flumequine (FLU)	S	S	S	S	S
Kanamicin (KAN)	S	S	S	S	S
Oxolinic Acid (OXO)	S	S	S	S	S
Gentamicin (GEN)	S	S	S	S	S
Enrofloxacin (ENR)	S	S	S	S	S
Apramycin (APR)	S	S	S	S	S
Nitrofurantoin (FUR)	S	S	S	S	S
Chloramphenicol (CMP)	S	S	S	S	S
Tetracycline (TET)	S	S	S	R	R
Rifampicin (RFA)	R	R	R	S	S
Doxycycline (DOT)	S9	S	S	R	R
No. (%) of isolates	16 (69.6)	7 (30.4)	29 (100)	4 (10.5)	34 (89.5)
Source of isolation	Humans (n = 23)		Cattle (n = 29)	Pigs (n = 38)	

[a]Drug resistance profiles were designated A, B, C, and D as described in Material and Methods
S – sensitive; R – resistant

Table 4. Multiple antimicrobial resistance among the 90 *E. coli* O157 isolates tested

No. of antimicrobials to which resistance was observed	No. of isolates					
	Total $n = 90$	STEC $n = 63$	Non-STEC $n = 27$	Humans $n = 23$	Cattle $n = 29$	Pigs $n = 38$
1	45	45	0	16	29	0
2	7	7	0	7	0	0
3	4	3	1	0	0	4
4	34	8	26	0	0	34

by testing the presence of the H7 antigen, production of enterohemolysin and Shiga toxins as well as analyzing biochemical properties and antimicrobial resistance. Moreover, the results of the previous PCR analyses of the respective genes of these *E. coli* O157 strains were included into the present study. It was shown that the H7 antigen was only present on O157 isolates of human and bovine origin. All human and bovine strains as well as 11 isolates recovered from swine, were classified as STEC, producing either Stx1, Stx2 or both toxins. Strains O157:H7$^+$ or O157:H$^-$ have been often isolated from cattle but there is little information concerning the prevalence and characteristic of this bacteria recovered from pigs (Beutin et al., 1993; Wray et al., 1993; Chapman et al., 1997; Heuvelink et al., 1999; Nakazawa et al., 1999). Wray et al. (1993) and Wittig et al. (1995) described a few O157 *E. coli* strains of swine origin and found that they were different from other O157 isolates in terms of Shiga toxin production (usually Stx-negative). Nakazawa et al. (1999) found 3 O157:H7$^+$ isolates among 221 porcine *E. coli* samples tested and they were Stx-positive. Moreover, Johnsen et al. (2001), testing as many as 1 976 bacterial samples from pigs, detected only 2 *E. coli* O157:H7-positive isolates, which were not further analyzed for toxin production. However, there is no information concerning the prevalence of other typical O157-associated markers (enterohemolysin and intimin) as well as biochemical and antimicrobial resistance of such strains and correlation of these phenotypic properties with the source of isolation. It was shown that most typical STEC strains possessed the *eaeA* marker whereas only few porcine O157:H$^-$ isolates were intimin-positive. On the other hand, as shown in the previous study (Osek et al., 2003), all these strains had another putative virulence gene (*lpfO113*) that was detected in some of bovine bacteria that were H7-negative. As determined before (Osek et al., 2003) the presence of the lpf_{O113} gene in *E. coli* O157 strains had been associated with the lack of the LEE pathogenicity island, harbouring the *eaeA* intimin marker.

The early studies of Whittam et al. (1988) demonstrated that *E. coli* O157:H7 recovered from different sources and analysed by the multilocus enzyme electrophoresis method were genotypically identical or nearly identical except the isolates recovered from swine. It was also shown in the present and previous studies (Osek et al., 2003) that porcine *E. coli* O157 were different from human and cattle STEC O157 in terms of virulence properties (presence of *stx1*, *stx2*, *eaeA*, and lpf_{O113} genes). Moreover, detailed phenotypic assays revealed that they also differed in several biochemical and antimicrobial resistance properties. The most typical characteristic of swine isolates was the ability to ferment sorbitol after 24 h whereas none of the bovine and human strain displayed this biochemical pattern. Only 4 isolates (classified into biochemical profile 3) were able to utilize sorbitol after 48 hours. This is an important observation from a public health point of view since many commercially available bacterial diagnostic tests (e.g. sorbitol-MacConkey agar) are based on utilization of D-sorbitol during 18–24 hours. It has to be noted that 11 of those 38 sorbitol-positive porcine isolates were able to produce Shiga toxin (Stx1 or Stx2) as determined using the VTEC-RPLA test and confirmed by the presence of the *stx* genes by PCR. Moreover, four of these isolates secreted enterohemolysin. Therefore, they seem to be potentially pathogenic for humans. On the other hand, during the last years sorbitol-fermenting (SF) STEC strains of serotype O157:H-negative have emerged as important causes of diarrhea and HUS in Germany and in the Czech Republic (Bielaszewska et al., 1998, 2000; Ammon et al., 1999; Karch et al., 1999). O157-positve but H-negative SF isolates have also

been recently detected (5.9%) among *E. coli* strains tested in Belgium (Leclercq et al., 2001). Until now, there is only one report concerning the isolation of SF O157:H7-positive strains (Ware et al., 2000) but such isolates were not detected in the present study. However, a study of Fratamico et al. (1993) has shown that isolates of serotype O157:H7 in sorbitol-containing foods can mutate from the SN form to a sorbitol-fermenting phenotype.

Recently, Ware et al. (2000) described several *E. coli* O157:H7 strains with aberrant biochemical properties. The most common atypical reaction observed was the inability to ferment one of several different carbohydrates analyzed in their study (rhamnose, sucrose, lactose, glucose). Also in the present study, the rhamnose-negative *E. coli* O157 strains (both H7-positive and H-negative) were found (9 isolates of cattle origin and 9 strains recovered from pigs). Interestingly, several isolates obtained from all three sources tested were sucrose-negative (as many as 41 strains).

Different antimicrobial resistance profiles of *E. coli* O157 strains analyzed in the present study were observed. All bovine isolates displayed one antibiogram pattern which was also characteristic for the majority of bacteria recovered from humans. The strains of porcine origin showed two diverse profiles that were different from human and cattle isolates in resistance to sulfamethizole, tetracycline, and doxycycline as well as in sensitivity to rifampicin. Overall, the *E. coli* O157 isolates displayed *in vitro* sensitivity to most of the drugs tested. Only the strains recovered from swine, especially those classified to the profile C, were resistant to 4 antimicrobials used, including streptomycin. This antibiotic was shown to be active *in vitro* to all other isolates analysed, including the 4 remaining strains of porcine origin. The opposite results were described by Meng et al. (1998) who found that streptomycin was the most common antibiotic to which *E. coli* O157:H7$^+$ and O157:H$^-$, isolated from animals, food, and humans, were resistant. Moreover, it has been also observed that seven human strains were resistant to cephalothin, the antibiotic which is used in human medicine. On the other hand, no strain of animal origin was resistant to this drug that is not approved for veterinary use.

It is difficult to compare the drug resistance profiles obtained in the present analysis with previous studies because of differences in bacteria selection and methods used for the sensitivity test. Galland et al. (2001) observed that all O157:H7 isolates from cattle were resistant to tilmicosin, an antibiotic that is commonly added to food for cattle. However, this drug was not included in the panel of antimicrobials analysed in the present study. On the other hand, all strains tested by Galland et al. (2001) were susceptible to ciprofloxacin, an antibiotic widely used in human medicine. Unfortunately, this antimicrobial has also not been tested in the present study, either. Recently, Schroeder et al. (2002) tested antimicrobial resistance of 361 *E. coli* O157 isolated from humans, cattle, swine, and food, using broth microdilution, a method similar to the test applied in the present analysis. They found that there was high prevalence of resistance to tetracycline, sulfamethoxazole, cephalothin, and ampicillin. However, the only drug analyzed in the present study was tetracycline and the resistant *E. coli* isolates were recovered from pigs. As in the present study, Schroeder et al. (2002) observed the highest prevalence of resistance among *E. coli* O157 from swine as compared to the strains isolated from other sources. Most porcine bacteria (74%) were resistant to sulfamethoxazole and to tetracycline (71%). Interestingly, many strains of porcine origin (54%) were resistant to cephalothin. In the present study, no *E. coli* isolate recovered from animals (pigs, cattle) was resistant to this antibiotic.

It was previously shown (Schmidt et al., 1998; Orden et al., 1999) that there was a marked difference among antimicrobial resistance observed in STEC and non-STEC O157 *E. coli* bacteria. Resistance among non-STEC isolates was much higher than in STEC for ampicillin, sulfamethoxazole, gentamycin, tetracycline and trimethoprime-sulfamethoxazole (in the present study – cotrimoxazole, TSU). Similar observations were made during the present study in the case of streptomycin and tetracycline only whereas the sensitivity to gentamycin was identical among STEC and non-STEC *E. coli* strains analyzed. The high prevalence of resistance to tetracycline among *E. coli* O157 strains isolated from pigs observed in the present study also agrees with previous reports.

Galland et al. (2001) found that among 57 *E. coli* O157 of cattle origin, 27 (47%) were resistant to amoxicillin-calvulanic acid (AMC). On the other hand, Schroeder et al. (2002) found that only 1 of 93 *E. coli* O157 exhibited AMC resistance. In the present study none of the 90 *E. coli* strains tested was resistant to AMC. These differences result of e.g. geographical differences or methods used for determination of bacterial sensitivity *in vitro* between

studies of Galland et al. (2001) and tests performed by Schroeder et al. and in the present studies.

The highest prevalence of antimicrobial resistance was observed among porcine O157 isolates, where 100% of strains were resistant to sulfamethizol, tetracycline and doxycycline. These data may suggest a correlation between antimicrobial use in swine and development of resistance to commonly applied drugs in these animals in Poland. Since antimicrobial resistant bacteria from pigs may enter the human population via the food chain or direct contact, it is possible that these resistant microorganisms may be transferred to humans (van den Bogaard and Sobberingh, 1999). Therefore, the emergence and dissemination of antimicrobial resistance in STEC and non-STEC O157 of animal origin may complicate therapeutic strategies of HC and HUS in humans.

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