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Porcine pathogenic *Escherichia coli* strains differ from human fecal strains in occurrence of bacteriocin types

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ABSTRACT

Enterotoxigenic and Shiga-toxigenic *Escherichia coli* (i.e., ETEC and STEC) are important causative agents of human and animal diseases. In humans, infections range from mild diarrhea to severe life-threating conditions, while infections of piglets result in lower weight gain and higher pig mortality with the accompanying significant economic losses.

In this study, frequencies of four phylogenetic groups, fourteen virulence- and thirty bacteriocin determinants were analyzed in a set of 443 fecal *E. coli* isolates from diseased pigs and compared to a previously characterized set of 1283 human fecal *E. coli* isolates collected in the same geographical region. In addition, these characteristics were compared among ETEC, STEC, and non-toxigenic porcine *E. coli* isolates.

Phylogenetic group A was prevalent among porcine pathogenic *E. coli* isolates, whereas the frequency of phylogroup B2, adhesion/invasion (*fimA*, *pap*, *sfa*, *afaI*, *ial*, *ipaH*, and pCVD432) and iron acquisition (*aer* and *iucC*) determinants were less frequent compared to human fecal isolates.

Additionally, porcine isolates differed from human isolates relative to the spectrum of produced bacteriocins. While human fecal isolates encoded colicins and microcins with a similar prevalence, porcine pathogenic *E. coli* isolates produced predominantly colicins (94% of isolates); especially colicins B (42.6%), M (40.1%), and Ib (34.0%), which are encoded on large conjugative plasmids. The observed high prevalence of these colicin determinants suggests the importance of large colicinogenic plasmids and/or the importance of colicin production in intestinal inflammatory conditions.

1. Introduction

Escherichia coli (*E. coli*) is a highly diverse species with respect to its gene content, phenotype, and virulence. Based on different virulence factors, *E. coli* strains can be classified into three main groups: commensal/nonpathogenic (NonPEC), intestinal pathogenic (InPEC), and extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson, 2000). Both pathogenic groups of *E. coli* differ from nonpathogenic commensal strains by the presence of virulence factors (Tenaillon et al., 2010).

ExPEC are associated with a spectrum of infections ranging from simple urinary tract infections to life-threatening bacteremia. ExPEC strains typically encode virulence factors that allow them to bind to eukaryotic cells (e.g., P-fimbriae, S-fimbriae), survive outside the gut (e.g., siderophores), and damage cells and tissues (e.g., hemolysin, cytotoxic necrotizing factor) (Russo and Johnson, 2000; Dale and Woodford, 2015).

InPEC strains are pathogens associated with diarrhea and there are six well-described pathotypes: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), and enterohemorrhagic *E. coli* (EHEC), which is subgroup of Shiga-toxigenic *E. coli* (STEC). In the case of ETEC and STEC, human and animal diseases are associated with the production of toxins (i.e., enterotoxins and Shiga toxins, respectively). In humans, ETEC are associated with infant diarrhea in developing countries and traveler's diarrhea, while STEC causes sporadic and epidemic foodborne infections, typically, with

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extremely low infection doses. In both cases, symptoms range from mild diarrhea to severe life-threating conditions. The pathogenesis, epidemiology, diagnosis, and clinical aspects of diarrheagenic *E. coli* are reviewed in Nataro and Kaper (1998) and Kaper et al. (2004). At the same time, intestinal infections with toxigenic *E. coli* are also important swine diseases (colibacillosis) resulting in lower weight gain of piglets and higher pig mortality, which is associated with significant economic losses worldwide. ETEC infections are responsible for neonatal and post-weaning diarrhea and the severity of diarrhea is affected by stress of weaning, lack of antibodies from the sow's milk, and dietary changes (Fairbrother et al., 2005). STEC infections are associated with edema disease of piglets; where STEC colonize the small intestine and produce Shiga toxin. The toxin enters the bloodstream and impairs blood vessels, which leads to edema, ataxia, and piglets' death (Tseng et al., 2014).

Another important characteristic of *E. coli* strains is the production of bacteriocins. *E. coli* strains are able to synthesize two types of bacteriocins – colicins (proteins) and microcins (peptides). Colicins and microcins differ in a number of additional parameters including operon organization, regulation of gene expression, export from producer cells, presence of post-translational modifications, antimicrobial activity, etc. (Cascales et al., 2007). The ecological role of bacteriocinogeny is not clear. Some bacteriocins specifically inhibit pathogenic bacteria (Šmajs and Weinstock, 2001; Patton et al., 2007; Bosák et al., 2013, 2016), and at the same time, production of some other bacteriocins have been associated with virulence in pathogenic *E. coli* strains, especially ExPEC strains and ExPEC-related virulence factors (Azpiroz et al., 2009; Šmajs et al., 2010; Budič et al., 2011; Micenková et al., 2014, 2016b).

In this study, the prevalence of phylogenetic groups, virulence- and bacteriocin determinants was analyzed in a set of 443 fecal *E. coli* isolates from diseased pigs and was compared to a previously characterized set of 1283 human fecal *E. coli* isolates collected in the same geographical region (Micenková et al., 2016a). In addition, these characteristics were compared among enterotoxigenic, Shiga-toxigenic, and non-toxigenic porcine *E. coli* isolates.

2. Methods

2.1. Bacterial strains

A set of 443 *E. coli* isolates originating from the gastrointestinal tract of pigs with clinical manifestation of diseases was isolated at the University of Veterinary and Pharmaceutical Sciences in Brno (Brno, Czech Republic) and at the Veterinary Research Institute (Brno, Czech Republic). Isolates originated from fecal samples (mostly rectal swabs), which were collected at different swine farms in the Czech Republic during the years 1996–2014. The list of pathogenic *E. coli* from pigs is shown in Table S1.

The set of *E. coli* strains isolated from diseased pigs was compared to a set of human fecal commensal *E. coli* (n = 1283), which had been collected in the same geographical region, and had been characterized in a previously published study (Micenková et al., 2016a; Table S2). All human participants gave written informed consent, their data were anonymized, and the study was approved by the ethics committee of the Faculty of Medicine, Masaryk University (Brno, Czech Republic).

Indicator strains used for screening of bacteriocin production (i.e., *E. coli* K12-Row, C6 (ϕ), B1, P400, S40, and *Shigella sonnei* 17) and the control strains for PCR detection of virulence- and bacteriocin genes were previously described in detail (Micenková et al., 2016a).

2.2. Phylogenetic classification of E. coli isolates

For classification of *E. coli* isolates to four phylogenetic groups (i.e., A, B1, B2, and D), triplex-PCR amplifying the *chuA*, *yjaA*, and TspE4.C2 genomic fragments was used (Clermont et al., 2000). In addition, revisited phylotyping method (Clermont et al., 2013) able to detect new phylogroups (i.e., C, E, F, and clade I) was used for all porcine isolates. Since human isolates were classified using triplex-PCR (Clermont et al., 2000) in previous study (Micenková et al., 2016a), four major phylogroups (i.e., A, B1, B2, and D) identified by triplex-PCR were used for comparisons.

2.3. PCR detection of genes encoding virulence factors

The presence of 18 virulence determinants encoding 17 diff ;erent virulence factors (i.e., *α-hly* – α-hemolysin; *afaI* – afimbrial adhesin; *aer* – aerobactin synthesis; *cnf1* – cytotoxic necrotizing factor; *sfa* – S-fimbriae; *pap* – P-fimbriae; pCVD432 – aggregative adherence plasmid; *ial* – locus associated with invasivity; *lt* – thermolabile enterotoxin; *st* – thermostable enterotoxin; *bfpA* – bundle-forming pilus; *eaeA* – intimin; *ipaH* – locus associated with invasivity, *iucC* – aerobactin synthesis; *fimA* – fimbriae type I; stx_1 – Shiga toxin 1; stx_2 – Shiga toxin 2; and *ehly* – enterohemolysin) was screened in all porcine *E. coli* isolates. These virulence determinants were previously detected in other studies analyzing fecal commensal and pathogenic *E. coli* strains with diverse origins (Micenková et al., 2014, 2016a, 2016b, 2017, 2018). For detection of virulence genes, specific primers and PCR profiles are shown in Table S3.

Toxin determinants *st*, *lt*, stx_1, and stx_2 were screened for classification of isolates (i.e., STEC, ETEC, and toxigenic) and were not used in comparisons due to partial selection of porcine isolates.

2.4. Bacteriocin production and PCR detection of bacteriocin types

Bacteriocin production was detected using a method described by Šmajs et al. (2010). Briefly, TY (tryptone-yeast) agar plates were inoculated with stabs from the porcine *E. coli* isolates and incubated at 37 °C for 48 h. The macrocolonies were killed using chloroform vapors (30 min. exposure) and each plate was overlaid with a thin layer of TY agar (0.7%, w/v) containing 10^8 cells of one of the six bacteriocin-indicator strains (see above). The plates were then incubated at 37 °C overnight and zones of growth inhibition were read.

For bacteriocin producers phenotypically identified using the agar stab method, PCR screening for bacteriocin determinants encoding 23 colicin types (i.e., A, B, D, E1-9, Ia, Ib, Js, K, L, M, N, S4, U, Y, and 5/10) and 7 microcin types (i.e., H47, M, B17, C7, J25, L, and V) was performed. The specific primers and PCR profiles are shown in Table S3. In addition, PCR products of closely related colicin-encoding genes (i.e., E2-9, Ia–Ib, and U–Y) were further sequenced and analyzed. Since microcins H47 and M are sensitive to chloroform, which was used during phenotype identification of bacteriocinogeny, all 443 porcine *E. coli* isolates were screened for the presence of genes encoding microcins H47 and M.

2.5. Statistical analysis

For statistical analysis of the incidence of genetic determinants of phylogenetic groups, virulence factors, and bacteriocins, either Pearson's Chi-squared test (for more than 2 groups) or two-tailed Fisher's exact test (for 2 × 2 contingency tables) was used. P-values less than 0.05 were considered statistically significant, and were denoted with asterisks (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$). The statistical analyses were performed in R 3.5.2 -software for statistical computing (R Core Team; https://www.R-project.org) and GraphPad Prism 5 software and GraphPad online platform (https://www.graphpad.com/quickcalcs/contingency1/). In order to study the effect of different distribution of virulence factors and bacteriocins between phylogroups, we performed the comparisons between porcine and human isolates within each of the phylogroups separately.

3. Results

3.1. Characterization of E. coli isolates

Pathogenic *E. coli* isolates were derived from diseased pigs, which originated from several different swine farms in the Czech Republic. A set of 166 isolates originated from diseased pigs was provided by the University of Veterinary and Pharmaceutical Sciences Brno. Among them, only 48 isolates were identified as STEC or ETEC; and set was enriched by 277 porcine *E. coli* isolates with confirmed STEC or ETEC pathotypes provided by the Veterinary Research Institute (Table S1). Because of partial selection of STEC and ETEC isolates, the virulence determinants for Shiga-toxins (*stx*$_1$ and *stx*$_2$) and enterotoxins (*st* and *lt*) were omitted from further comparisons.

The set of *E. coli* isolates from diseased pigs (n = 443) was compared to a set of human fecal commensal *E. coli* (n = 1283) from the same geographical region. These isolates were characterized in our previous study (Micenková et al., 2016a; Table S2).

3.2. Comparison of pathogenic E. coli from pigs and human fecal E. coli isolates

Among the 443 porcine isolates, phylogenetic group A was the most frequent (59.4%), followed by phylogroups D, B1, and B2 (22.8%, 12.0%, and 5.9%, respectively). Compared to healthy human microflora, the frequency of phylogroups A and B1 was higher ($p < 0.001$ and $p < 0.01$, respectively), while the phylogroup B2 was less frequent among porcine isolates ($p < 0.001$; Fig. 1A and Table S4).

While hemolysin determinant (*α-hly*) was more frequent among porcine pathogenic *E. coli* isolates than in human fecal microflora, the frequency of adhesion/invasion (*fimA*, *pap*, *sfa*, *afaI*, *ial*, *ipaH*, and pCVD432) and iron acquisition (*aer* and *iucC*) determinants were less frequent compared to human isolates. Statistically significant differences in the frequency of virulence determinants are shown in Fig. 1A.

Production of bacteriocins was identified in 60.3% of porcine isolates, which was a slightly higher frequency than in the human fecal microflora (54.2%, $p < 0.05$). Compared to the set of human bacteriocinogenic *E. coli* strains producing colicins and microcins equally, production of colicins was more prevalent among porcine pathogenic *E. coli* (93.8%, $p < 0.001$). Genes encoding colicins B, M, and Ib were the most abundant among bacteriocinogenic *E. coli* from diseased pigs (42.6, 49.1, and 34.0%, respectively); these colicins, together with colicin E2 (4.9%), were statistically more frequent among porcine isolates compared to healthy human fecal isolates ($p < 0.001$). On the other hand, genetic determinants encoding colicins K (1.1%) and Js (0.0%), and microcins B17 (0.8%), C7 (0.0%), H47 (7.2%), J25 (0.0%), M (0.0%), and V (0.0%) were more frequent in healthy human fecal isolates. Statistically significant differences in the frequency of bacteriocin determinants are shown in Fig. 1A.

In addition, we analyzed associations of bacteriocins with phylogroups in porcine pathogens and human commensals separately and also associations of bacteriocins with *E. coli* origin within the phylogroups. We found associations of bacteriocins with phylogroups and these associations were different for porcine and human *E. coli* (for more details see Table S5). Out of 12 bacteriocins, which significantly differed between human and porcine isolates (Fig. 1A), five bacteriocins did not shown association with phylogroups (i.e., E2, K, mC7, mJ25, and mV) and other seven bacteriocin determinants showed association with the origin of isolates, and in few cases, associations with *E. coli* phylogroups. While determinants colJs, colIb, mH47, and mM were associated with origin of isolates (i.e., more frequent in pigs (colIb) or humans (colJs, mH47, and mM) regardless of phylogroups), prevalence of colB, colM, and mB17 determinants reflected also associations with *E. coli* phylogroups (Fig. S1 and Table S5).

3.3. Comparison of toxigenic and non-toxigenic E. coli isolates from diseased pigs

Based on the presence of four toxin determinants (i.e., *st*, *lt*, *stx*$_1$, and *stx*$_2$), the set of porcine *E. coli* isolates was classified into two groups: toxigenic and non-toxigenic isolates (n = 325 and n = 118, respectively).

While phylogroups A and D were abundant among the toxigenic isolates, the non-toxigenic *E. coli* isolates contained a significantly higher frequency of phylogroups B1 and B2 ($p < 0.001$, Fig. 1B).

Only four virulence determinants *fimA* (66.0%), *α-hly* (51.1%), *ehly* (0.3%), and *pap* (0.3%) were detected among the toxigenic *E. coli* and at the same time, the set of non-toxigenic *E. coli* isolates contained a significantly higher frequency of virulence determinants including *fimA*, *α-hly*, *ehly*, *pap*, *aer*, *cnf1*, *eae*, *iucC*, and *sfa*. Statistically significant differences in the frequency of virulence determinants are shown in Fig. 1B.

In both groups, the frequency of bacteriocinogeny was similar (58.5% for toxigenic and 65.3% for non-toxigenic, p = n.s.); however, the bacteriocin mono-producers among non-toxigenic *E. coli* were more abundant compared to the toxigenic isolates (54.5% and 32.1%, respectively; $p < 0.001$). In addition, non-toxigenic *E. coli* contained a significantly higher frequency ($p < 0.001$) of colicin E1 (52.0%) and microcin V (13.3%) determinants, while determinants for colicins B (53.7%), M (55.8%), and Ib (39.5%), and microcin H47 (9.5%, $p < 0.05$) were more frequent in the toxigenic isolates (Fig. 1B, Table S4).

3.4. Comparison of Shiga-toxigenic and enterotoxigenic E. coli isolates

Among the set of 325 toxigenic *E. coli* isolates, 91 isolates harbored genes for Shiga toxins, 203 isolates encoded enterotoxins, and 31 isolates were positive for both types of toxins and these isolates were omitted from a further comparison of STEC and ETEC isolates.

Phylogroup A was abundant in both group of isolates (61.5% for STEC and 68.3% for ETEC, p = n.s.); however, ETEC isolates had a significantly higher frequency of phylogroup B1 ($p < 0.05$) and STEC isolates had an elevated frequency of phylogroup D ($p = 0.01$, Fig. 1C).

Two virulence determinants were detected (Fig. 1C, Table S4), whereas *α-hly* was more frequent in the ETEC group (56.7%, $p < 0.01$) and *fimA* was typical for the STEC group (94.5%, $p < 0.001$).

ETEC and STEC isolates differed significantly in the frequency of bacteriocinogeny, which was more prevalent among ETEC isolates (71.4% and 31.9%, respectively; $p < 0.001$). The low number of bacteriocinogeny among STEC isolates resulted in a high frequency of mono-producers and lower frequency of double- and multi-producers compared to the ETEC group (Fig. 1C). Among ETEC isolates, colicins B and M were the most abundant (64.1.9% and 66.9%, respectively) and were significantly more frequent than in the STEC group ($p < 0.001$). In addition, STEC isolates frequently encoded colicins Ia and Ib (41.4% and 48.3%, respectively) and colicin Ia was detected significantly more often in the STEC group ($p < 0.001$, Fig. 1C and Table S4).

4. Discussion

In this study, 443 pathogenic porcine *E. coli* isolates, including 325 toxigenic isolates, were collected and analyzed. STEC and ETEC strains are important veterinary pathogens in swine breeding and intestinal infections caused by them result in increased pig mortality and in significant economic losses. In humans, ETEC cause diarrhea in developing countries and STEC cause sporadic and epidemic foodborne infections worldwide (Nataro and Kaper, 1998; Fairbrother et al., 2005; Tseng et al., 2014).

The pathogenic *E. coli* isolates from diseased pigs clearly differed from human fecal isolates. The majority of porcine isolates belonged to phylogenetic group A, while phylogroup B2, which is typical for human

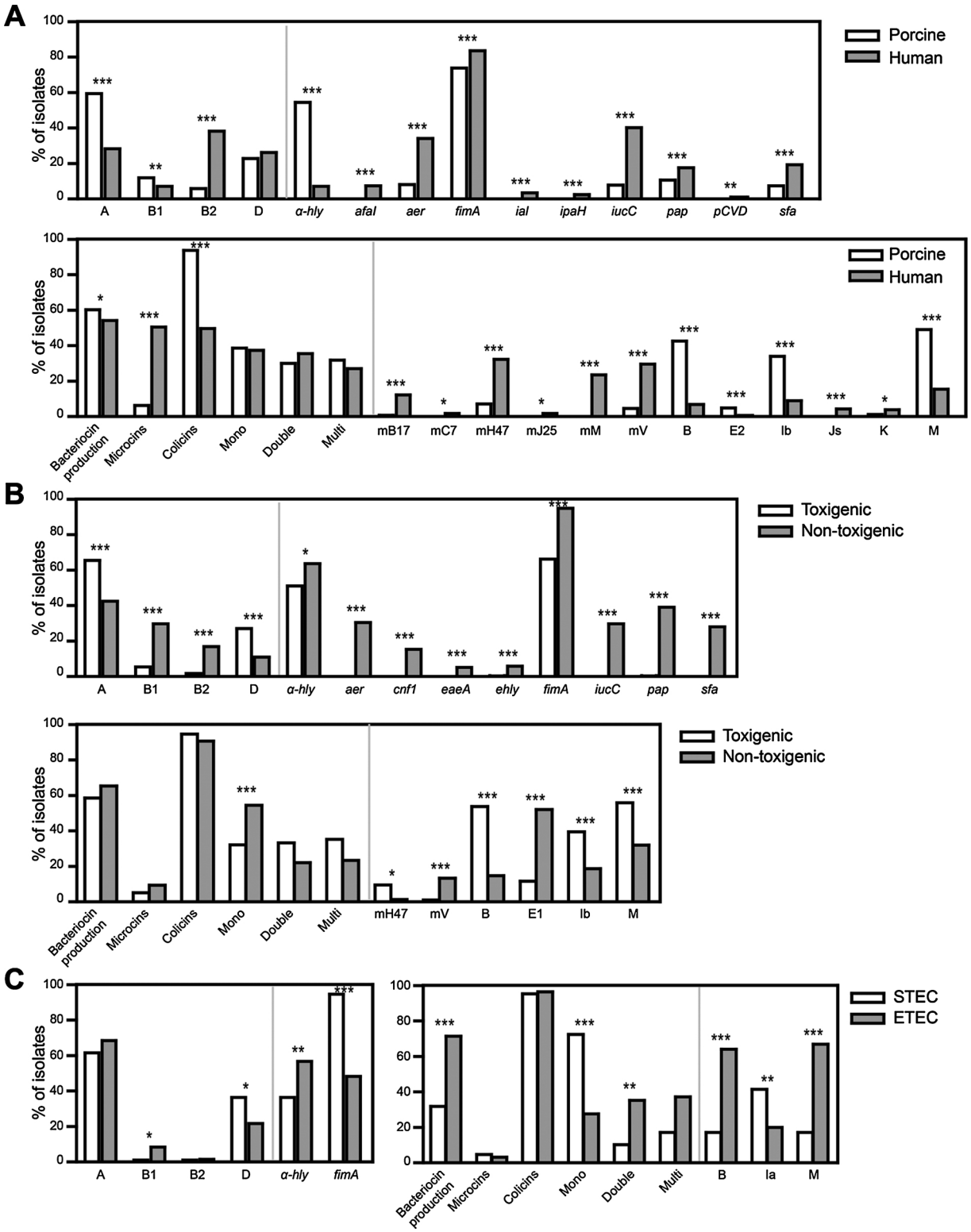

Fig. 1. Characterization of *E. coli* isolates originating from diseased pigs. The distribution of phylogroups, virulence- and bacteriocin genes was determined among porcine isolates (n = 443) and compared to human fecal *E. coli* isolates (n = 1283) **(A)**. Among porcine isolates, the prevalence of identified determinants were compared between toxigenic (n = 325) and non-toxigenic isolates (n = 118) **(B)** and between STEC (n = 91) and ETEC isolates (n = 203) **(C)**. For each comparison (A–C), the prevalence of phylogroups and virulence determinants is shown in the top panel, while the prevalence of bacteriocinogeny and distribution of bacteriocin types among bacteriocin producers is shown in the bottom panel. Bacteriocinogenic isolates were classified as mono-producers, double-producers, and multi-producers. In case of individual virulence- and bacteriocin determinants, only statistically significant differences are shown (for all results see Table S4). The two-tailed Fisher's exact test was used for calculation of statistical significance (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

isolates (Escobar-Páramo et al., 2006; Micenková et al., 2016a), was less frequent. The abundance of group A among porcine isolates has also been observed in several other studies (Ding et al., 2012; Šmajs et al., 2012). Additionally, the majority of ExPEC infections in humans have been found to be caused by strains belonging to groups B2 and D (Micenková et al., 2017), while InPEC infections in mammals have been found to be mostly caused by strains related to groups A and B1, and to a lesser extent by group D strains (Rolland et al., 1998).

Using revisited phylotyping method (Clermont et al., 2013), we revealed that 10.2% of porcine isolates belonged to new phylogroups C (6.8%), E (2.0%), F (0.2%), and clade I (1.1%) (Table S1). This is in accordance with the work of Clermont et al. (2013) and our previous study (Micenková et al. 2016a), where 13% of human fecal isolates belonged to new phylogroups. This suggests that additional typing to newly described phylogroups would result in no or in limited alteration of results with respect to bacteriocinogeny and distribution of bacteriocin determinants in phylogroups.

In this study, porcine and human isolates differed significantly relative to the spectrum of detected virulence determinants, but the average virulence score was similar for both groups (i.e., 2.9 and 2.2 virulence determinants per isolate, respectively; calculated from porcine nonselected isolates obtained from University of Veterinary and Pharmaceutical Sciences Brno). While the virulence score of ExPEC isolated from various sources ranged from 3.6 to 4.3 (Micenková et al. 2017), the value of the virulence score for IBD-derived *E. coli* strains was 2.7 which was almost identical with that observed in our set of porcine pathogens.

Among *E. coli* isolates from diseased pigs, we found a high prevalence of α-hemolysin, which was found to enhance the virulence and colonization capacity of porcine diarrheal strains (Elliott et al., 1998). On the other hand, they have rarely been found to encode virulence determinants, such as genes for adhesion or for iron acquisition systems, which are typical for human intestinal and extraintestinal *E. coli* isolates (Micenková et al., 2014, 2016b).

As documented in many studies, about a half of human fecal *E. coli* isolates encode bacteriocins (Šmarda and Obdržálek, 2001). In this study, bacteriocinogeny of porcine isolates was slightly higher (60.2%; $p < 0.05$) compared to human fecal isolates (54%). Abraham et al. (2014) found a similar rate of bacteriocinogeny (65.7%) among porcine ETEC isolates in Australia. Other studies have shown bacteriocinogeny to be more frequent among pathogenic than non-pathogenic *E. coli* strains, and more frequent among veterinary isolates than human isolates (Karama et al., 2009; Micenková et al., 2016b).

The spectrum of bacteriocin types encoded by porcine isolates was completely different from those encoded by human fecal *E. coli* isolates. While human fecal isolates produced colicins and microcins with a similar prevalence, porcine pathogenic *E. coli* isolates predominantly produced colicins (94%) and only rarely microcins (6%). Higher numbers of colicin determinants have also been found among isolates from healthy (Abraham et al., 2012; Šmajs et al., 2012) and diseased pigs (Abraham et al., 2014). On the other hand, a higher prevalence of microcin determinants was found among human pathogenic isolates, especially ExPEC strains (60%) (Micenková et al., 2016b, 2017). In addition, these findings are in accordance with Micenková et al. (2016a), who found that the production of microcins was associated with the B2 phylogroup and colicins were more frequent among phylogroups A and B1.

In this study, determinants for colicins B, M, Ib, and E2 were significantly more frequent among fecal isolates from diseased pigs compared to normal fecal isolates from healthy humans. These colicins were previously described to be common among animals (Christenson and Gordon, 2009; Abraham et al., 2012; Šmajs et al., 2012). Moreover, colicins B, M, Ia/Ib, E3, and E7 were found to be common among porcine ETEC isolates from Australia (Abraham et al., 2014). On the other hand, colicin E1 and microcins H47 and V have been found frequently among healthy control pigs (Abraham et al., 2012; Šmajs et al., 2012).

In addition, we revealed associations of bacteriocins with phylogroups, and these associations differed within human and porcine isolates. For the most bacteriocins, the differences in the prevalence of bacteriocin determinants between human and animal strains corresponded to origin of samples rather than to *E. coli* phylogroup. Colicins B and M were significantly more frequent among pathogenic porcine isolates belonging to phylogroup A, and at the same time, colicin M and microcin B17 were associated with phylogroup B2 regardless of *E. coli* origin. The associations of bacteriocins with *E. coli* phylogroups were previously described for human commensal isolates (Micenková et al., 2016a), especially production of microcins H47 and M in B2 phylogroup.

Among porcine isolates, we found 118 non-toxigenic isolates. They belonged to phylogroups A and B1, and harbored genetic determinants typical for the human fecal microflora. For example, they encoded iron transports (*aer*, *iucC*), fimbriae (*sfa*, *pap*), and cytotoxic necrotizing factor (*cnf1*). These isolates were also associated with colicin E1 and microcin V, which have been previously associated with human ExPEC strains (Azpiroz et al., 2009; Šmajs et al., 2010; Budič et al., 2011). In general, non-toxigenic porcine isolates were more similar to human commensal *E. coli* strains compared to toxigenic porcine strains.

Porcine associated colicins B, M, and Ib belong to group B colicins (Cascales et al., 2007), which are encoded on large conjugative plasmids (> 50 kb, with a one or few copies per cell), containing additional virulence genes. For example, plasmids encoding colicin B harbored an average of 7 additional virulence factors, including genes for iron transport, genes encoding hemolysins, genes involved in the overproduction of outer membrane vesicles, serum survival genes, etc. (Christenson and Gordon, 2009; Micenková et al., 2018). The observed association between group B colicins and veterinary isolates could thus reflect an association between certain groups of large plasmids and toxigenic porcine isolates.

Contrary to the described differences between porcine pathogenic isolates and human fecal and ExPEC isolates (Fig. 2), colicins from group B have also been associated with fecal isolates of patients with inflammatory bowel diseases (IBD) (Micenková et al., 2018). Although the biological relevance of this finding is not clear, it could suggest the potential involvement of these large plasmids (and the relevant encoded genes) in the development of IBD.

Colicin gene expression has been shown to be tightly regulated by the SOS-response and iron-limitation (Gillor et al., 2008; Bosák et al., 2018), which are often associated with dysbiosis and gut inflammation. For example, Nedialkova et al. (2014) showed that colicin Ib synthesis and activity is increased under gut inflammatory conditions. Since infections with toxigenic *E. coli* in pigs as well as human IBD display inflammation (Fairbrother et al., 2005; Dubreuil, 2017; Man, 2018), the observed high prevalence of inflammatory-controlled colicins from group B suggests the importance of colicins in these intestinal conditions.

5. Conclusion

Characterization of genetic determinants among *E. coli* isolates from diseased pigs revealed that porcine pathogenic *E. coli* strains differ from human strains relative to the prevalence of phylogroup-, virulence-, and bacteriocin determinants. Most importantly, porcine pathogens frequently encoded colicins, especially colicins B, M, and Ib, which are encoded on large conjugative plasmids. The observed high prevalence of these colicin determinants suggests the importance of large colicinogenic plasmids and/or even the importance of colicin production in intestinal inflammatory conditions.

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	A	B1	B2	D	α-hly	afaI	aer	cnf1	fimA	iaI	iucC	pap	sfa
Porcine (n=443)	59	12	6	23	54	0	8	4	74	0	8	11	7
Human (n=1283)	28	7	38	26	7	7	34	4	84	3	40	18	19
ExPEC (n=721)	14	8	56	20	16	12	65	9	93	1	65	36	27
IBD (n=178)	24	4	37	30	10	5	43	11	91	9	46	28	19

	Bacteriocinogeny	mB17	mH47	mM	mV	B	E1	Ia	Ib	K	M	Microcins	Colicins	Mono producers	Double producers	Multi producers
Porcine (n=443)	60	1	7	0	5	43	23	26	34	1	49	6	94	39	30	32
Human (n=1283)	54	12	32	23	30	7	20	30	9	4	15	50	50	37	36	27
ExPEC (n=721)	60	5	37	41	26	5	16	29	4	6	11	58	42	32	49	19
IBD (n=178)	67	8	24	24	23	23	13	35	12	3	18	44	56	38	41	21

Fig. 2. Comparison of prevalence (%) of genetic determinants among porcine and human isolates. Sets of *E. coli* isolates from pathogenic conditions (i.e., porcine, ExPEC, and IBD) were compared to a set of human fecal commensals. Compared to the prevalence among human commensals (grey), higher prevalence among pathogens is shown as a darker color and lower prevalence is shown as a lighter color. In addition to set of porcine pathogens, previously published data sets from Micenková et al. (2016a, 2016b, 2017, 2018) were used for comparisons. Because of the set of isolates from hemocultures (Micenková et al., 2017) was highly similar to the set of other ExPEC isolates (Micenková et al., 2016b), both sets were combined for this analysis. In addition, only determinants with prevalence higher than 5% (in one or more sets) are shown.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.04.003.

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