Determinants encoding fimbriae type 1 in fecal Escherichia coli are associated with increased frequency of bacteriocinogeny

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Abstract

Background: To screen whether E. coli strains encoding type 1 fimbriae, isolated from fecal microflora, produce bacteriocins more often relative to fimA-negative E. coli strains of similar origin.

Methods: PCR assays were used to detect presence of genes encoding 30 bacteriocin determinants (23 colicin- and 7 microcin-encoding genes) and 18 virulence determinants in 579 E. coli strains of human and animal origin isolated from hospitals and animal facilities in the Czech and Slovak Republic. E. coli strains were also classified into phylogroups (A, B1, B2 and D).

Results: fimA-negative E. coli strains (defined as those possessing none of the 18 tested virulence determinants) were compared to fimA-positive E. coli strains (possessing fimA as the only detected virulence determinant). Strains with identified bacteriocin genes were more commonly found among fimA-positive E. coli strains (35.6 %) compared to fimA-negative E. coli strains (21.9 %, p < 0.01) and this was true for both colicin and microcin determinants (p = 0.02 and p < 0.01, respectively). In addition, an increased number of strains encoding colcin E1 were found among fimA-positive E. coli strains (p < 0.01).

Conclusions: fimA-positive E. coli strains produced bacteriocins (colicins and microcins) more often compared to fimA-negative strains of similar origin. Since type 1 fimbriae of E. coli have been shown to mediate adhesion to epithelial host cells and help colonize the intestines, bacteriocin synthesis appears to be an additional feature of colonizing E. coli strains.

Keywords: Escherichia coli, Colicin, Microcin, Bacteriocin, Type 1 fimbriae, Phylogenetic group

Background

Escherichia coli (E. coli) is a common, variable, aerobic bacterial species that inhabits the gut of vertebrates [1]. Strains of E. coli differ in a number of important characteristics including genome size [2], gene content and virulence [3]. E. coli strains are classified into four phylogroups; E. coli strains of phylogroups A and B1 contain smaller genomes and are frequently non-pathogenic, while strains of phylogroups B2 and D encode more genes and are more often pathogenic [2, 4–6].

Approximately 80 % of all E. coli strains of fecal origin are able to produce type 1 fimbriae, which are encoded by the chromosomal fim operon [7]. This operon consists of fimAICDFGH genes [8], where fimA encodes the major fimbrial subunit (FimA). FimA is arranged in a helical manner along with minor components, one of which includes FimH (an adhesin that mediates attachment of type 1 fimbriae to mannose-containing receptors) [9]. E. coli type 1 fimbriae have been shown to mediate adhesion to a number of host cell types (e.g. epithelial and endothelial cells), thus potentially increasing virulence of fimA-positive E. coli strains [10].

E. coli strains are able to synthesize two types of bacteriocins - colicins and microcins. While microcins are low molecular weight oligopeptides, colicins are proteins...
with molecular weights between 30–70 kDa. 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. [11–13]. However, this classification is not strict, since colicin J1 [14, 15] is known to share features of both bacteriocin types. Several bacteriocins (i.e. colicins E1, E2, and S4, and microcins B17, E492, H47, I47, M and V) have been found to be associated with virulence factors (i.e. with aer, cnf1, fyuA, hlyA, iroCDN, iucC, papCG, sfa, tcpC and usp determinants) in E. coli strains [16–20].

Previous studies have found associations between several genes encoding bacteriocin types and several virulence determinants, however, no association between type 1 fimbrae and bacteriocin genes has been identified [16–20]. The role of fimbrae type 1 in the E. coli virulence is not clear [21, 22]. This situation is likely a result of very frequent presence of type 1 fimbrae among E. coli strains (over 80 %) and the fact that in most E. coli strains the type 1 fimbrae are combined with other virulence determinants. Therefore we collected a set of fecal E. coli strains encoding type 1 fimbrae as the only detected virulence determinant (out of 18 tested) and a set of E. coli strains with no virulence determinants (out of 18 tested).

In this communication, we studied prevalence of bacteriocin production and prevalence of bacteriocin types in both sets of E. coli strains to assess association of fimbrae type 1 encoding genes and bacteriocin determinants in E. coli strains. In addition, we also tested association between type 1 fimbrae determinants and other factors including biochemical profiles and E. coli phylogroups.

Results

Characteristics of E. coli strains

Fecal E. coli strains used in this study (n = 579) (Fig. 1) either tested negative for all 18 virulence determinants (pCVD432, α-hly, afal, aer, cnf1, sfa, pap, ial, lt, st, bfpA, eaeA, ipaH, iucA, fimA, stx1, stx2 and ehy) and were, therefore, considered fimA-negative, or the strains were fimA-positive, while still testing negative for all other virulence determinants. To assess presence of other genes of the fimA cluster, a fimH determinant, encoding adhesin mediating attachment of type 1 fimbrae, was tested on all fimA-positive strains. Except of 10 isolates, fimA-positive isolates were also positive for fimH determinants (98.3 % showed both determinants for type 1 fimbrae). Within groups of strains with different origins (Table 1), a relatively small proportion (≈17 %) of E. coli strains had no detected virulence determinants. Therefore, all strains were analyzed without regard for their origin.

Biochemical analysis

E. coli isolates (n = 579) were positive for the following biochemical reactions: TRE (99.1 %), MAN (99.0 %), SOR (97.8 %), ONP (97.6 %), IND (97.4 %), LYS (93.6 %), SUC (51.8 %), ORN (50.4 %), ESL (14.2 %), ADO (8.8 %), H2S (3.6 %), CEL (2.2 %), SCI (2.1 %), MAL (1.0 %), URE (0.7 %), INO (0.7 %) and PHE (0.2 %). The utilization of substrate showed variable results in ORN and SUC reactions between fimA-positive and fimA-negative E. coli strains. Biochemical analysis showed that the production of ornithine decarboxylase was significantly higher among fimA-positive E. coli strains (n = 254; 52.6 %) compared to fimA-negative strains (n = 38; 39.6 %, p < 0.03). In addition, production of succinate dehydrogenase was significantly higher among fimA-positive E. coli strains (n = 269; 55.7 %) compared to fimA-negative E. coli strains (n = 31; 32.3 %, p < 0.01).

Detection of phylogenetic groups in E. coli strains

Phylogenetic analysis of 579 E. coli strains revealed that fimA-negative E. coli strains contained a significantly higher prevalence of phylogenetic group A (66.7 %) compared to fimA-positive E. coli strains (39.1 %, p < 0.01). Phylogroups B1 and B2 were found less frequently among fimA-negative E. coli strains (p = 0.01 and p = 0.04, respectively), while the prevalence of phylogroup D was similar in both groups of E. coli strains (Table 2). Frequency of bacteriocin production in E. coli strains belonging to phylogenetic groups A, B1, B2 and D, respectively, was not significantly different between fimA-positive and fimA-negative E. coli strains (Additional file 1: Table S1).

Detection of bacteriocin-encoding determinants

Genetic determinants encoding 30 bacteriocin types including 23 colicins (A, B, D, E1, E2-9, Ia, Ib, Js, K, L, M, N, S4, U, Y and 5/10) and 7 microcins (H47, M, B17, C7, J25, L and V) were tested in all 579 E. coli strains used
in this study. Strains with identified bacteriocin genes were more frequently found among fimA-positive E. coli strains (35.6 %) compared to fimA-negative E. coli strains (21.9 %, \( p < 0.01 \)). Altogether, 3 microcin types and 8 colicin types were identified among fimA-negative E. coli strains while all 7 tested microcin types and 14 of the colicin types were found among fimA-positive E. coli strains (Tables 2 and 3). All of the identified bacteriocin determinants found among fimA-negative E. coli strains were also found among the fimA-positive E. coli strains.

A higher number of microcin determinants was found among fimA-positive E. coli strains (22.4 %) compared to fimA-negative E. coli strains (9.4 %; \( p < 0.01 \)). Similarly, a higher number of detected colicin determinants was also found among fimA-positive E. coli strains (38.7 %) compared to fimA-negative E. coli strains (26.0 %; \( p = 0.02 \)). In addition, an increased number of strains encoding colicin E1 was found among fimA-positive E. coli strains (8.1 %) compared to fimA-negative E. coli strains (1.0 %; \( p < 0.01 \)).

In silico analysis of E. coli genomes

A set of 1951 publicly available E. coli genomes including 121 completed genomes in the NCBI database was analyzed for the presence of virulence determinants tested in our study. Out of 1951 genomes, 490 genomes (25 %) met the criteria of our study (i.e. absence of tested virulence determinants or the sole presence of fimA determinant). In this set, 286 (58 %) genomes contained type 1 fimbriae as the only detected virulence determinant (fimA-positive E. coli strains) and 204 (42 %) genomes did not contain any of the 18 tested virulence factors (fimA-negative E. coli strains). No significant difference was found in the prevalence of bacteriocin determinants in the group of fimA-positive E. coli strains (15.4 %) compared to fimA-negative E. coli strains (14.7 %).

In addition, the subset of 121 complete genomes was analyzed. Out of them, 64 genomes were suitable for our study (i.e. contained no tested virulence determinants or contained only fimA determinant). While 50 (78 %) genomes belonged to fimA-positive group, 14 (22 %) genomes were fimA-negative. Bacteriocin genes were detected in only three fimA-positive genomes.

Discussion

The fimA-negative E. coli, as well as fimbriae type I-possessing E. coli strains, used in this study were found to have similar frequencies in both humans and animals. A relatively small proportion (\( \approx 17 \) %) of E. coli strains had no detected virulence factors. These results are in accordance with other published data where 83 – 100 % of fimA-positive E. coli strains were found [7, 23]. Detection of both fimA and fimH determinants in majority of strains suggests that the complete fimA cluster is present in most of the tested strains.

In silico analysis of 121 complete genomes identified fimA determinant in 78 % of E. coli complete genomes. This finding is in accordance to previous studies [7, 23] and also with the experimental results of this work, where fimA virulence determinant was identified in more than 80 % of isolates. On the other hand, the fimA determinant was identified only in 25 % of 1830 draft genomes suggesting that the fimA determinant likely remained unsequenced in a number of draft genomes.

### Table 1

| Origin of E. coli strains | fimA-negative E. coli strains (\( n = 96 \)) | fimA-positive E. coli strains (\( n = 483 \)) | Total |
|--------------------------|---------------------------------------------|---------------------------------------------|-------|
| University hospitals Brno | 63 (15.8 %) | 336 (84.2 %) | 399 (100 %) |
| University teaching hospital Hradec Králové | 9 (20.5 %) | 35 (79.5 %) | 44 (100 %) |
| Strains isolated from pigs | 18 (18.0 %) | 82 (82.0 %) | 100 (100 %) |
| Strains isolated from non-human primates | 6 (16.7 %) | 30 (83.3 %) | 36 (100 %) |
| Total | 96 (16.7 %) | 483 (83.4 %) | 579 (100 %) |

### Table 2

| Prevalence of phylogroups and bacteriocin determinants | fimA-negative E. coli strains (\( n = 96 \)) | fimA-positive E. coli strains (\( n = 483 \)) | \( p \)-value |
|--------------------------------------------------------|---------------------------------------------|---------------------------------------------|--------------|
| Phylogroup A                                            | 64 (66.7 %) | 189 (39.1 %) | \( p < 0.01 \) |
| Phylogroup B1                                           | 18 (8.3 %)  | 92 (19.0 %)  | \( p = 0.01 \) |
| Phylogroup B2                                           | 9 (9.4 %)   | 89 (18.4 %)  | \( p = 0.04 \) |
| Phylogroup D                                            | 15 (15.6 %) | 113 (23.4 %) | - |
| Bacteriocinogeny                                        | 21 (21.9 %) | 172 (35.6 %) | \( p < 0.01 \) |
| Microcin determinants                                   | 9 (9.4 %)   | 108 (22.4 %) | \( p < 0.01 \) |
| Colicin determinants                                    | 25 (26.0 %) | 187 (38.7 %) | \( p = 0.02 \) |
| Colicin E1 determinants                                 | 1 (1.0 %)   | 39 (8.1 %)   | \( p < 0.01 \) |
strains were in fact due to differences in the urine infections and deletion of E. coli 4 (Phylogenetic Using Analysis Parsimony). Using et al. BMC Microbiology gene cluster was not correlated with delty used probiotic strain, gene cluster from the virulent H31), which has been ap-

strains without a isolates in up B2 (and also D) consists 

ess type 1 fimbriae as well phylogroups. Gordon Distribution of bacteriocin encoding genes among isolates and fimbriae type I-possessing E. coli strains are usually in 

nterestingly, our set of fimA strains, it is 

phylogroups [40]. isolates where over 50 % of 

fimA strains produced bacteriocins [17, 24]. These findings are in agreement with the observa-

tion that non-pathogenic E. coli strains are usually in phylogroup A and B1 [1, 39]. Interestingly, our set of strains also contained strains from group B2 (17 %). Moreover, no virulence factors were detected in of the 9 strains from this phylogroup, which indicates that the relationship between E. coli phylogroup and the presence of virulence factors is not exclusive.

Since prevalence of phylogroups among fimA-negative strains differed from fimA-positive E. coli strains, it is possible that the observed differences in the prevalence of bacteriocin determinants between both groups of E. coli strains were in fact due to differences in the E. coli phylogroups. However, there is no clear association between bacteriocinogeny and E. coli phylogroups. Gordon and O’Brien (2006) detected 4 phylogenetic groups and 19 bacteriocin types in a set of 266 fecal E. coli strains and did not find significant differences in the frequency of bacteriocinogeny in different E. coli phylogroups [40].

Table 3 Distribution of bacteriocin encoding genes among fimA-negative and fimA-positive E. coli strains

| Bacteriocin types | fimA-negative E. coli (%) (n = 96) | fimA-positive E. coli (%) (n = 483) |
|------------------|----------------------------------|-----------------------------------|
| Colicin A        | -                                | -                                 |
| Colicin B        | 2 (2.1)                          | 13 (2.7)                          |
| Colicin D        | -                                | -                                 |
| Colicin E1       | 1 (1.0)                          | 39 (8.1)                          |
| Colicin E2       | -                                | 1 (0.2)                           |
| Colicin E3       | -                                | -                                 |
| Colicin E4       | -                                | -                                 |
| Colicin E5       | -                                | -                                 |
| Colicin E6       | -                                | -                                 |
| Colicin E7       | 2 (2.1)                          | 2 (0.4)                           |
| Colicin E8       | -                                | 1 (0.2)                           |
| Colicin E9       | -                                | -                                 |
| Colicin Ia       | 10 (10.4)                        | 56 (11.6)                         |
| Colicin Ib       | 2 (2.1)                          | 32 (6.6)                          |
| Colicin K        | 1 (1.0)                          | 3 (0.6)                           |
| Colicin L        | -                                | -                                 |
| Colicin M        | 6 (6.3)                          | 30 (6.2)                          |
| Colicin N        | -                                | 2 (0.4)                           |
| Colicin S4       | -                                | 2 (0.4)                           |
| Colicin U        | -                                | -                                 |
| Colicin Y        | -                                | 2 (0.4)                           |
| Colicin 5/10     | -                                | 1 (0.2)                           |
| Colicin Js       | 1 (1.0)                          | 3 (0.6)                           |
| Microcin B17     | -                                | 11 (2.3)                          |
| Microcin C7      | -                                | 2 (0.4)                           |
| Microcin H47     | 5 (5.2)                          | 42 (8.7)                          |
| Microcin J25     | -                                | 1 (0.2)                           |
| Microcin L       | -                                | 1 (0.2)                           |
| Microcin M       | 1 (1.0)                          | 33 (6.8)                          |
| Microcin V       | 3 (3.1)                          | 18 (3.7)                          |

Similar underrepresentation was found also for bacteriocin determinants (24 % and 38 % of all complete and draft genomes contained bacteriocin determinants, respectively; data not shown). This is in contrast to experimentally determined prevalence of bacteriocinogeny among human E. coli isolates where over 50 % of E. coli strains produced bacteriocins [17, 24]. These findings suggest that in silico analysis of draft genomes is of lim-

ited value in this and similar studies.

To assess clonal character of isolates, the obtained data from biochemical screening and analysis of phylogenetic groups and bacteriocin determinants were analyzed using Paup* 4 (Phylogenetic Using Analysis Parsimony). Using this approach, 52 and 226 individual strain types (data not shown) were identified in the groups of 96 and 483 fimA-negative E. coli isolates and fimA-positive E. coli isolates, respectively, indicating that E. coli isolates in this study were not predominantly of clonal character.

E. coli type 1 fimbriae mediate adhesion to a number of host cell types including epithelial, endothelial and lymphoid cells [10, 25, 26], where they recognize mannoscontaining glycoproteins and activate epithelial cells via Toll-like receptor 4 [27]. Type 1 fimbriae are expressed by both uropathogenic and fecal E. coli strains. In murine models, type 1 fimbriae have been shown to be important in the persistence of E. coli urine infections and deletion of the fim gene cluster from the virulent E. coli strain O1:K1:H7 has been shown to decrease the virulence of this strain in the urinary tract infection model [22, 28]. However, several other studies have demonstrated that the presence of the fim gene cluster was not correlated with uropathogenicity in humans [21, 29–33]. Additionally, E. coli strain A0 34/86 (O83:K24:H31), which has been approved as live oral vaccine preparation for infants in the Czech and Slovak Republic, was shown to possess type 1 fimbriae [34, 35]. Another widely used probiotic strain, E. coli Nissle 1917, is known to possess type 1 fimbriae as well as other adhesins (e.g. F1C fimbriae) [36, 37]. These examples demonstrate that type 1 fimbriae are primarily important for attachment to eukaryotic cells and could be, in certain strains, important also with regard to virulence.

While phylogroup A (and also B1) consists mostly of commensal strains, phylogroup B2 (and also D) consists mainly of extraintestinal pathogenic E. coli strains [4–6, 38]. The majority of tested strains in this study (61 %) belonged to A and B1 phylogenetic groups, which was the direct result of sampling E. coli strains without a specific set of virulence factors or E. coli strains harboring fimA determinant as the only detected virulence gene. These findings are in agreement with the observation that non-pathogenic E. coli strains are usually in phylogroup A and B1 [1, 39]. Interestingly, our set of strains also contained strains from group B2 (17 %). Moreover, no virulence factors were detected in of the 9 strains from this phylogroup, which indicates that the relationship between E. coli phylogroup and the presence of virulence factors is not exclusive.
In our previous study, we have found that prevalence of colicinogenic strains was higher in phylogroups A and D compared to phylogroups B2 [17]. In contrast, the study of Budić et al. (2011) revealed increased bacteriocinogeny in the phylogroup B2 among 105 uropathogenic strains [18]. In this study, no differences in the prevalence of bacteriocinogeny were found among fimA-negative *E. coli*, while an increased bacteriocinogeny was found in the phylogenetic group B2 compared to phylogenetic group A in the set of fimA-positive *E. coli* strains. Although more frequent phylogenetic group B2 could be the reason of increased prevalence of bacteriocinogeny among fimA-positive *E. coli* strains, increased prevalence of bacteriocin genes were found in all tested *E. coli* phylogroups (statistically not significant; Additional file 1: Table S1), suggesting the association between bacteriocinogeny and the fimA gene cluster.

In humans, two types of commensal *E. coli* strains (resident and transient) are known to exist. They differ in their ability to persist in the human intestine. While resident strains are present in the intestines of an individual for months at a time, transient strains only persist for days to weeks [41–43]. In addition, it has been shown that the ability of *E. coli* strains to persist in the human intestines is associated with several virulence factors, especially various fimbriae [44, 45]. Since the *E. coli* strains in phylogroup B2 are typical for resident flora [39, 46, 47] and *E. coli* of phylogroup A is typical for transient strains [39] the fimA-negative *E. coli* isolates in this study appear to be more frequently transient strains.

The fimA-positive *E. coli* strains were more often positive for activity of ornithine decarboxylase compared to fimA-negative *E. coli*. Activity of ornithine decarboxylase, which results in production of polyamines (e.g. putrescine), helps to cope with stress conditions, such as oxidative radicals [48] and low pH [49]. In addition, polyamines play an important role in biofilm formation [50]. There is a relationship between cellular adherence and biofilm formation in certain strains of *E. coli* [51].

This study has shown that fecal fimA-positive *E. coli* strains produced bacteriocins more often compared to similar, but fimA-negative, strains. Bacteriocin synthesis appears to be important in microbial communities because of its potential invasive and defensive roles [52]. Moreover, antimicrobial effect of individual bacteriocin types showed differences with respect to their activity on *E. coli* strains [18]. In previous studies, the occurrence of several bacteriocin genes was found to be associated with several genes encoding virulence factors [16–20] and the results of this study extend the original findings. Bacteriocin types and their sequences have been shown to be host population-specific [53], indicating that bacteriocin-encoding determinants mainly spread among and within hosts. Since virulence genes likely evolved and are being maintained to improve inter-host persistence of commensal bacteria [54, 55], bacteriocin synthesis may further promote stable colonization of the gut. Similar findings were published by Gillor et al. 2009 [56], in which they reported that bacteriocinogeny plays a significant role in the colonization of *E. coli* in the intestinal tract. As with type 1 fimbriae, which were shown to increase virulence in the urinary tract infection model [26, 27], synthesis of colicin E1 was found to be associated with uropathogenic strains [17].

Conclusions

In summary, fimA-positive *E. coli* strains of animal origin were isolated more often posi-tive for ornithine decarboxylase, succinate de-hydrogenase and bacteriocin synthesis more frequently than fimA-negative *E. coli* strains. All these findings are consistent with increased adherence to intestinal epithelium, increased bacterial virulence, and increased ability to survive in the intestine.

Methods

Bacterial strains

The origins of *E. coli* strains used in this study are shown in Table 1. *E. coli* strains were collected between 2007 and 2012 from intestinal microflora of patients at two University Hospitals in Brno (*n* = 399) and one University Teaching Hospital in Hradec Králové (*n* = 44), Czech Republic. Strains were collected from feces of patients without bacterial gut infection. The patients were admitted for a number of concerns including infectious and parasitic diseases (*n* = 165); neoplasms (*n* = 60); blood diseases (*n* = 2); endocrine, nutritional and metabolic diseases (*n* = 42); mental and behavioral disorders (*n* = 4); diseases of the nervous system (*n* = 5); diseases of the circulatory system (*n* = 7); diseases of the respiratory system (*n* = 4); diseases of the digestive system (*n* = 73); diseases of the skin and subcutaneous tissue (*n* = 5); diseases of the musculoskeletal system and connective tissue (*n* = 3); diseases of the genitourinary system (*n* = 6); symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (*n* = 30); injury, poisoning and certain other consequences of external causes (*n* = 5); and factors influencing health status and contact with health services (*n* = 32). An International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10)-2015-WHO Version for 2015, was used for the classification of diseases. In addition, fecal *E. coli* strains of animal origin were isolated from pigs (*n* = 100) and non-human primates (NHP) (*n* = 36). The animal isolates were included into the study because of their availability and because there was no statistically significant difference in the ratio of fimA-negative to fimA-positive strains between human and animal *E. coli*.
strains. *E. coli* strains from pigs were isolated during 2010–2012 in Hradec Králové [57, 58]. *E. coli* strains isolated from NHP feces were collected in 2012 from 7 zoological gardens in the Czech and Slovak Republic (Zoological Garden Hodonin (48°51′52.06′′N, 17°6′24.52′′E), Zoological Garden Jihlava (49°23′51.834′′N, 15°35′57.872′′E), Zoological Garden Košice (48°47′00.8′′N, 21°12′13.6′′E), Zoological Garden Liberec (50°46′34.038′′N, 15°4′32.655′′E), Zoological and Botanical Garden Přešov (49°45′27.85′′N, 13°21′35.90′′E), The Prague Zoological Garden (50°7′0.099′′N, 14°24′39.676′′E) and Zoological Garden Zlín - Lešná (49°16′20.048′′N, 17°42′54.118′′E)). From each patient or animal, a single *E. coli* strain was isolated using selective diagnostic ENDO agar. Metabolic profiles of isolates were obtained during determination of *E. coli* among isolates using commercial screening kit EnteroTest 16 (test for the presence of several metabolic reactions (H₂S, LYS, IND, ORN, URE, PHE, ESI, SCI, MAL, INO, ADO, CEL, SUC, SOR, TRE and MAN) (Lachema, Brno, CZ) and ONP test for detection of β-galactosidase (Lachema, Brno, CZ). The obtained metabolic profiles were compared with the database (TNW ProAuto 7 software) for classification of isolates.

All human data used in the study were anonymized and the study was approved by the Joint Ethical Committee (Charles University in Prague, Faculty of Medicine at Hradec Králové & University Teaching Hospital Hradec Králové) and the ethics committee of the Faculty of Medicine, Masaryk University, Czech Republic. All clinical samples were collected after patients gave written informed consent for participation in the study and for their samples to be used for research. For children under the age of 18, consent was obtained from parents. The animal part of the study (i.e. *E. coli* strains isolated from pigs) was approved by the Institutional Review Board of the Animal Care Committee of the Institute of Experimental Biopharmaceutics, Academy of Sciences of the Czech Republic, Record Number 1492006. NHP fecal samples were collected after presentation of a preliminary research plan that specified the agreement between particular ZOO zoologists or veterinarians. We obtained all the required permits needed to collect the samples, which were collected during routine cages cleaning, without direct contact or interaction with animals.

**Detection of virulence determinants**

*E. coli* strains were tested for the presence of 18 virulence determinants (α-hly, afal, aer, cnf1, sfa, pap, pCVD432, ial, lt, st, bfpA, eaeA, ipaH, iucC, fimA, stx1, stx2 and ehly). Primer pair sequences and PCR product lengths are shown in Additional file 2: Table S2; the PCR protocols were previously described [59–67]. Positive controls for detection of virulence genes were taken from the laboratory stock and comprised following strains: *E. coli* B2917 (pCVD432), *E. coli* B3428 (α-hly), *E. coli* B3406 (afal), *E. coli* B3427 (aer), *E. coli* B3410 (cnf1), *E. coli* B3418 (sfa), *E. coli* B3406 (pap), *E. coli* B3430 (ial), *E. coli* B2541 (st), *E. coli* B2802 (lt), *E. coli* B1804 (bfpA), *E. coli* B2905 (eaeA), *E. coli* B2987 (ipaH), *E. coli* B3411 (iucC), *E. coli* B3404 (aer), *E. coli* B3423 (fimA) and *E. coli* B2871 (ehly). To assess presence of other genes of the *fimA* cluster, a *fimH* determinant, encoding adhesin mediating attachment of type 1 fimbriae, was tested on a set of *fimA*-positive strains. *E. coli* B3423 strain was used as a positive control for *fimH* gene. *E. coli* strains with none of the 18 tested virulence determinants were used as control strains (i.e. *fimA*-negative *E. coli* strains), while the experimental strains consisted of *E. coli* strains encoding only fimbriae type I (i.e. *fimA*-positive *E. coli* strains).

**Detection of bacteriocinogeny and bacteriocin determinants**

*E. coli* strains were cultivated (37 °C for 48 h) in parallel on (i) TY agar and (ii) nutrient broth agar plates. The TY agar consisted of yeast extract (Hi-Media, Mumbai, India) 5 gl⁻¹, tryptone (Hi-Media) 8 gl⁻¹, sodium chloride 5 gl⁻¹, and a 1.5 % (w/v) of agar (Hi-Media). Nutrient broth agar contained a Nutrient Agar (HiMedia) 28 gl⁻¹. The bacteria were then killed using chloroform vapors and each plate was then overlaid with a thin layer of soft TY agar (0.7 %; w/v) containing 10⁷ cells ml⁻¹ of an indicator strain. The plates were then incubated at 37 °C overnight and bacteriocin producers were identified [17, 20]. Indicator strains *E. coli* K12-Row, C6 (ϕ), B1, P400, and *Shigella sonnei* 17 and *E. coli* S40 were used to detect bacteriocin production [17, 20]. The set of these strains is capable to detect all known colicin types and most of the microcin types.

Altogether, 30 bacteriocin types were detected among tested strains (23 colicin and 7 microcin genes) using methods previously described [17, 20, 40]. Isolated DNA (using DNAzol reagent, Invitrogen, Carlsbad, CA, according to the manufacturer’s protocol) was diluted 100-fold in sterile distilled water. Alternatively, one bacterial colony of each *E. coli* strain was resuspended in 100 µl of sterile distilled water and 1 µl of this suspension was added to the PCR mix. A list of primers is shown in Additional file 2: Table S2. Cycling conditions were 94 °C (2 min); 94 °C (30 s), 60 °C (30 s), 72 °C (1 min), 30 cycles; and 72 °C (7 min). For colony PCR, the initial step was set for 5 min. For identification of bacteriocin determinants among tested strains,
known bacteriocin producers were used as positive controls: *E. coli* BZB2101pColA - CA31, BZB2102 pColB - K260, BZB2103 pColD - CA23, BZB2107 pColE4 - CT9, BZB2108 pColE5 - 099, BZB2150 pColE6 - CT14, BZB2120 pColE7 - K317, BZB2279 pColLa - CA53, BZB2202 Colib - P9, BZB2116 pColK - K235, PAP1 pColM - BZB22, BZB2123 pColN - 284 (original source: A. P. Pugsley), *E. coli* 189BM pColE2 - P9 (B. A. D. Stocker), *E. coli* 385/80 pColE1, pColV (H. Lhotová), *E. coli* 185 M4 pColE3 - CA38 (P. Fredericq), *E. coli* W3110 pColE8, W3110 pColE9 (J. R. James), *E. coli* K-12 pColS4 (D. Šmajs), *S. boydii* M592 (serovar 8) pColU (V. Horák), *E. coli* K339 pColY (D. Friedman), *Shigella sonnei* (colicinotype 7) pColJs (J. Šmarda), *E. coli* pColA and *E. coli* pCol10 (H. Pilsl).

As microcin control producers, the following bacterial strains were used: *E. coli* 449/82 pColX (microcin B17); *E. coli* 313/66 pColG (microcin H47); *E. coli* 363/79 pColV (microcin V, original source: H. Lhotová); *E. coli* TOP10F' pDS601 (microcin C7); *E. coli* D55/1 (microcin J25); *E. coli* B1239 (microcin L, D. Šmajs). *E. coli* B3423 strain was used as a positive control for *fimH* gene detection. Because of sensitivity of microcins H47 and M to chloroform vapours, all *E. coli* strains were tested by PCR method for the presence of mH47 and mM genes [36]. PCR products of related bacteriocin types (colicins E2-9, Ia-Ib, U-Y) were sequenced using dideoxy-terminator sequencing with amplification primers. Sequence analyses were carried out using Lasergene software (DNASTAR, Inc., Madison, WI).

**Phylogenetic analysis of *E. coli* strains**

A previously described triplex PCR method [68] was used to assign *E. coli* strains to one of four main phylogenetic groups (A, B1, B2 and D).

**Statistical analyses**

The statistical analyses of the prevalence bacteriocin and phylogroups used standard methods derived from the binomial distribution, including the two-tailed Fisher’s exact test. STATISTICA software, version 8.0 (StatSoft, Tulsa, OK), was used for calculations.

**In silico analysis of *E. coli* genomes**

In total, 121 complete and 1830 draft genome sequences of *E. coli* strains were downloaded as FASTA files from ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/ and ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria_DRAFT/ NCBI public databases, respectively, using ncbi ftp download script (available at: https://github.com/aleimba/bac-genomics-scripts/). For determination of the presence/absence of virulence determinants and bacteriocin encoding genes in downloaded genome sequences, DNA comparison using Smith-Waterman algorithm [69], implemented in a cross-match software (unpublished) was used. Identity scores higher than 75% were used.

**Availability of supporting data**

The data set supporting the results of this article is included in the Additional file 1: Table S1. The data set of colicin gene sequences supporting the results of the article has been deposited in the GenBank/EMBL/DDBJ. Accession numbers for colicin sequences are shown in the Additional file 3: Table S3.

**Additional files**

Additional file 1: Table S1. Complete data set presented in this article. (DOCX 19 kb)

Additional file 2: Table S2. DNA primers used for PCR detection of colicin and microcin encoding genes and genes encoding virulence factors. (XLSX 167 kb)

Additional file 3: Table S3. Colicin gene sequences deposited in the GenBank/EMBL/DDBJ. (XLSX 19 kb)

**Abbreviations**

*E. coli*: Escherichia coli; TRE: Trehalose; MAN: Mannitol; SOR: Sorbitol; ONP: Beta-galatosidase; IND: Indole; LVS: Lysine; SUC: Sucrose; ORN: Ornithine; ESL: Esculin; ADO: Adonitol; HS: Hydrogen sulphide; CEL: Cellobiose; SCI: Simmons citrate; MAL: MAL; URE: Urease; NIQ: Inositol; PHE: Phenylalanine.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

DS designed the study and together with BS, LM and JB wrote the manuscript. BS, LM and JB performed bacteriocin and virulence testing of *E. coli* strains. DS and LM analyzed the data. KH, ES, MV, AS, DK, VW and JB contributed to isolation and characterization of the bacterial strains and gathered data. All authors read and approved the final manuscript.

**Authors’ information**

Not applicable.

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