Colicin insensitivity correlates with a higher prevalence of extraintestinal virulence factors among *Escherichia coli* isolates from skin and soft-tissue infections

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Colicins are toxic proteins with a narrow killing spectrum that are produced by colicinogenic *Escherichia coli* strains. The aim of this study was to analyse systematically whether extra-intestinal virulence potential is linked to colicin (in)sensitivity. In total, 102 well-characterized *E. coli* isolates from skin and soft-tissue infections (SSTIs) were exposed to 17 single-colicin-producing strains, and the correlation between insensitivity to colicin and phylogenetic group as well as the extra-intestinal virulence potential of the SSTI strains was examined. The results showed that SSTI strains belonging to the B2 phylogenetic group were statistically significantly associated with insensitivity to at least ten colicins, and several colicin insensitivities were correlated with virulence factors. As far as is known, this is the first study to report such correlations.

**INTRODUCTION**

*Escherichia coli* is a diverse bacterial species found naturally in the intestinal tract of humans and many other animal species. However, some strains are pathogenic and cause a wide variety of different intestinal as well as extra-intestinal diseases (Marrs *et al.*, 2005). Extra-intestinal pathogenic *E. coli* (ExPEC) isolates are a medically significant group of pathogens responsible for significant morbidity, mortality and cost to the healthcare system as a result of urinary tract infections (UTIs), diverse intra-abdominal infections, pneumonia, surgical-site infections, meningitis, osteomyelitis, skin and soft-tissue infections (SSTIs) and bacteremia (Russo & Johnson, 2006). As the causative agent of ~90% of all UTIs among ambulatory female patients (Johnson & Stamm, 1989), it is not surprising that the UTI subgroup of ExPEC has been studied the most extensively. Among SSTIs, *E. coli* is the third most prevalent species isolated (Moet *et al.*, 2007). Nevertheless, strains from these infections have not been characterized extensively. All ExPEC strains have a common phylogenetic background deriving typically from phylogenetic groups B2 and D, and share the same spectrum of virulence determinants (Russo & Johnson, 2000).

Colicins are narrow-killing-spectrum bacteriocins produced by *E. coli*. Each colicin is unique in its characteristics (see Table 1 for some examples) and they do not share the same killing efficiencies (Feldgarden & Riley, 1998). Whilst it has been shown that colicins prevent the growth of intestinal and uropathogenic *E. coli* isolates (Rijavec *et al.*, 2007; Schamberger *et al.*, 2004; Stahl *et al.*, 2004), a high prevalence of insensitivities to colicins among microbial populations isolated from animals, humans and patients with UTIs has also been reported (Feldgarden & Riley, 1998; Rijavec, 2010; Riley & Gordon, 1992). To our knowledge, the correlation between colicin insensitivity and virulence potential has not yet been investigated. Therefore, the prevalence of colicin insensitivity among *E. coli* SSTI isolates with known virulence potential was determined systematically by exposing 102 strains to 17 different colicins. Furthermore, for the first time, the correlation between colicin insensitivity, virulence potential and phylogenetic background was investigated.

**METHODS**

**Bacterial strains.** A collection of 102 previously described SSTI isolates was studied. The investigated strains had been screened previously for antibiotic resistance, phylogenetic grouping and certain ExPEC virulence factors (VFes) (Petkovšek *et al.*, 2009).

A collection of previously described colicinogenic strains (Pugsley, 1985) was used for colicin production. In Table 1, the colicinogenic strains used and the characteristics of the colicins produced are presented.

**Colicin sensitivity.** An unseeded agar plate was stab inoculated with strains of the colicinogenic collection. Following overnight incubation at room temperature, colicin synthesis was induced by exposure to...
UV light for 25 s. Subsequently, the plates were incubated for several hours at 37 °C, exposed to chloroform vapour and aerated. Clinical isolates cultivated in lysogeny broth were added to soft agar and overlaid on the chloroformed and aerated plates. The plates were incubated overnight at 37 °C. Each strain was tested at least in duplicate. As UV irradiation was used to induce colicin synthesis, microcins that are not regulated by the SOS response (i.e. colicin V; Gillor et al., 2004) were excluded from our study.

Statistical analysis. Fisher’s exact test (two-tailed) (http://www.langsrud.com/fisher.htm) and Bonferroni correction were used to analyse the data. The threshold for statistical significance after Bonferroni correction was set at \( P < 0.05 \).

RESULTS AND DISCUSSION

Prevalence of colicin insensitivities

The colicinogenic strains differed greatly in their ability to prevent growth of SSTI E. coli strains from our collection (Table 2). Whilst 95 of the 102 clinical isolates (93 %) were insensitive to ColA, only two strains (2 %) were insensitive to ColK. Not a single strain was insensitive to all of the tested colicins. As colicins differ in their binding and translocation characteristics, as well as in their mechanism of action (Table 1), it is not surprising that the activity of colicins varied from one strain to another and also with regard to colicin type.

Colicin insensitivity and phylogenetic group

Examination of the association of colicin insensitivity with phylogenetic group showed that strains classified in the B2 group were in general significantly associated with insensitivity to the tested colicins. Only group B2 strains exhibited insensitivity against 12 or more of the tested colicins. The correlation between B2 group and insensitivity to at least ten colicins was statistically significant \((P<0.001)\), whilst insensitivity to five or fewer colicins was statistically associated with non-B2 groups \((P<0.001;\) data not shown). As the B2 group is generally recognized as comprising ExPEC strains (Picard et al., 1999), further analysis was performed to investigate the association of colicin insensitivity with ExPEC VF genes.

Colicin insensitivity and VFs

For ten of the 17 colicins tested, at least two statistically significant associations between insensitivity to a specific colicin and specific VFs were found. In all ten cases of colicin insensitivity, a correlation was found with cnf and hly. Insensitive cases, associations with sfa and usp were also determined, and in three cases, in addition to all the above-mentioned determinants, an association with papGIII was also found. All traits found to be associated with colicin insensitivity are considered important ExPEC VFs, even though the exact function of Usp remains unclear. Due to its high sequence similarity with certain colicins, it has been proposed to act as a bacteriocin (Parret & De Mot, 2002). Other statistically correlated VF sequences enable either adhesion \((papGIII and sfa)\) or toxin synthesis \((cnf and hly)\) (Oelschlaeger et al., 2002; Zhang & Foxman, 2003). Strains insensitive to zero to eight colicins possessed on average 3.2 VFs, whilst strains insensitive to nine to 16 colicins possessed on average 4.8 VFs. Contrary to our expectations, no correlation between the presence of ompT sequences and colicin insensitivity was

Table 1. The colicinogenic strains used in this study and characteristics of the colicins produced (adapted from Cascales et al., 2007 and Arnold et al., 2009)

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Colicin</th>
<th>Receptor</th>
<th>Translocation system</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZB2101</td>
<td>ColA</td>
<td>BtuB</td>
<td>Tol</td>
<td>Pore formation</td>
</tr>
<tr>
<td>BZB2102</td>
<td>ColB</td>
<td>FepA</td>
<td>Ton</td>
<td>Pore formation</td>
</tr>
<tr>
<td>BZB2103</td>
<td>ColD</td>
<td>FepA</td>
<td>Ton</td>
<td>Translation block</td>
</tr>
<tr>
<td>BZB2104</td>
<td>ColE1</td>
<td>BtuB</td>
<td>Tol</td>
<td>Pore formation</td>
</tr>
<tr>
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<td>ColE2</td>
<td>BtuB</td>
<td>Tol</td>
<td>DNA endonuclease</td>
</tr>
<tr>
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<td>BtuB</td>
<td>Tol</td>
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</tr>
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<td>BZB2107</td>
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<tr>
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<td>Tol</td>
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<td>BtuB</td>
<td>Tol</td>
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<td>BtuB</td>
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<tr>
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<td>ColE8-J</td>
<td>BtuB</td>
<td>Tol</td>
<td>DNA endonuclease</td>
</tr>
<tr>
<td>BZB2114</td>
<td>ColIa</td>
<td>Cir</td>
<td>Ton</td>
<td>Pore formation</td>
</tr>
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<td>BZB2115</td>
<td>ColIb</td>
<td>Cir</td>
<td>Ton</td>
<td>Pore formation</td>
</tr>
<tr>
<td>BZB2116</td>
<td>ColIc</td>
<td>Tse</td>
<td>Tol</td>
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</tr>
<tr>
<td>PAP1</td>
<td>ColM</td>
<td>FhuA</td>
<td>Ton</td>
<td>Inhibition of murein synthesis</td>
</tr>
<tr>
<td>BZB2123</td>
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<td>OmpF</td>
<td>Tol</td>
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</tr>
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<td>PAP2</td>
<td>ColS4</td>
<td>OmpW</td>
<td>Tol</td>
<td>Pore formation</td>
</tr>
</tbody>
</table>

*Designation as used by Pugsley (1985).
itivity could also be regarded as an additional fitness factor, increased adaptation to their products. A higher insensitivity to non-B2 phylogenetic groups, whilst in our case colicin synthesis was SOS-induced as a result of exposure to UV irradiation, resulting in massive production of ColE1 (and other colicins), which probably overflowed the overlaid cells and concealed their OmpT activity.

The association of colicin insensitivity with a higher prevalence of VFs was unexpected, as several studies have reported that strains resistant to certain antibiotics (e.g. fluoroquinolones) exhibit a lower extra-intestinal virulence potential (Horcajada et al., 2005; Starčič Erjavec et al., 2007) or, as for ampicillin resistance, a comparable extra-intestinal virulence potential among susceptible and resistant strains (Johnson et al., 2005). Recently, it has been reported that higher numbers of virulence genes and antibiotic resistances can be positively associated (Olesen et al., 2009; Johnson et al., 2010). However, strains exhibiting an antibiotic-resistant phenotype are predominantly linked to non-B2 phylogenetic groups, whilst in our case colicin insensitivity was associated solely with the B2 group.

A correlation between colicin insensitivity and a higher prevalence of VFs could be explained by several hypotheses. VFs promote persistence at sites of infection or in the intestinal tract (Nowrouzian et al., 2001, 2003), resulting in prolonged exposure to other microbes and consequently increased adaptation to their products. A higher insensitivity could also be regarded as an additional fitness factor, enabling pathogenic strains to persist successfully in a competitive environment.

In conclusion, for the first time, the association between colicin insensitivity and phylogenetic group B2, and colicin insensitivity and extended virulence of clinical SSTI E. coli isolates, was shown. As we showed in a previous study (Petkovšek et al., 2009) that E. coli isolates from SSTI share virulence characteristics as well as phylogenetic and antibiotic resistance profiles with ExPEC isolates (e.g. urinary tract E. coli) and since several other published reports state that E. coli strains isolated from different extraintestinal sites are similar with respect to phylogenetic background and combinations of extraintestinal virulence factors (Russo & Johnson, 2000; Obata-Yasuoka et al., 2002; Johnson et al., 2003), we believe that the results of the presented study reveal an important aspect of the entire ExPEC group. When considering practical applications of colicins, this fact should be kept in mind.

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**REFERENCES**


