Invasin gimB found in a bovine intestinal *Escherichia coli* with an adherent and invasive profile

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Abstract

The invasin gimB (genetic island associated with human newborn meningitis) is usually found in ExPEC (Extraintestinal Pathogenic *Escherichia coli*) such as UPEC (uropathogenic *E. coli*), NMEC (neonatal meningitis *E. coli*) and APEC (avian pathogenic *E. coli*). In NMEC, gimB is associated with the invasion process of the host cells. Due to the importance of *E. coli* as a zoonotic agent and the scarce information about the frequency of gimB-carrying strains in different animal species, the aim of this study was to investigate the presence of gimB in isolates from bovine, swine, canine and feline clinical samples. PCR was conducted on 196 isolates and the identity of the amplicons was confirmed by sequencing. Of the samples tested, only *E. coli* SB278/94 from a bovine specimen was positive (1/47) for gimB, which represents 2.1% of the bovine isolates. The ability of SB278/94 to adhere to and invade eukaryotic cells was confirmed by adherence and gentamicin-protection assays using HeLa cells. This is the first study that investigates for gimB in bovine, canine and feline *E. coli* isolates and shows *E. coli* from the intestinal-bovine samples harboring gimB.

Key words: gimB, adherence, invasiveness, zoonotic potential, livestock, companion animals.

Introduction

*Escherichia coli* is a facultative anaerobic bacterium commonly found in the intestinal microbiota of most animal species (Gyles and Fairbrother, 2010). Although typically commensal, various *E. coli* strains cause intestinal and extraintestinal diseases due to the presence of a range of virulence factors (Kaper et al., 2004). The invasin gimB (genetic island associated with newborn meningitis) consists of a sequence of approximately 5,200 bp with six ORFs (Open Reading Frame). It was firstly found by subtractive hybridization in NMEC (neonatal meningitis *E. coli*) (Bonacorsi et al., 2003). In NMEC, approximately 60% of the strains harbor the gimB sequence, which has been associated with the high levels of bacteremia and ability to the bacteria to invade endothelial cells (Bonacorsi et al., 2003; Ewers et al., 2007). The presence of gimB has also been reported in other ExPEC (extraintestinal pathogenic *E. coli*) strains, with frequencies of 9% in UPEC (uropathogenic *E. coli*) and 24% in APEC (avian pathogenic *E. coli*) (Ewers et al., 2007, Barbieri et al., 2013). Recently, gimB-carrying *E. coli* strains were isolated from pigs that displayed symptoms of diarrhea as well as asymptomatic pigs. gimB appeared in approximately 3% of both groups. While a 3% frequency is relatively low, this study showed that the gimB virulence factor may be more frequent and specific in ExPEC strains (Schierack et al., 2011).

Due to the importance of this bacterium as a zoonotic agent and the scarce studies regarding the frequency of gimB-carrying *E. coli* in different animal species, the aim of this work was to investigate the presence of gimB in *E. coli* strains isolated from a variety of animal species.

Material and Methods

*E. coli* isolates and PCR

In order to detect the presence and origin of gimB in *E. coli* from different animal species, PCR was performed on DNA isolated from clinical samples of swine, cattle, dogs and cats stored in the LABAC’s collection, UFSM/RS (Table 1). These samples were taken between 1990 and
the aim of analyzing the adherence (association) profile of the gimB-positive SB278/94 strain, a confluent monolayer of HeLa cells was infected with the bacteria at a multiplicity of infection (MOI) of ~100 cfu per cell in high glucose Dulbecco’s modified Eagles medium (DMEM) (Gibco, Grand Island, NY) plus fetal calf serum (Gibco, Grand Island, NY, USA). After 2 h of incubation at 37 °C under 5% CO2 (Thermo Fisher Scientific, Asheville, NC, USA), the medium was removed, and the cells were washed three times with Phosphate Buffered Saline (PBS) and lysed with 1% (v/v) Triton X-100 (Sigma, Steinheim, Germany) at room temperature. Serial dilutions of the lysate in PBS were plated on Luria Bertani agar (Himedia, Mumbai, India) for cfu determination. The experiment was performed at least three separate times with quadruplicates of each strain.

For the invasion assay (gentamicin protection assay), HeLa cells were infected with bacteria in the same way as described for the adhesion assay and then washed three times with PBS after 2 h of incubation to allow interaction.

Statistical analysis

Student’s t-test was carried out for multiple comparisons (GraphPad Prism Package 5) of adhesion- and invasion-assay results. P < 0.05 was considered statistically significant.

Results and Discussion

Two strains (SB31/94 and SB278/94) out of 196 total E. coli isolates were PCR-positive for gimB. The amplicons from these strains were sequenced to confirm that the sequences corresponded to gimB (Genbank: AJ810519.1). Thus, 0.5% of the 196 isolates, were gimB-positive, which represents 2.1% of the bovine isolates.

The presence of gimB is relatively well documented in avian and human species (Ewers et al., 2007; Ewers et al., 2009; Matter et al., 2011; Barbieri et al., 2013); however, few studies have determined the frequency of gimB in other animal species. Schierack et al. (2008; 2009; 2011; 2013) found 3.2% (2/62) of the diarrhea isolates and 2.7% (1/37) of E. coli from healthy animals carrying gimB in a study of ExPEC-genes in hemolytic E. coli from swine. None of the non-hemolytic E. coli from healthy pigs con-
tained \textit{gimB} (Schierack \textit{et al.}, 2011). In our study none of the 136 swine isolates carried \textit{gimB}.

We also did not find \textit{gimB} in the isolates from canine and feline samples, despite the relatively common presence of this genetic island in extraintestinal-infection isolates (Ewers \textit{et al.}, 2007, Barbieri \textit{et al.}, 2013). This result is likely due to the small sample numbers of these groups.

The SB278/94 strain was isolated in 1994 from the intestinal lumen of a calf with diarrhea that died from peritonitis. There are no data about bovine \textit{E. coli} harboring \textit{gimB} in the literature. The only data regarding intestinal \textit{gimB}-containing \textit{E. coli} are those describing isolates from swine (Schierack \textit{et al.}, 2011) and human samples (GenBank accession number: CP002167.1). The human intestinal \textit{E. coli} is an adherent and invasive \textit{E. coli} (AIEC) pathotype that is associated with Crohn’s disease, a form of inflammatory bowel disease (IBD). AIEC can adhere to and invade enterocytes and can replicate inside macrophages (Krause \textit{et al.}, 2011). According to recent studies, AIEC strains share many genetic and phenotypic features with ExPEC strains (Moulin-Schouleur \textit{et al.}, 2006; Martinez-Medina \textit{et al.}, 2009; Krause \textit{et al.}, 2011). The prevalence and importance of this island for this \textit{E. coli} pathotype is still unknown.

The SB278/94 strain is also capable of adhering to and invading eukaryotic cells such as HeLa cells, at levels comparable to the positive control MT78 strain (Figures 1 and 2). Although our data suggest that this isolate is an AIEC, genetic characterization and \textit{in vivo} studies with macrophages and enterocytes are still required. \textit{In vitro} and \textit{in vivo} studies will also be necessary to investigate the mechanism by which \textit{gimB} contributes to adherence and invasion in this strain.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Capacity of \textit{E. coli} strains to adhere to HeLa cells. Data represent the average and standard deviation of at least three assays done in quadruplicates for each strain. MT78 and DH5α strains represent the strain with high and low adherence level. Statistical analysis has showed significant difference among the three strains (p < 0.05).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Capacity of \textit{E. coli} strains to invade HeLa cells. Results are shown as UFC/mL. Data represent the average and standard deviation of at least three assays done in quadruplicates for each strain. MT78 and DH5α strains represent the positive and negative controls for invasiveness. Statistical analysis showed significant difference between MT78 and 278/94 (p < 0.05). None bacterium was recovered from inside HeLa cells after gentamicin protection assay with DH5α strain.}
\end{figure}
In summary, this study has revealed that *E. coli* from clinical bovine sources can also harbor *gimB*. Future studies should be performed to determine the actual clinical impact of this finding and the role of *gimB* in the pathogenesis of intestinal pathotypes.

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