Genotypic Prevalence of the Adhesin Involved in Diffuse Adherence in Escherichia coli Isolates in Pre-weaned Pigs with Diarrhoea in Korea

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With 1 table

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Summary
A total of 1002 Escherichia coli strains isolated from pre-weaned pigs with diarrhoea on 1114 swine farms were screened for the presence of the adhesin involved in diffuse adherence (AIDA) gene by polymerase chain reaction (PCR). Escherichia coli isolates that carried AIDA genes were also tested by PCR for the detection of five fimbriae (F4, F5, F6, F18 and F41), heat-stable (STa, STb) and heat-labile (LT) enterotoxin, enteroaggregative E. coli heat-stable enterotoxin 1 (EAST1), and Shiga toxin 2 oedema disease (Stx2e) genes. Twenty-three (2.3%) of the 1002 E. coli isolates carried the gene for AIDA. Among 23 isolates shown to carry genes for AIDA, three carried the AIDA gene as the only shown virulence factor. Other isolates carried other virulence factor genes in addition to AIDA. Four isolates carried genes for at least one of the fimbrial adhesins and enterotoxins. Sixteen isolates carried genes for enterotoxins only. The AIDA may represent an additional virulence determinant in pre-weaned pigs with diarrhoea.

Introduction
Enteropathogenic Escherichia coli (EPEC) strains were classified only by their O:K serotype (Robins-Browne, 1987). Later, EPEC is more often used to denote E. coli strains that cause diarrhoea but do not produce heat-stable (ST) or heat-labile (LT) enterotoxins and are not invasive (Nataro and Kaper, 1998). The EPEC strains were further classified as EPEC, enteroaggregative E. coli (EAEC), and diffusely adherent E. coli (DAEC) by their ability to produce distinct patterns of adherence to cultured epithelial cells in vitro: localized, aggregative, and diffuse. The EPEC strains bind to host cells in a pattern called localized adherence (LA), whereby bacteria attach and form microcolonies in distinct areas of the cell surface (Scaletsky et al., 1984). The EAEC strains bind in an aggregative adherence (AA) pattern, whereby aggregated bacteria attach to the cell (Nataro and Kaper, 1998). The DAEC strains are defined by pattern of diffuse adherence (DA), whereby bacteria adhere evenly to the whole cell surface (Scaletsky et al., 1984). The DA phenotype of DAEC is mediated by the adhesin involved in diffuse adherence (AIDA) gene (Benz and Schmidt, 1989). The AIDA gene is consisted of two open reading frames, aah gene (previously known as orfA) and aidA gene (previously known as orfB) (Benz and Schmidt, 1992a, 2001). However, the AIDA gene is not restricted to the DAEC strain. Recently, the AIDA gene is also identified in porcine ETEC strains isolated from pigs with weaned diarrhoea or oedema disease (Niewerth et al., 2001).

The purpose of this study was to investigate the occurrence of the AIDA gene in E. coli isolated from pre-weaned pigs with diarrhoea by the polymerase chain reaction (PCR), and determine the frequency of occurrence of selected fimbrial (F4, F5, F6, F18 and F41) enterotoxin (STa, STb, LT) enteroaggregative E. coli heat-stable enterotoxin 1 (EAST1), and Shiga toxin 2 oedema disease (Stx2e) genes in strains that possess the gene for AIDA.

Materials and Methods
Escherichia coli strains
Between 1995 and 2002, 1002 E. coli were isolated from 1114 pre-weaned pigs with diarrhoea from across Korea to the Department of Veterinary Pathology, Seoul National University. Seventy-eight were isolated in 1995, 129 were isolated in 1996, 151 were isolated in 1997, 159 were isolated in 1998, 126 were isolated from 1999, 127 were isolated in 2000, 115 were isolated in 2001, and 117 were isolated in 2002. All isolates were taken from epidemiologically unrelated pigs. The isolates originated from cultures of jejunum and ileum from pre-weaned pigs with diarrhoea. Colonial characteristics of each isolate were noted, including haemolytic reaction on blood agar and texture (smooth, rough, mucoid) on Tergitol-7 agar (Difo Laboratories, Detroit, MI, USA). Infections were considered potentially dual or multiple when colonies with different haemolytic patterns or textures were observed. Biochemical analyses were performed at 37°C with fermentations in glucose, adonitol, and raffinose and by the indole test. All different colonies were selected to perform the PCR.

Reference strains
Escherichia coli O141:K85ab strain AS12 (negative control), E. coli O126:H7 (AIDA+), E. coli K12ab (F4+), E. coli O9:K99 (F5+), E. coli O1413 (F6+), E. coli O15:H11 (LT+), E. coli O78:H− (STa+ and STb+), E. coli O126:H7 (EAST1+), and E. coli O15:H11 (LT+) were used as controls (Benz and Schmidt, 1992b; Franklin et al., 1996; Kwon et al., 1999; Choi et al., 2001a,b).
Detection of AIDA gene by PCR

A total of 1002 isolates were tested for the aah and aidA genes by PCR as previously described (Niewerth et al., 2001). Lyed bacteria were obtained by suspending a colony of bacteria grown overnight on trypticase soy agar in 50 μl of H2O and boiling at 100°C for 10 min. Bacterial DNA amplification was performed using 5 μl of the supernatant of lyed bacteria, 1 μl (1 nm) of each of oligonucleotide primers, 0.2 mm each of dATP, dGTP, dCTP, and dTTP, 10 mm Tris-HCl (pH 8.8), 1.5 mm MgCl2; 50 mm KCl, and 1 unit of Taq polymerase, all in 50 μl of distilled water. The PCR profile used included a denaturing step at 94°C for 30 s, followed by annealing of the primers at 55°C for 30 s, with an extension step at 72°C for 1 min. The 30 cycles of this 3-step procedure were performed in a thermal cycler, followed by 10 min extension step at 72°C. The PCR reactions were performed in triplicate. Control DNAs from reference strains were included in each reaction.

Detection of fimbrial and toxin genes by PCR

Escherichia coli isolates that carried either aah or aidA genes were also examined for fimbrial (F4, F5, F6, F18 and F41) and toxin (STa, STb, EAST1, LT and Stx2e) genes. The PCR for were also examined for fimbrial (F4, F5, F6, F18 and F41) and toxin (STa, STb, EAST1, LT and Stx2e) genes. The PCR for fimbrial and toxins was carried out as previously described with slight modification (Imberechts et al., 1992; Woodward et al., 1992; Ojienyi et al., 1994; Yamamoto et al., 1997). Lyed bacteria were obtained by suspending a colony of bacteria grown overnight on trypticase soy agar in 50 μl of H2O and boiling at 100°C for 10 min. Bacterial DNA amplification was performed using 5 μl of the supernatant of lyed bacteria, 1 μl (1 nm) of each of oligonucleotide primers, 0.2 mm each of dATP, dGTP, dCTP and dTTP, 10 mm Tris-HCl (pH 8.8), 1.5 mm MgCl2; 50 mm KCl, and 1 unit of Taq polymerase, all in 50 μl of distilled water. The PCR profile used included a denaturing step at 94°C for 1 min, followed by annealing of the primers at T1 for 2 min, with an extension step at 72°C for 1 min. T1 of each primer was determined by T1 = 4(G + C) + 2(A + T), where G, C, A, and T indicate the number of corresponding nucleotides in the oligonucleotide. The 30 cycles of this 3-step procedures were performed in a thermal cycler, followed by 10 min extension step at 72°C.

Results

Primer pairs for AIDA genes yielded PCR products of the expected size for control strains and field isolates. Twenty-three (2.3%) of the 1002 E. coli isolates carried the genes for AIDA. The PCR products from all 23 AIDA-positive E. coli isolates were sequenced and their identity was confirmed as AIDA gene. There was 100% homology among the obtained sequences from all 23 AIDA-positive E. coli isolates (data not shown). All 23 isolates carried both aah and aidA genes. There was a positive association between AIDA and EAST1. Seventeen (73.9%) of 23 AIDA-positive E. coli possess EAST1 gene. Among 23 isolates known to carry genes for AIDA, one was isolated in 1995, one was isolated in 1996, four were isolated in 1997, five were isolated in 1998, four were isolated in 1999, two were isolated in 2000, three were isolated in 2001, and three were isolated in 2002.

Table 1. Prevalence and relationship of AIDA, fimbrial adhesin, and enterotoxin genes of 23 Escherichia coli* isolated from pre-weaning pigs with diarrhoea as determined by polymerase chain reaction

<table>
<thead>
<tr>
<th>Toxins</th>
<th>AIDA only</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of toxins</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>STa + STb + EAST1</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>STa + LT + EAST1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>STa + STb + LT + EAST1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AIDA, adhesin involved in diffuse adherence; ST, heat-stable enterotoxin; LT, heat-labile enterotoxin; EAST1, enteric aggregative Escherichia coli heat-stable enterotoxin 1.

*Two isolates recovered from the same pig have the same virulence gene profile.

Among 23 isolates known to carry genes for AIDA, three carried the AIDA gene as the only known virulence factor. Other isolates carried other virulence factor genes in addition to AIDA. Among the 20 isolates known to carry genes for the fimbrial adhesins or enterotoxins, 16 carried genes for the enterotoxin gene only and four carried genes for at least one of the fimbrial adhesins and enterotoxins.

Among the 16 isolates shown to carry genes for one or more of the enterotoxins, nine also carried STa gene, eight also carried STb gene, 14 also carried EAST1 gene, and two also carried LT gene. Among the four isolates shown to carry for one or more of the fimbrial adhesins, three also contained genes for F4 and one also contained genes for F5 (Table 1).

Discussion

The results of the present study demonstrated that E. coli carrying the AIDA gene was detected in pre-weaned pigs with diarrhoea. Twenty-three (2.3%) of the 1002 E. coli isolates carried the gene for AIDA. The prevalence of AIDA-positive E. coli isolated from pre-weaned pigs with diarrhoea is lower than that of AIDA-positive E. coli isolated from post-weaned pigs with diarrhoea and oedema disease (Ha et al., 2003).

The proportion of AIDA-positive strains that were ETEC were 17.4% (four of 23, ETEC being enterotoxin- and fimbrial-positive strains) or 69.6% (16 of 23, ETEC being enterotoxin-positive strains). The present study indicates that the AIDA gene is not restricted to DAEC strains. The AIDA gene was also found in porcine origin DAEC strains. Hence, AIDA gene may possess wider patho-biological significance. As the commercial vaccine against AIDA-positive strains is not available yet, the antimicrobial susceptibility profiles of a given bacterium can help to select appropriate treatment for the infections caused by AIDA-positive E. coli-like east1 E. coli (Han et al., 2002).

The AIDA gene is associated with F18 and Stx2e in E. coli isolated from post-weaned pigs with diarrhoea and oedema disease (Niewerth et al., 2001) and from piglets at weaning time (Mainil et al., 2002). In contrast, AIDA-positive E. coli isolated from pre-weaned pigs with diarrhoea was not carried genes for F18, Stx2e and ST. In the present study, strains
carrying the AIDA gene also possessed EAST1 and ST genes, suggesting that AIDA, EAST1, and ST genes are related. These data were in general agreement with those of a previous study (Ngeleka et al., 2003). However, it has not yet been determined whether porcine *E. coli* strains carrying AIDA, EAST1, and ST genes may be diarrhoeagenic in pre-weaned pigs.

The genes encoding AIDA are located on two open reading frames, *aah* and *aidA* genes (Benz and Schmidt, 1992a, 2001). The latter codes for the autotransporter adhesin AIDA-1 system and *aah* gene (*orfA*) encodes for a 45-kDa cytoplasmic protein which is required to modify AIDA-1 such that it adheres to target cells (Benz and Schmidt, 1989, 1992b). Therefore, both *aah* and *aidA* genes are required to adhere to target cells completely. Although the detection of *aah* and *aidA* genes by PCR does not necessarily indicate functional activity of AIDA gene in *vivo*. Further study is needed to determine the correlation between PCR results in *vitro*, functional activity of AIDA gene in *vivo*, and the virulence of corresponding AIDA strains in pigs.

The classical method for detection of AIDA is the HeLa and HEp-2 cells in tissue culture as model system (Scaletsky et al., 1984; Nataro and Kaper, 1998) but it is expensive and time-consuming. The PCR test is sensitive and can be completed quickly, giving results such as the identification of gene of AIDA within one working day. Therefore, the PCR system, used in this study, can be used as an alternative to the tissue culture techniques used to confirm AIDA of *E. coli*.

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