Pathotyping of swine *Escherichia coli* strains

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**Abstract**

*Escherichia coli* can exist as harmless commensals in the intestinal tracts of humans and animals while others can be pathogenic. Pathogenic forms of *E. coli* can cause a variety of diarrhoeal diseases where EHEC and ETEC are the most common pathotypes found in pigs. A total of 527 presumptive *E. coli* were obtained from swine farms located in two states in Malaysia. Theswine *E. coli* isolates were subjected to PCR confirmation targeting at the housekeeping gene (*phoA*) and pathotyping was performed for those *phoA*-positive *E. coli*. A total of 357 *E. coli* isolates (67.7\%) were confirmed to be *E. coli* and ten isolates were verocytotoxin (*VT*) -positive but *eae* (attaching and effacing) gene negative. All VTEC (verocytotoxin-producing *E. coli*) isolates were not *E. coli* serogroup O157. All VTEC were isolated in farms located in Penang and were found in three types of swab samples (nasal, rectal and tongue). No significant correlation was found between the health conditions of pigs with the presence of *VT* gene. This report indicates that *eae*-negative VTEC was the most common *E. coli* pathotype isolated from local swine samples. Although the VTEC isolates did not possess *eae* gene which is needed for expression of full virulence, however, its presence should not be overlooked as *eae*-negative VTEC has been reported worldwide.

**Introduction**

*Escherichia coli* (*E. coli*) is a common inhabitant in the gastrointestinal tract of humans and animals. They can exist as harmless commensals in the intestinal tract while others can be highly versatile and pathogenic (Kaper et al., 2004). Pathogenic forms of *E. coli* can cause a variety of diarrhoeal diseases in hosts due to the presence of specific colonisation factors, virulence factors and pathogenicity associated genes. Six pathotypes are now recognised, namely the enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteraggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) (Kaper et al., 2004). Swine has been reported to be one of the reservoirs of pathogenic *E. coli* with EHEC and ETEC as the most commonly found pathotypes (Botteldoorn et al., 2003). *E. coli* serogroup O157 is currently the most common EHEC reported in many regions of the world (Bouvet et al., 2002). Hence, the objectives of this study were to determine the prevalence of different *E. coli* pathotypes in Malaysian swine samples and to detect the presence of *E. coli* serogroup O157.

**Materials and methods**

A total of 527 presumptive *E. coli* isolates were obtained from swine farms in Perak and Penang which were previously identified by streaking on selective medium Chromagar *E. coli*. (CHROMagar, Paris, France). Three types of swabs were sampled (nasal, tongue and rectal). Boiled suspensions of bacterial cells for the 527 isolates were used as DNA templates. All *E. coli* isolates were subjected to PCR confirmation targeting at the housekeeping gene (*phoA*) of *E. coli* (Yu and Thong, 2009). For *E. coli* isolates that were positive for *phoA* gene, a multiplex PCR to identify the virulence genes of pathogenic *E. coli* was performed. The virulence genes include *VT*, *LT1*, *LT2*, *ST* and *eaeA* genes which are associated with three pathotypes ETEC, EPEC and EHEC (Yu and Thong, 2009). Three positive strains for were included (kind gifts of Prof. Dr Kong (City University of Hong Kong) (Figure 2). For VTEC, specific PCR detection of serogroup O157 was carried out with inclusive of a positive control.

**Results and discussion**

A total of 357 *E. coli* isolates (67.7\%) were confirmed to be *E. coli* isolates as they were positive for the *phoA* housekeeping gene. None of the isolates were positive for *LT1*, *LT2*, *ST* and *eaeA* genes while ten isolates were positive for *VT* (verocytotoxin) gene. All VTEC (verocytotoxin-producing *E. coli*) were isolated from swine farms (G, H and I) located in Penang. Five of them were isolated from healthy pigs while another five were from unhealthy pigs. No significant correlation was found between the health conditions of swine with the presence of *VT* genes (*p > 0.05*). Majority (n=7/10) of the VTEC isolates were sampled from farm G. This can be due to the hygiene in the respective swine farm. Equal numbers of VTEC were obtained from rectal and tongue swabs (n=4).

Although pathogenic *E. coli* detected in this study were known to be associated with diarrhoeal diseases, however, VTEC isolates were found in nasal and tongue samples. Bacterial typing should be carried out to determine the diversity of these VTEC isolates. All VTEC isolated were *eae* -negative and non-O157, which has been reported (Bouvet et al., 2002). *eae* gene plays an important role in
the colonization of the colon by attaching and effacing, and is required for the expression of full virulence of VTEC. 

*ea*e negative- VTEC were thought to be less virulence than classical EHEC which possess the *ea*e gene (Kaper et al., 2004). However, *ea*e- negative VTEC should not be overlooked since it has been reported worldwide (Kaper et al., 2004).

Conclusions

*ea*e- negative VTEC (verocytotoxin-producing *E. coli*) was the most common *E. coli* pathotype found in the swine samples and *E. coli* with serogroup O157 was not detected.

References


