Materials and Methods

Bacterial isolation from swine and human samples

A total of 617 *E. coli* isolates from pig rectal swabs and human fecal samples were included in this study. One hundred and eighteen isolates were obtained from 50 diarrheal piglets from 5 farms, 314 isolates were from 36 healthy sows, 19 healthy piglets, and 50 healthy market pigs representing 10 farms. Additionally, 42 isolates were obtained from stool samples of 9 pig farmers (healthy males, age range of 21-32) on a single farm where all classes of swine were present, and for comparison, 143 isolates were similarly obtained from 41 healthy individuals (21 males and 20 females, age range 12-46) in the same region, but without contact with pig farms. All collections took place in southern Thailand during the period of March 2004 to June 2005. All isolates were recovered from fecal swabs using standard microbiological procedures (Forbes et al., 2002). Briefly, a fecal sample on a cotton swab was streaked for isolation directly on MacConkey agar (Difco) and incubated for 24 h at 35°C. Five lactose-positive colonies of different colony morphology were picked and re-streaked onto EMB agar (Merck) and incubated for 24 h at 35°C. Colonies with a metallic sheen were streaked onto TSA for purity. Biochemical confirmation of the strains was performed according to Bacteriological Analytical Manual completed test for *E. coli* (Feng et al., 1998). All isolates appearing as Gram-negative, nonspore-forming rods, fermenting lactose with gas production within 48 h at 35°C, demonstrating IMViC patterns of ++-- , urease negative and hydrogen sulfide negative were considered to be *E. coli*.

Bacterial isolation from retail pork

A total of 147 *E. coli* isolates from raw retail pork loins from local markets and supermarkets in Hat Yai Municipal area were also included in this study. Each sample was collected in a separate clean plastic bag. Samples were transported to the laboratory immediately after collection in an ice box. A 25 gm sample was taken aseptically by scapel excision and placed in a sterile stomacher bag containing 225 ml

of peptone water. Samples were stomached for 2 min. Isolation of *E. coli* was performed as described above.

PCR detection of class 1 integrons

Integrons were detected using a multiplex PCR (MP-PCR) targeting three conserved sequences of class 1 integrons (*intI1, qacEΔ1 sul1*), as adapted from Ebner et al. (2004). The *intI1* gene encoding the integrase which catalyzes site-specific recombination of adjacent gene cassette is located within the 5' conserved region. The *qacEΔ1* gene encoding low level resistance to quaternary ammonium compounds and the *sul1* gene encoding sulfonamide resistance are located within the 3' conserved segment. Primer pairs were purchased from a commercial source (QIAGEN Operon GmbH, Cologne, Germany). Primers, (reported from 5' to 3') included, GGTTCGAATGTCGTAACCGC and ACGCCCTTGAGCGGAAGTATC for amplification of the *intI1* gene, ATCAGACGTCGTGGATGTCG and CGAAGAACCGCACAATCTCG for amplification of the *sul1* gene and GAGGGCTTTACTAAGCTTGC and ATACCTACAAAGCCCCACGC for amplification of the *qacEΔ1* gene. Template DNA was prepared by boiling overnight cultures. Boiled cultures were cooled on ice for 5 min and 1 µl volumes were used immediately for PCR using a PTC-100™ Peltier thermocyler, MJ Research, Inc. (Waltham, MA, USA) with the following cycle: (i) one cycle of 94°C for 4 min; (ii) 10 "touchdown" cycles of 94°C for 1 min, 65°C for 30 s (decreasing 1°C/cycle), 70°C for 2 min; (iii) 24 cycles of 94°C for 1 min, 55°C for 30 s, 70°C for 2 min; and (iv) one final cycle of 70°C for 5 min. *Salmonella enterica* Typhimurium DT104, a known carrier of a class 1 integron, was used as a positive control. The PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis (Fig. 1). Prevalence of class 1 integrons was based on the presence of *intI1* gene sequences.

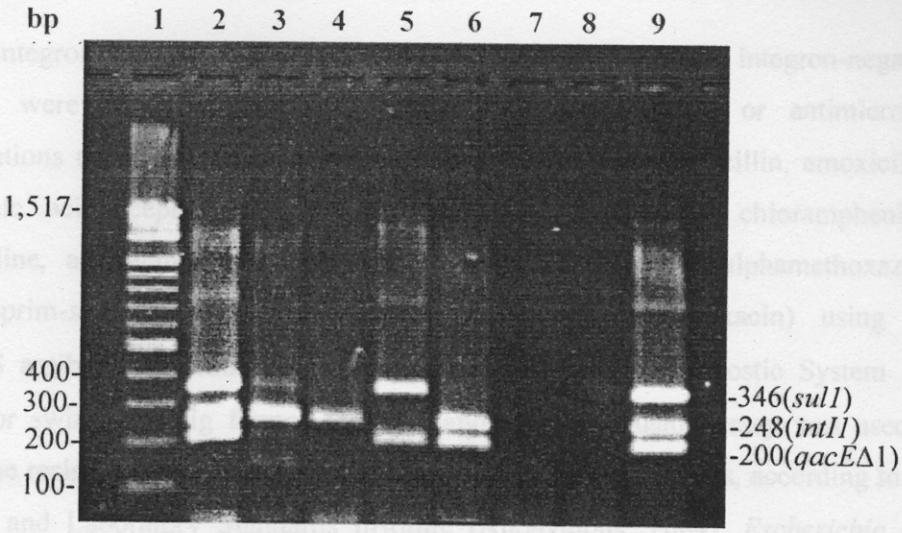

Fig.1. MP-PCR detecting class 1 integron gene sequences.

Lane 1, 100 bp DNA ladder

Lane 2-7, wild-type isolates

Lane 8, negative control

Lane 9, positive control (*Salmonella enterica* Typhimurium DT104)

Antimicrobial susceptibility testing

Integron-positive and a subsample of randomly selected integron-negative bacteria were tested for susceptibility to 16 antimicrobials or antimicrobial combinations used in either human or veterinary medicine (ampicillin, amoxicillin-clavulanic acid, cephalothin, ceftiofur, cefoxitin, ceftriaxone, chloramphenicol, tetracycline, amikacin, gentamicin, kanamycin, streptomycin, sulphamethoxazole, trimethoprim-sulphamethoxazole, nalidixic acid, and ciprofloxacin) using the NARMS antibiotic microdilution plates (Sensititre®, Trek Diagnostic System Inc, USA) for swine and pig farmer samples, and a disc diffusion assay was used to determine resistance patterns for pork and non-farm human samples, according to the Clinical and Laboratory Standards Institute (Anonymous, 2004). *Escherichia coli* ATCC25922 was used as reference strain.

Statistical analysis

Comparisons of integron frequency were made using a Chi-square test, one-way ANOVA and Post Hoc test using SPSS program. Antimicrobial resistances were compared by a Chi-square test. Differences were considered significant at $P<0.05$.