Discussion

It is well established that class 1 integrons play an important role in antibiotic resistance in enteric bacteria. Our results show that class 1 integrons are widespread in multidrug resistant *E. coli* isolated from both healthy and diarrheal pigs as well as from humans, with and without contact with farms. The prevalence of integrons we noted in swine isolates for this study (74.5 %) compares with that reported by Hsu et al. (2006), who characterized integron prevalence in E. coli from swine in Taiwan using primers detecting *intl1* and variable region sequences. That study reported 78.9% of isolates were positive for class 1 integrons. Maynard et al. (2003), who characterized integron prevalence in O149:K9 *E. coli* from diarrheal pigs through detection of a 3' conserved sequence, *qacEΔ1- sul1,* found that 60% of isolates were positive for class 1 integrons. It should be noted; however, that only a small subset (11.5%) of isolates in our study simultaneously contained *intl1, qacEΔ1,* and *sul1* gene sequences, which have been reported to be conserved in class 1 integrons. This result is similar to that reported by Mathew et al. (2005). That group determined class 1 integron prevalence by detecting the three conserved genes sequences, *intl1, qacEΔ1,* and *sul1l.* Sunde (2006), reported that a high portion of class 1 integrons found in *E. coli* lacked the *sul1* gene, suggesting that use of specific primers for conserved segments can produce false negatives when those specific gene components are not present in otherwise functional integrons.

We found that the prevalence of integrons in porcine isolates from commercial farms was significantly higher than that from smaller farms. It may be that higher pig densities of commercial farms require more frequent use of antibiotics, and/or those farms have more rigorous herd health programs that include antibiotics. Ebner (2003) noted a higher integron prevalence in *E. coli* from swine on farms where antibiotics were used, compared to those from farms where antibiotics were not used. In agreement with Campbell et al. (2005) we noted a higher prevalence of integrons in isolates from younger pigs compared to isolates from finishing swine. As antibiotics are more frequently used in younger swine to maintain health and resist disease challenge, intestinal bacteria may be under a greater selection pressure to acquire and maintain resistance genes. We also noted a higher prevalence of integrons in isolates

collected from sows. It may be that as sows spend a longer period of time on farms (three to five years, compared to six months for a market hog), they have more opportunity for exposure to antibiotics, and thus present a greater opportunity for a resistant microflora to develop.

With the exception of market pigs, we observed a greater prevalence of integrons in isolates from swine groups compared to isolates from pig farmers and from humans without contact with pig farms. Our findings are in agreement with Campbell et al. (2005) who also noted a greater integron prevalence in swine compared to human isolates. However, those investigators observed a much lower prevalence of *intI1* in both swine (9.7%) and human (5.5%) isolates compared to our study (74.5% and 37.9% for swine and human isolates, respectively).

As in the US, a wide array of antibiotics, including Beta-lactams, aminoglycosides, and tetracyclines, are used in swine production in Thailand (Jaroenpoj, 2004). However, chloramphenicol has been banned from animal feed in Thailand since 1999, and nitrofurazone, furazolidone, dimetridazole, ronidazole were banned in 2002 (Anonymous, 2002).

We noted a significant level of resistance to nearly all test antibiotics in both integron-positive and integron negative isolates. This is in agreement with Kang et al. (2005) who noted that while integrons play a major role in the spread of resistance, much resistance is not spread through integrons but rather through other exchange mechanisms such as plasmids, transposons, and bacteriophages. In our study only chloramphenicol and ampicillin resistance levels differed between integron-positive and integron-negative isolates, with integron-positive having a greater prevalence of resistance to those antibiotics. Harada et al. (2006) noted a high correlation between chloramphenicol resistance genes and integrons in *E. coli* isolates from cattle and pigs. In that study, it was also found that cattle isolates carrying chloramphenicol genes were often also resistant to ampicillin. In our study, co-resistance between those two antibiotics was noted in integron-positive isolates, with 52.2% of isolates from swine and swine farmers containing resistance to both antibiotics; whereas only 28% of integron-negative isolates demonstrated resistance to both antibiotics. Harada et al. (2006) did not observe co-resistance between those two antibiotics in swine isolates.

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All integron-positive and integron-negative isolates from swine and pig workers were multidrug resistant. Resistance to sulphamethoxazole, tetracycline, trimethoprim-sulphamethoxazole, streptomycin and ampicillin was most common, in agreement with other studies (Guerra et al., 2003, Kijima-Tanaka et al., 2003, Lanz et al., 2003, Maynard et al., 2003, Scott et al., 2005, Stephan and Schumacher, 2001, Sunde et al., 1998, Van den Bogaard et al., 2000). Moreover, resistance to chloramphenicol, kanamycin, and streptomycin was significantly higher in porcine isolates than in those from pig farmers. These results are similar to those of Nijsten et al. (1996) and may be due to the fact that these drugs were often widely used for prevention and treatment of infectious diseases in pigs. Hanson et al. (2002) conducted a pilot study to determine the prevalence and antimicrobial resistance of Salmonella and *E. coli* in swine, broiler chickens and human workers from farms and abattoirs in northern Thailand. That group reported high levels of resistance in *E. coli* to tetracycline (91.5%), nalidixic acid (67.4%), ampicillin (61.6%), florfenicol (51.8%), and lower levels of resistance to enrofloxacin (28.7%), ciprofloxacin (12.5%), ceftiofur (4.9%) and ceftriaxone (1.5%). Isolates from farm workers showed high levels of resistance to tetracycline, florfenicol, nalidixic acid, ampicillin and enrofloxacin. On both swine and chicken farms, the proportions of multi-resistant *E. coli* were higher in animals than in humans. In our study, the antimicrobial susceptibility of isolates from non-farmers was tested by disc diffusion method with 11 antimicrobials. The most frequent patterns in this group were TET (19.6%) and SUL-SXT-AMP-TET-STR (19.6%) (data not shown).

When comparing isolates from pigs and farm workers on a single farm we found that resistance patterns differed, with no common patterns occurring between isolates from those two hosts. While resistance patterns were somewhat similar between swine and human isolates, swine isolates typically lacked resistance to cefoxitin and amoxicillin-clavulanic acid; whereas human isolates demonstrated resistance to those antibiotics. Although commensal *E. coli* from pigs may represent a considerable reservoir of antibiotic resistance genes that might be transferable, transmission of resistance from pigs to humans involved in their husbandry is uncertain. Nijtsen et al. (1994) found that only 4% of E. coli from pigs and pig farmers on the same farm had the same antibiotic resistance patterns. However, concern continues to be expressed regarding the possibility of transfer of resistant commensal microflora from meat

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animals to workers and other humans via environmental and/or foodborne transfer. Hsu et al. (2006) reported that of 80 resistance phenotypes, 13 patterns were shared between *E. coli* from humans and swine from southern region of Taiwan. They further noted that the most prevalent resistance pattern, AMP-CAR-TET-STR-GEN-KAN-NEO-APR-SPT-CHL-SUL-SXT-CEF-NAL-CIP-NOR-OFX, was one of those shared between the two hosts, occurring in 10.9% of the human and 44.3% of the swine isolates.

Box et al. (2005) reported that at least five different class 1 integron types were found in multidrug resistant *E. coli* from slaughter animals and meat, and three types were shared between hospitalized patients, humans in the community, meat, and slaughter animals. However, the original source(s), and direction of transfer for those genetic resistance elements was not definitively characterized in that study. Hunter et al. (1994) surveying isolates from a farm in the United Kingdom noted that *E. coli* exhibiting resistance to apramycin, a drug used to prevent colibacillosis in poultry and swine, were isolated from both pigs, a farm manager and the manager's wife. As apramycin is not used in human medicine, they concluded that enteric bacteria from swine likely transferred the resistance genes into the human bacterial populations. In this study, however, we were not able to see clear evidence of transfer between commensal *E. coli* of swine and those of workers who had direct contact with the swine. Given that specific antibiotic uses may vary across the countries where the above works were conducted (Netherlands, United Kingdom, Taiwan) it may be difficult to compare those results directly to our study, or generalize regarding the risks associated with resistance transfer between production animals and humans.

Of additional concern, however is possibility that class 1 integrons may have the capability to integrate nearly all known antimicrobial resistance genes, including β-lactams, aminoglycosides, phenicols, sulphonamides, macrolides, rifampicin, and trimethoprim, to more effectively disseminate multidrug resistance in bacteria (Jaroenpoj, 2004, Levesque et al., 1995, Peters et al., 2001, Recchia and Hall, 1995).

Resistant bacteria from the intestine of food animals may be transferred to retail meat products as a result of fecal contamination during the slaughter process and subsequent handling of animal tissues. We found that 38.8% of the isolates had class

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1 integrons and 90% of randomly selected *E. coli* isolates from retail pork were multidrug resistant. This study shows that *E. coli* in retail pork can contain integrons responsible for multidrug resistance. Bacteria in pork may therefore be a source of exposure of human bacteria to integrons. Proper hygiene during slaughter and food-processing routines are needed. In addition, it was recently demonstrated that meat and meat products can also be contaminated with pathogenic extraintestinal *E. coli* (Johnson et al., 2005). Meat may therefore be an important vehicle for dissemination of both antibiotic resistant *E. coli* and pathogenic *E. coli*.

Our study shows that integrons and multidrug resistant commensal bacteria are common and appear to be a significant aspect of current microbial communities associated with pigs and humans. However, integron prevalence data and antibiotic resistance patterns of *E. coli* from the various classes of swine and from humans in our study do not clearly demonstrate a high rate of transfer of resistance genes between the commensal *E. coli* of swine and pig farmers. As foodborne risks are highly relevant to food industries and consumers, continued research to characterize sources and routes of transfer of antibiotic resistance genes, including integrons, will be required to determine appropriate strategies for control.