RESEARCH NOTE

WIDESPREAD PRESENCE OF *dfrA12* AND ITS ASSOCIATION WITH *dfrA12-aadA2* CASSETTE IN *SALMONELLA ENTERICA* ISOLATES FROM SWINE

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Abstract. One hundred and eighty-nine *Salmonella* isolates from swine were tested for susceptibility to nine antimicrobial agents, presence of *dfrA12* and class 1 integrons containing *dfrA12*-orfF-*aadA2* cassette. All isolates were multidrug resistant and exhibited highest resistance prevalence to trimethoprim (93%). Most isolates (89%) were *intl1*-positive and 107 isolates (57%) carried *dfrA12*, all of which were resistant to trimethoprim. Forty-eight *dfrA12*-harboring strains (45%) were *intl1*-positive together with *dfrA12-aadA2* gene cassette. Fifteen isolates contained *dfrA12* but not *intl1* and *dfrA12-aadA2* cassette. The results indicated a wide distribution of *dfrA12* and its role in dissemination of trimethoprim resistance among *Salmonella* isolates from fattening pigs.

Keywords: *Salmonella enterica, dfrA12, dfrA12-aadA2* cassette, swine

INTRODUCTION

Salmonella enterica plays an important role as food borne pathogen worldwide and food animals, including fattening pigs, are considered major reservoirs of this pathogen (Hsu *et al*, 2006; Padungtod and Kaneene, 2006). In the past two decades, antimicrobial-resistant *Salmonella* increasingly has emerged as a serious threat to global health causing high mortality and morbidity (Randall *et al*, 2004; O'Mahony *et al*, 2005). The pathogen has become resistant to several drugs conventionally used for infection treatment including trimethoprim. This antibiotic in combination with sulphonamide drug has been widely used for both prophylaxis and treatment of bacterial diseases in swine production.

Trimethroprim is an analog of dihydrofolate and competitively inhibits dihydrofolate reductase (DHFR), which catalyses the reduction of dihydrofolate to tetrahydrofolate in the DNA synthesis pathway (Huovinen *et al*, 1995). High

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trimethoprim resistance is most commonly due to overproduction of trimethroprim-resistant DHFR encoded by *dfr*. More than 30 DHFR have been described so far and are usually associated with integrons, mobile genetic elements, capable of capturing several resistance gene cassettes (Skold, 2001). Integrons are usually located on transposons and/or plasmids leading to an efficient horizontal transfer of antimicrobial resistance among bacteria (Collis and Hall, 1992). A variety of *dfr*-containing gene cassettes have been identified in *Salmonella* (Peirano *et al*, 2006) and different *dfr*s have been detected in isolates from different sources and regions (Guerra *et al*, 2000; Chen *et al*, 2004). Various *dfr* combinations have been identified in class 1 integrons, *eg*, *dfrA16-aadA2* in isolates from food animals in Spain (Riano *et al*, 2006) and *dfrA1-aadA* and *dfrA17-aadA5* in isolates from humans in Hungary (Nogrady *et al*, 2005). In Thailand, previous studies have demonstrated that *dfrA12- aadA2* cassette is prevalent among isolates from poultry, pigs, pork and humans (Khemtong and Chuanchuen, 2008; Wechsiri *et al*, 2011).

Pigs are the major food animals for people worldwide including Thailand and trimethoprim resistance is very common among the bacterial foodborne pathogens. However, epidemiology of *dfrA12* has not hitherto been well studied. In this report, we investigated the prevalence of *dfrA12* and its relationship to integrons in *Salmonella* isolates from fattening pigs.

MATERIALS AND METHODS

Bacterial strains, media and growth conditions

One hundred and eighty-nine *Salmonella* isolates were obtained from clinically healthy fattening pigs and their farm environment in Chiang Mai and Lamphun Districts, Thailand during 2000-2006. All strains were recovered by standard methods as previously described (Hanson *et al*, 2002; Dorn-In *et al*, 2009). Briefly, fecal and swab samples were inoculated into Rappaport and Vasilidis (RVS) broth and incubated overnight at 42ºC. A loop of inoculum from RVS broth was streaked on Brilliant Green (BG) agar and incubated for 24 hours at 37ºC. Two colonies with *Salmonella* appearance were selected from each plate and inoculated on triple-sugar iron (TSI) agar. Colonies exhibiting an alkaline slant, an acid butt and H_2O_2 production were further examined for their biochemical characteristics. Only a single colony was collected from each positive sample and was stored in 20% glycerol at -80ºC.

Determination of antimicrobial susceptibility

Antimicrobial susceptibility to 6 antimicrobials: ceftiofur (30 g), chloramphenicol (30 g), enrofloxacin (5 g), erythromycin (15 g), gentamicin (10 g) and tetracycline (30 g) were determined using disk diffusion method (NCCLS, 2002). Susceptibility to 3 other antimicrobials, spectinomycin, streptomycin and trimethoprim was determined as minimum inhibitory concentration using a two-fold agar dilution technique (NCCLS, 2002). Multidrug resistance (MDR) was defined as an isolate being resistant to three or more different classes of antibiotics. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29212 were used as control organisms.

Detection of *intl1*, *dfrA12* and *dfrA12-aadA2*

The presence of *intl1* was detected using either PCR or DNA dot-blot hy-

bridization. The primer set used for *intl1* amplification was INT1F, 5′-AAGGATCGGGCCTTGATGTT-3′ and INT1R, 5′-CAGCGCATCAAGCGGTGAGC-3′ (Pongpech *et al*, 2008). The *intl1* insert in pCTF202 was labeled and used as probe for detection of *inlt1* by dot-blot hybridization using random primed DNA labeling kit (Roche, Mannhein, Germany) (Tribuddharat and Fennewald, 1999). The presence of *dfrA12* was examined by PCR using specific primers dfrA12-F, 5′-TTCGCAGACTCACTGAGGG-3′ and dfrA12-R, 5′-CGGTTGAGACAAGCTCGAAT-3′ (Chuanchuen and Padungtod, 2009). The existence of *dfrA12-aadA2* was determined using DNA dot-blot hybridization employing a labeled probe amplified with a primer pair of 5′-CS, 5′-GGCATCCAAGCAGCAAG-3′ and 3′-CS, 5′-AAGCAGACTTGACCTGA-3′ from class 1 integrons (Levesque *et al*, 1995).

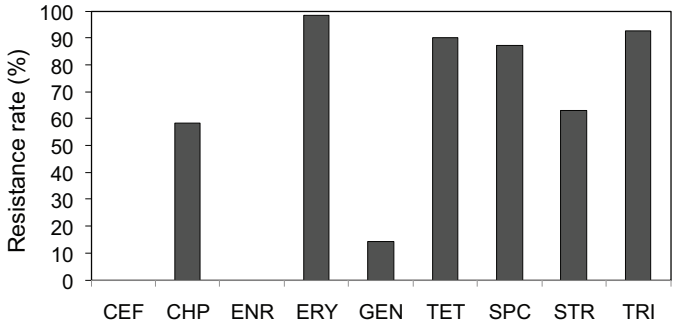

Fig 1–Frequency of resistance to nine antimicrobial agents in 189 *Salmonella enterica* from fattening pigs. CEF, ceftiofur; CHP, chloramphenicol;ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; TET, tetracycline; SPC, spectinomycin; STR, streptomycin; TRI, trimethoprim

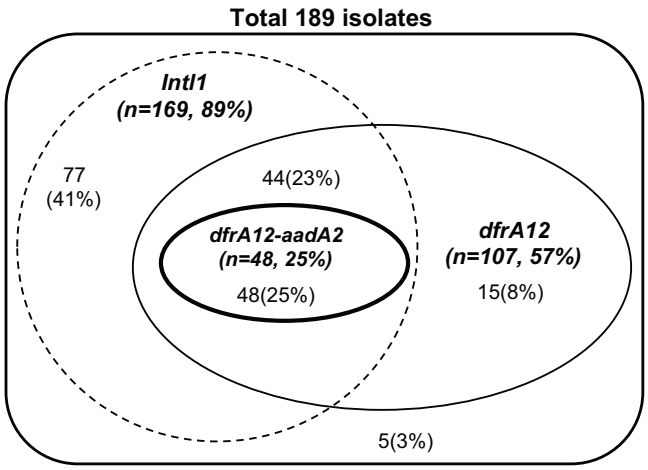

Fig 2–Venn diagram of presence of *intl1*, *dfrA12* and *dfrA12-aadA2* cassettes among 189 *Salmonella* isolates from fattening pigs. Genes and their total numbers are indicated in bold.

RESULTS

Antimicrobial susceptibility

All the *Salmonella* strains in this study were multidrug resistant (Fig 1). Resistance frequency to chloramphenicol, erythromycin, gentamicin, tetracycline, spectinomycin, streptomycin and trimethoprim was 58, 98, 14, 90, 87, 63 and 93%, respectively. All the isolates were susceptible to ceftiofur and enrofloxacin.

Detection of *intl1*, *dfrA12* and *dfrA12-aadA2*

One hundred and sixty-nine isolates (89%) were *intl1*-positive and 107 isolates (57%) were found to contain *dfrA12* (Fig 2). All *dfrA12*-containing isolates exhibited resistance to trimethoprim. Of all *intl1*-positive *Salmonella* strains, 48 isolates (28%) harbored *dfrA12-aadA2* cassette and 44 isolates (26%) carried *dfrA12*

without *dfrA12-aadA2* cassette. Among the *dfrA12*-harboring strains, 48 isolates (45%) contained *dfrA12-aadA2* cassette. Fifteen isolates (3%) contained *dfrA12* but not *intl1*.

DISCUSSION

All the *Salmonella* isolates in this study were resistant to multiple drugs, in agreement with previous reports in the isolates from food animals (Randall *et al*, 2004; Riano *et al*, 2006; Khemtong and Chuanchuen, 2008). This is most likely the result of the extensive and long-term use of antimicrobial agents in pig production for three main purposes: treatment of infection, prevention of disease and growth promotion. Resistance to trimethoprim was very common and high exposure to this antibiotic-selective pressure could be the reason. Ceftiofur resistance is currently a particular concern because it is closely related to ceftriaxone, a drug of choice for treatment of invasive *Salmonella* infections in children (Fey *et al*, 2000). However, resistance to ceftiofur was not observed among the strains in this study. Similarly, none of the *Salmonella* isolates were resistant to enrofloxacin. The explanation could be the limited use of these two antibiotics in pig production.

In this study, the widespread presence of *dfrA12* was observed with a frequency that was higher than the previous study (47%) of isolates from poultry and pigs (Khemtong and Chuanchuen, 2008). All the *dfrA12*-carrying isolates were resistant to trimethoprim, indicating the association between *dfrA12* and trimethoprim resistance phenotype. However, it cannot be concluded that *dfrA12* is the only explanation for trimethoprim resistance in these isolates as several *dfr* genes and multidrug efflux systems have been previously characterized (Eaves *et al*, 2004). However, their contribution in trimethoprim resistance was not tested in this study.

The *dfrA12* gene is frequently found to be associated with class 1 integrons where it usually exists as *dfrA12*-orf-*aadA2* cassette (Hsu *et al*, 2006; Khemtong and Chuanchuen, 2008). This is consistent with the present study showing that up to 45% of *dfrA12* were associated with *dfrA12* -orf-*aadA2* cassette. This resistance gene array was previously identified in class 1 integrons in food animal isolates in different countries, *eg*, cattle, pigs and poultry in Germany (Miko *et al*, 2005), poultry and pig in Thailand (Khemtong and Chuanchuen, 2008) and pig in Taiwan (Hsu *et al*, 2006). Such resistance gene combination also has been isolated from other bacterial strains, *eg*, *Escherichia coli* (Yang *et al*, 2009), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Gu *et al*, 2007). The presence of a specific gene combination in different *Salmonella* serovars and different bacterial species from different animal hosts and different geographic area indicates horizontal transfer of the resistance gene cassette in the clinical settings (Khemtong and Chuanchuen, 2008). The *dfrA12*-orf-*aadA2* cassette has been described previously on conjugative plasmids (Khemtong and Chuanchuen, 2008) but horizontal transfer of *dfrA12* was not examined in this study.

Although *dfrA12* has been shown to be combined predominantly with *aadA2*, several studies have demonstrated different combination of *dfr* and *aad*, *eg*, *dfrA1-aadA1* (Miko *et al*, 2005), *dfrA16-aadA2* (Riano *et al*, 2006), *dfrA14-aadA1a* (Guerra *et al*, 2000), *dfrA17-aadA*5 (Hsu *et al*, 2006). It is still unclear of existence of a specific combination in a certain geographic area.

The different antibiotic uses within geographically distinct regions could provide an explanation (Khemtong and Chuanchuen, 2008).

In summary, this study demonstrates the high prevalence of trimethoprim-resistance encoding *dfrA12* and its association with class 1 integrons among *Salmonella* isolates from fattening pigs. This confirmed that *dfrA12* plays an important role in trimethoprim-resistance among the *Salmonella* isolates and reinforced that pigs serve as major carriers of mobile genetic elements carrying resistance determinants. Strategies to prevent distribution of antimicrobial resistance among pigs and also other food animals must be encouraged. The prudent use of antimicrobials in food animal production should be advised and discontinuation of the antimicrobial abuse in livestock is mandatory. Antimicrobial resistance monitoring and surveillance programs among bacteria from food animals should be routinely performed.

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