International Scholars Journals

African Journal of Pig Farming ISSN 2375-0731 Vol. 5 (8), pp. 001-007, August, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antibiotic resistance, integrons and *Salmonella* genomic island 1 among *Salmonella* Schwarzengrund in broiler chicken and pig

Yu-Chih Wang[1], Yi-Chih Chang[1,6], Hsiao-Li Chuang[2], Chien-Chao Chiu[2], Kuang-Sheng Yeh[4], Chao-Chin Chang[3], Shih-Ling Hsuan[5] and Ter-Hsin Chen[3,5]*

[1]Department of Veterinary Medicine, National Chung Hsing University, Taichung, 402, Taiwan.
[2]National Laboratory Animal Center, National Applied Research Laboratories, Taipei, 115, Taiwan.
[3]The Graduate Institute of Microbiology and Public Health, College of Veterinary Medicine, National Chung Hsing University, Taichung, 402, Taiwan.
[4]Department of Microbiology and Immunology, School of Medicine, Taipei Medical University, Taipei, 402, Taiwan. [5]Graduate Institute of Veterinary Pathobiology, College of Veterinary Medicine, National Chung Hsing University, Taichung, 402, Taiwan.
[6]Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung, 402, Taiwan.

Accepted 04 March, 2017

***Salmonella* Schwarzengrund is an infrequent serovar isolated from humans in Taiwan. However, this serovar is which is highly invasive, is found in humans as report by two independent epidemiologic surveys. The present study delineated the widespread resistance to fluoroquinolone in *S*. Schwarzengrund isolated from both poultry and swine carcesses in Taiwan. We conducted the present study to investigate the prevalence and characteristics of *S*. Schwarzengrund isolated from broiler chicken in central Taiwan. A total of 187 *S*. Schwarzengrund isolates was gotten from slaughtered chicken and pigs in central Taiwan between June 2006 and March 2007. The percentages of resistance were as follows: ampicillin (90.74%), chloramphenicol (69.75%), florfenicol (24.07%), streptomycin (91.98%), trimethoprim and sulfamethoxazole (91.36%), nalidixic acid (96.30%), ciprofloxacin (8.02%), tetracycline (95.68%), amikacin (8.64%), ceftiofur (0.62%) and ceftriaxone (0%). Four types of class 1 integrons were detected: 1.2 kb carried *blaPSE-1*, *aadA2* gene (*n* = 2), 1.2 kb carried *blaPSE1*, *dfraA1* gene (*n* = 2), 1.0/1.2 kb carried *blaPSE-1*, *aadA2* (*n* = 2) and 1.9 kb carried *dfrA12*, *aadA2* gene (*n* = 152). *Xba*I-digested PFGE patterns generated related clusters implicated in the dissemination of integron. Thirteen ciprofloxacin-resistant isolates were all detected in 5 identical mutations in the QRDR of *gyrA*, *parC* and *parE*. Our results suggest that there is a risk of transmitting multidrug resistant *Salmonella* between pig and chicken farms, and also suggest that there is a need to prevent the transmission of this organism from a neighboring contaminated farm.**

Key words: Integron, multidrug resistance, *Salmonella* Schwarzengrund.

INTRODUCTION

Salmonella enterica is one of the most common causes of human foodborne infection. Most serovars of *Salmonella* infections result to self-limited gastroenteritis which is usually recovered from without treatment. However some lead to severe invasive infections. Invasive *Salmonella* infections can be life threatening and usually require hospitalization and antibiotics treatment. Antimicrobial- resistant *Salmonella* were associated with an increased rate of hospitalization compared with outbreaks caused by multidrug resistant (MDR) *Salmonella* strains (Boyd et al., 2002) . In Taiwan, *Salmonella* Schwarzengrund was the 3rd or 4th frequent serotypes (Chiu et al., 1999; Lauderdale et al., 2006), and the high

*Corresponding author. E-mail: thc@dragon.nchu.edu.tw. Tel: 886 4 22840894. Ext: 407.

prevalence of resistance to extended-spectrum cephalosporins or fluoroquinolones found in *Salmonella* Schwarzengrund (Chen et al., 2006;_Lauderdale et al., 2006; Yan et al., 2005) has become an important concern. This serovar showed highly invasive rate (25 and 12.5%, respectively, for) report by two independent epidemiologic surveys (Lauderdale et al., 2006; Vugia et al., 2004).

Multidrug resistance of *Salmonella* is linked to the presence of class 1 integrons (Leverstein et al., 2003). The acquisition of integrons via horizontal gene transfer allows bacteria to rapidly evolve. Class 1 integrons are the most common integrons found in clinical *Salmonella* isolates (Fluit 2005; Kwon et al., 2002) . *Salmonella* genomic island 1 (SGI1) is a 43 kb genomic island, which contains a complex integron. SGI1 is mobile and can be transferred between di erent *Salmonella* serovars and other bacteria in the presence of a helper plasmid (Doublet et al., 2005; Khemtong and Chuanchuen, 2008).

Studies suggest that broiler chickens are a major reservoir for *Salmonella* Schwarzengrund (Aarestrup et al., 2007; Bangtrakulnonth et al., 2004). However, epidemiology data of MDR *Salmonella* Schwarzengrund among chickens are limited. One potential health hazard associated with the veterinary use of antibiotics is the transmission of antibiotic-resistant pathogens from animals to humans, this study examined antibiotic resistance of *Salmonella* Schwarzengrund isolated from broiler and swine in Taiwan.

MATERIALS AND METHODS

Sample isolates

A total of 187 *Salmonella* Schwarzengrund isolates were obtained from slaughtered chickens from 5 chicken slaughterhouses (N = 159) in 2006 - 2007 and 2 pigs slaughterhouses (N = 28) in 2005 - 2006 in central Taiwan. Isolates were serotyped by using commercial *Salmonella* O and H antisera purchased from S&A Reagents Laboratory (Bangkok, Thailand) and Denka Seiken (Tokyo, Japan). All isolates were stored in 20% glycerol at -80°C. Bacteria were grown on Tryptic Soy agar/broth (Difco, MI, U.S.A.) at 37°C.

Antibiotic susceptibility test

Antimicrobial susceptibility was tested by a standard disk diffusion method, and *Escherichia coli* ATCC 25922 was used for control. The antimicrobial agents used were ampicillin, chloramphenicol, florfenicol, streptomycin, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin, amikacin, ceftiofur, ceftriaxone and tetracycline. Susceptible and resistant isolates were defined according to the criteria suggested by the Clinical and Laboratory Standards Institute.

Genotyping by pulsed-field gel electrophoresis (PFGE)

Genotypes of all *Salmonella* Schwarzengrund isolates were determined by pulsed-field gel electrophoresis (PFGE) analysis using the restriction endonuclease *Xba*I to digest total genomic DNA. The procedure for the PFGE was performed according to the standard protocol developed by the Centers for Disease Control and Prevention (Barrett et al., 2006). The digested DNA was separated by the use of CHEF DR II (Bio-Rad) in 0.5x Tris-borate-EDTA at 14°C for 20 h.

PCR analysis, DNA purification and DNA sequencing

DNA templates used for PCR were prepared by boiling bacterial cultures or by using the QIAGEN Genomic-tip System (QIAGEN). Amplifications were performed in 25 L reaction mixtures containing 2.5 L of DNA, 2.5 L 10X PCR buffer, 1.5 M MgCl2, 200 M each deoxynucleoside triphosphate, 2.5 U of Taq DNA Polymerase (Promega) and 1 M each primer. To amplify fragments larger than 3 kb, Blend Taq-Plus polymerase (TOYOBO) was used instead of Taq DNA polymerase. The PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis. Amplification products were purified with the QIAquick PCR Purification Kit (Qiagen) for sequencing. The resulting DNA sequence data were compared to the GenBank Database using the Blast algorithm available at the National Center for Biotechnology for Biotechnology information website (www.ncbi.nlm.nih.gov).

Class 1 integrons were screened by PCR with primers 5' -CS and 3' -CS as described previously (Lévesque et al., 1995). Detection of *Salmonella* genomic island 1 was done using primers corresponding to left and right junctions as described previously (Cloeckaert et al., 2000). PCR mapping of the typical antibiotic resistance genes associated with SGI1 was performed using conditions and primers described previously (Boyd et al., 2002; Levings et al., 2005).

For the detection of resistance-mediating mutations in the DNA gyrase, topoisomerases IV and plasmid- mediated quinolone resistance genes, the quinolone-resistance determining region (QRDR) of *gyrA*, *gyrB*, *parC*, *parE*, *qnrA, qnrB and qnrS* genes was amplified by PCR oligonucleotide primers which were amplified and sequenced. The sequences of the primers and PCR condition was described previously (Casin et al., 2003; Gay et al., 2006; Ling et al., 2003).

RESULTS

Antimicrobial susceptibility testing

All isolates were sensitive to ceftriaxone and only one isolate was resistant to ceftiofur. Eighty-six isolates (45.99%) displayed the pentaresistance phenotype (ACSSuT). Resistance to nalidixic acid, tetracycline, streptomycin, trimethoprim- sulfamethoxazole and ampicillin was found in 96.30, 95.68, 91.98, 91.36 and 90.74% of the isolates, respectively. Isolates from chickens resistant to ampicillin, trimethoprim-sulfamethoxazole, nalidixic acid and tetracycline were more than the isolates from pigs. However, resistance to florfenicol, ciprofloxacin and amikacin of the isolates from pigs was higher than isolates from chickens (Table 1).

PCR detection of integrons and gene cassettes Class 1 integrons were positive in 84.49% (158/187)

Table 1. Percentages of *Salmonella* Schwarzengrund isolates resistant to antibiotics from broiler chickens and pigs.

Antibiotic	% of resistant isolates from		
	Broiler(n = 159)	Pig (n = 28)	Overall (n = 187)
Ampicillin	93.06 (134)	72.22 (13)	90.74
Chloramphenicol	69.44 (100)	72.22 (13)	69.75
Florfenicol	18.75 (27)	66.67 (12)	24.07
Streptomycin	91.67 (132)	94.44 (17)	91.98
Trimethoprim-Sulfamethoxazole	94.44 (136)	66.67 (12)	91.36
Nalidixic acid	97.92 (141)	83.33 (15)	96.30
Ciprofloxacin	4.17 (6)	38.89 (7)	8.02
Tetracycline	96.53 (139)	88.89 (16)	95.68
Amikacin	1.39 (2)	66.67 (12)	8.64
Ceftiofur	0.69 (1)	0	0.62
Ceftriaxone	0	0	0

Table 2. Characteristics of the serovar Schwarzengrund strains tested in this study.

Size(kb) of integron I and identity of resistance gene	Variant genomic island name	Isolated from		No. of strains
		Broiler	pig	
None	-	10	4	10
1.2- *blaPSE-1*, *aadA2*	SGI1	1	1	2
1.2- *blaPSE1*, *dfraA1*	SGI1-F	1	1	2
1.2- *blaPSE-1*, 1.0- *aadA2*	SGI1	2	0	2
1.9- *dfrA12*, *aadA2*	-	130	22	152

isolates with 3 PCR amplification product patterns: 1,900 (152 isolates), 1,200 (4 isolates) and 1,000 + 1,200 bp (2 isolates) detected. Sequence analysis of the integron PCR products showed the presence of classic gene cassettes in the integrons, including *aadA2* (which confer resistance to streptomycin), the dihydrofolate reductase gene cassettes *dfrXII*, *dfrA1* (which confer resistance to trimethoprim) and the beta-lactamase gene bla_{PSE1} (which confers resistance to ampicillin) (Table 2). Six isolates were positive to the SGI1. PCR mapping of the antibiotic resistance gene cluster demonstrated that 4 isolate contain the SGI1 and 2 isolate contain SGI1-F (Table 2).

Genotype of PFGE

Eleven PFGE profiles were observed from the 187 isolates in the study when total genomic DNA was digested with *Xba*I. Genotype 2D was seen in 84 isolates (44.92%) and genotype 2B was represented by 44 isolates (22.53%) . The third common genotype was 1A which was found in 15 isolates (8.02%). A dendrogram generated from Dice coefficients of similarity indicated that all isolates were highly related with Dice values greater than 84% (Figure 1). The predominant genotypes (1A, 2B and 2D) were observed in isolates from both chicken and pig sources.

For all isolates of *Salmonella* Schwarzengrund, screening gave negative results for *qnrA*, *qnrB* and *qnrS* genes. And 13 ciprofloxacin-resistant isolates were all detected as double mutations in the QRDR of *gyrA* (S83F and D87G), double mutations in the QRDR of *parC* (T57S and S80R) and an additional mutation in the QRDR of *parE* (S458P). These 13 ciprofloxacin-resistant *Salmonella* Schwarzengrund isolates were isolated from 2 different chicken slaughterhouses and 2 different pig slaughterhouses. All these isolates had the same genotype as 2D.

DISCUSSION

Nontyphoidal *Salmonella* which causes foodborne diseases has become an important public health problem worldwide. *Salmonella* Enteritidis and *Salmonella* Typhimurium were the most common causes of non-typhoidal salmonellosis. *Salmonella* Schwarzengrund is a less common cause of human salmonellosis worldwide. However, the incidence of *Salmonella* Schwarzengrund has increased in recent years (Bangtrakulnonth et al., 2004; Boyd et al., 2002). This serovar is commonly found from invasive salmonellosis patients in Taiwan

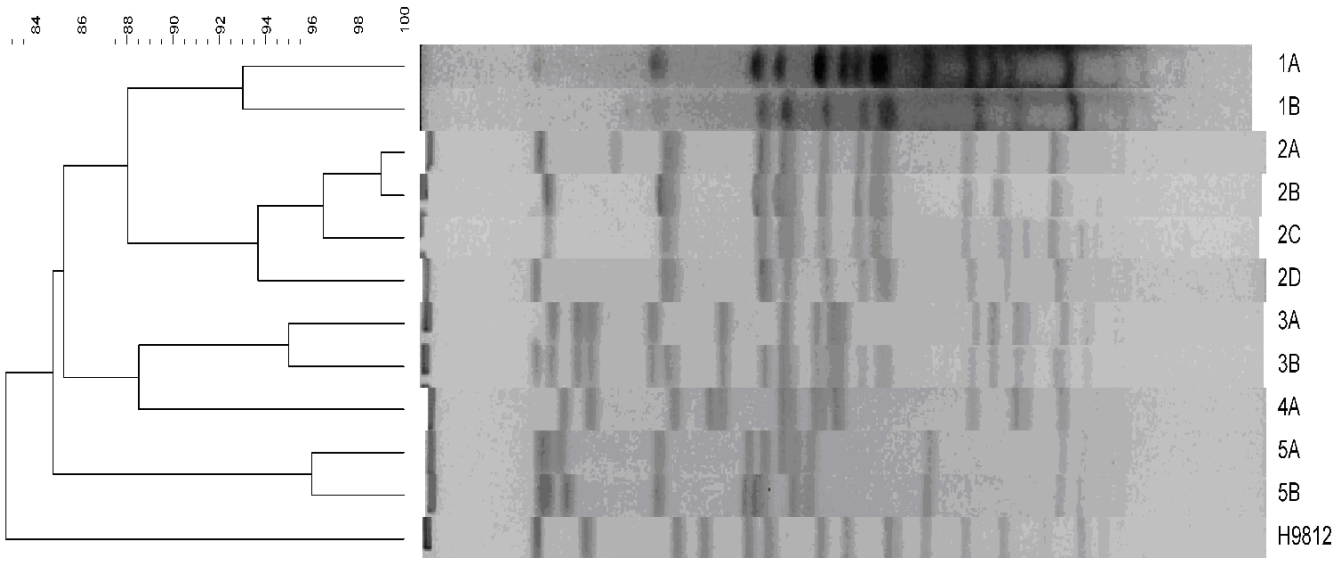

Figure 1. PFGE patterns of XbaI-digested chromosomal DNA of *S. enterica* serovar Schwarzengrund. The dendrogram was constructed by use of the UPGMA algorithm and the Dice similarity coefficient by using Gelcompare II software with 1% position tolerance.

(Boyd et al., 2002).

This study showed a high frequency of antimicrobial drug resistance, including ampicillin, trimethoprim-sulfamethoxazole, among *Salmonella* Schwarzengrund isolates from slaughtered chickens. In contrast, the frequency of resistance to florfenicol, ciprofloxacin and amikacin of isolates from pigs was higher. It may correlate the antibiotics used in the pig farms and broiler farms. 13 ciprofloxacin-resistant *Salmonella* Schwarzengrund isolates found in 2 different chicken slaughterhouses and 2 different pig slaughterhouses had the same mutations in the QRDRs of *gyrA*, *par* and identical PFGE pattern. Molecular characterization suggests an epidemiological relationship between the swine and chicken *Salmonella* Schwarzengrund isolates. Our results suggest that there is a risk of transmitting multidrug resistant *Salmonella* between pig and chicken farms, and also suggest that there is a need to prevent the transmission of this organism from a neighboring contaminated farm.

This study also found the widespread occurrence of class 1 integrons and SGI1 variants in multidrug resistant *Salmonella* Schwarzengrund isolates from broiler chickens and pigs. Genes encoding for resistance to trimethoprim (*dfrA12*) and aminoglycosides (*aadA2*) were most commonly found. The presence of this 1.9 kb integrons has been found in many serovars worldwide (Doublet et al., 2005; Fluit 2005; Gay et al., 2006; Hsu et al., 2006). The transfer of conjugative plasmids is a common mechanism for genetic exchange between bacteria, transfer can occur both within bacterial species and between different species. Therefore, the presence of the integron-carrying *Salmonella* isolates detected in this study could have contributed not only to the spread of drug-resistant strains but also to the spread of mobile elements carrying drug resistance genes across bacterial species.

REFERENCES

Aarestrup FM, Hendriksen RS, Lockett J, Gay K, Teates K, McDermott PF, White DG, Hasman H, Sørensen G, Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, Angulo FJ, Gerner-Smidt P (2007). International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerg. Infect. Dis. 13: 726 - 731.

Bangtrakulnonth A, Pornreongwong S, Pulsrikarn C, Sawanpanyalert P, Hendriksen RS, Wong LF, Aarestrup FM (2004). *Salmonella* serovars from humans and other sources in Thailand, 1993–2002. Emerg. Infect. Dis. 10: 131 - 136.

Barrett TJ, Gerner-Smidt P, Swaminathan B (2006). Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog. Dis. 3: 20 - 31.

Boyd D, Cloeckaert A, Chaslus-Dancla E, Mulvey MR (2002). Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. Antimicrob. Agents Chemother. 46: 1714 - 1722.

Casin I, Breuil J, Darchis JP, Guelpa C, Collatz E (2003). Fluoroquinolone resistance linked to GyrA, GyrB, and ParC mutations in *Salmonella enterica* typhimurium isolates in humans. Emerg. Infect. Dis. 9: 1455 - 1457.

Chen TH, Wang YC, Chen YT, Yang CH, Yeh KS (2006). Serotype occurrence and antimicrobial susceptibility of *Salmonella* isolates recovered from pork carcasses in Taiwan (2000 through 2003). J. Food Prot. 69: 674 - 678.

Chiu CH, Lin TY, Ou JT (1999). Predictors for extraintestinal infection of non-typhoidal *Salmonella* in patients without AIDS. Int. J. Clin. Pract. 53: 161 - 164.

Clinical and Laboratory Standards Institute (2007). Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement, M100-S17. CLSI, Wayne, Pennsylvania.

Cloeckaert A, Sidi BK, Flaujac G, Imberechts H, D'Hooghe I, Chaslus-Dancla E (2000). Occurrence of a *Salmonella enterica* serovar Typhi-

murium DT104-like antibiotic resistance gene cluster including the *floR* gene in *S. enterica* serovar Agona. Antimicrob. Agents Chemother. 44: 1359 - 1361.

Doublet B, Boyd D, Mulvey MR, Cloeckaert A (2005). The *Salmonella* genomic island 1 is an integrative mobilizable element. Mol. Microbiol. 55: 1911 - 1924.

Fluit AC (2005). Towards more virulent and antibiotic-resistant *Salmonella*? FEMS Immunol. Med. Microbiol. 43: 1 - 11.

Gay K, Robicsek A, Strahilevitz J, Park CH, Jacoby G, Barrett TJ, Medalla F, Chiller TM, Hooper DC (2006). Plasmid-mediated quinolone resistance in non-Typhi serotypes of *Salmonella enterica*. Clin. Infect. Dis. 43: 297 - 304.

Hsu SC, Chiu TH, Pang JC, Hsuan-Yuan CH, Chang GN, Tsen HY (2006). Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. Int. J. Antimicrob. Agents 27: 383 - 391.

Khemtong S, Chuanchuen R (2008). Class 1 Integrons and *Salmonella* Genomic Island 1 Among *Salmonella enterica* Isolated from Poultry and Swine. Microb. Drug Resist. 14: 65 - 70.

Kwon HJ, Kim TE, Cho SH, Seol JG, Kim BJ, Hyun JW, Park KY, Kim SJ, Yoo HS (2002). Distribution and characterization of class 1 integrons in *Salmonella enterica* serotype Gallinarum biotype Gallinarum. Vet. Microbiol. 89: 303 - 309.

Lauderdale TL, Aarestrup FM, Chen PC, Lai JF, Wang HY, Shiau YR, Huang IW, Hung CL, TSAR hospitals (2006). Multidrug resistance among different serotypes of clinical *Salmonella* isolates in Taiwan. Diagn. Microbiol. Infect. Dis. 55: 149 - 155.

Leverstein-van Hall MA, Blok M, Donders AR, Paauw A, Fluit AC, Verhoef J (2003). Multidrug resistance among *Enterobacteriaceae* is strongly associated with the presence of integrons and is independent of species or isolate origin. J. Infect. Dis. 187: 251 - 259.

Lévesque C, Piché L, Larose C, Roy PH (1995). PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. 39: 185 - 191.

Ling JM, Chan EW, Lam AW, Cheng AF (2003). Mutations in topoisomerase genes of fluoroquinolone-resistant *salmonella*e in Hong Kong. Antimicrob. Agents Chemother. 47: 3567 - 3573.

Levings RS, Lightfoot D, Partridge SR, Hall RM, Djordjevic SP (2005). The Genomic Island SGI1, Containing the Multiple Antibiotic Resistance Region of *Salmonella enterica* Serovar Typhimurium DT104 or Variants of It, Is Widely Distributed in Other *S. enterica* Serovars. J. Bacteriol. 187: 4401 - 4409.

Varma JK, Greene KD, Ovitt J, Barrett TJ, Medalla F, Angulo FJ (2005). Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984-2002. Emerg. Infect. Dis. 11: 943 - 946.

Vo AT, van Duijkeren E, Fluit AC, Wannet WJ, Verbruggen AJ, Maas HM, Gaastra W (2006). Antibiotic resistance, integrons and *Salmonella* genomic island 1 among non-typhoidal *Salmonella* serovars in The Netherlands. Int. J. Antimicrob. Agents 28: 172 - 179.

Vugia DJ, Samuel M, Farley MM, Marcus R, Shiferaw B, Shallow S, Smith K, Angulo FJ (2004). Emerging Infections Program FoodNet Working Group. Invasive *Salmonella* infections in the United States, FoodNet, 1996-1999: incidence, serotype distribution, and outcome. Clin. Infect. Dis. 38: S149 - 156.

Yan JJ, Chiou CS, Lauderdale TL, Tsai SH, Wu JJ (2005). Cephalosporin and ciprofloxacin resistance in *Salmonella*, Taiwan. Emerg. Infect. Dis. 11: 947 - 950.