Characterization of 13 multi-drug resistant *Salmonella* serovars from different broiler chickens associated with those of human isolates

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Abstract

**Background:** *Salmonella* are frequently isolated from chickens and their products. Prevalent serogroups and serovars of *Salmonella* as well as their genotypes and antibiograms were determined for cloacal samples from 1595 chickens. To understand the possible serovar and H antigens for transmission between chicken and human, serovars and their H antigens of 164 chicken and 5314 human isolates were compared.

**Results:** Prevalence of *Salmonella* differed among chicken lines and ages. Chicken and human isolates belonged mainly to serogroup B, C1, C2-C3, D, and E. 13 serovars and 66 serovars were identified for chicken and human isolates respectively. The common serovars for chicken and human isolates were *S*. Typhimurium, *S*. Enteritidis, *S*. Albany, *S*. Derby, and *S*. Anatum and shared common H1 antigens "g complex; i; e;h; and z4,z24" and H2 antigens "1 complex and -". In human isolates, H1 antigen "i" and H2 antigen "-" were common in all serogroups. In chicken, antimicrobial susceptibility differed among serogroups, serovars and three counties. All isolates were susceptible to cefazolin and ceftriaxone, but highly resistant to ampicillin, chloramphenicol, flumequine, streptomycin, sulfamethoxazole-trimethoprim, and tetracycline. Except those isolates of serogroup C1 of Chick group and serogroup G, all isolates were multi-drug resistance. Only *S*. Kubacha, *S*. Typhimurium, *S*. Grampian, and *S*. Mons were resistant to ciprofloxacin and/or enrofloxacin.

**Conclusion:** In chicken, prevalent serogroups and serovars were associated with chicken ages, lines and regions; and fluoroquinolone-resistant and MDR isolates emerged. H1 antigens "g complex and i" and H2 antigens "1 complex and -" might be important for transmission of *Salmonella* between chicken and human.

**Background**

*S*. Enteritidis and *S*. Typhimurium, as two main zoonotic and broad-host-range pathogens that cause human salmonellosis, have been frequently isolated from poultry and their products [1-8]. Prevalence of *Salmonella* differs between layers and broilers [9,10]. Factors influencing the prevalence of chicken-associated *Salmonella* are feeds and growth environment [11], transportation process [12,13], and chick sources [14]. Moreover, age-associated prevalence has been reported in layers, maximal prevalence at 18 weeks before egg production and gradually decreases with aging [15]. In broiler the prevalence differed depending on sale sites from 17.9% in slaughterhouses [16] and up to nearly 100% in the open markets and supermarkets [17]. Appearance of monophasic variants such as in *S*. Typhimurium [4,5,12:1:-] [18,19] increases the problem in serotyping. Therefore, molecular methods have been developed to differentiate the serovars based on the nucleotide sequence variations in flagellar structural genes *flIC* and *flJB* [20-22] and PFGE analysis [15,23,24]. Prevalent serovars differ between chickens and ducks [25] and are associated with chicken lines and geographic area [15,25-27]. In Taiwan, we reported that *Salmonella* serogroup C1 and B, especially *S*. Typhimurium, were predominant *Salmonella* in duck and geese [7,8]. In another study of duck, the prevalence of *Salmonella* was 4.6% and *S*. Potsdam, *S*. Dusseldorf, and...
S. Indiana were the predominant serovars [28]. Therefore, we analyzed the prevalence of *Salmonellae* among different chicken sources and determined serotypes by PFGE analysis first, followed by traditional agglutination test of each genotype. After characterizing antibiograms and genomic variations in chromosome and plasmid of chicken isolates, flagellar antigens of chicken and human isolates were compared to understand the common antigens possibly for transmission of *Salmonella* between human and chicken.

**Methods**

**Sample collection and enrichment**

Totally 1595 chickens of 1-year-old broiler breeder, 1-day-old chickens (Chick) and 9-week-old chickens (NHC) of Taiwan broiler chicken, 1-year-old layers and 3-week-old broiler were sampled by 108C Amies Agar Gel - Single plastic swab (Copan Diagnostic Inc. Murrieta CA 92562 USA) from cloaca of each chicken fed at different farms in Chiayi of Taiwan from 2002 to 2003. Layers and broilers were fed in commercial cage and house farm respectively. The sampled swabs were grown in 9 mL of gram-negative broth (GN, Difco 0486) at 37°C for 24 h. Over-night GN bacterial broth was streaked on xylose lysine deoxycholate (XLD, Difco 0788) plates, which were incubated at 37°C for 24 h. Black colonies were further examined by biochemical tests including triple sugar iron agar (TSI), Christensen’s urea agar (URE), Simmons’ citrate agar (CIT), sulfide-indole-motility medium (SIM), Voges-Proskauer medium (VP), Møller’s ornithine decarboxylase medium (ORN), lysine iron agar (LIA) and mobility-indole-ornithine agar (MIO) purchased from Merck (Taiwan). At least two positive isolates from each plate were maintained on brain heart infusion agar (BHIA). In addition, *Salmonella* from 9-week-old NHC in Tainan (36 isolates) and Pintung (30 isolates) at same period were also analyzed.

**Serogroup and serotype identification**

*Salmonella*-positive isolates were further serogrouped by the slide agglutination test with the use of O-antiserum and serotyped by the tube agglutination test with the use of H-antigen antisera. Both antisera were purchased from Difco (Becton Dickinson Co., Franklin Lakes, NJ, USA). In addition, 5314 *Salmonellae* were collected from 19 medical centers and district hospitals located throughout the countries from 2003 to 2005 and serotyped in the *Salmonella* Reference Laboratory of Centers for Disease Control (CDC), Department of Health, Taiwan, with antisera purchased from S&A Reagents Lab (Bangkok, Thailand), Denka Seiken (Tokyo, Japan), Statens Serum Institut (Copenhagen, Denmark), and a local biotech company, LTK Biolo laboratories (Taoyuan, Taiwan). Phase induction was performed using a paper-bridged method developed in the laboratory of Taiwan CDC [29].

**Antimicrobial susceptibility test**

Each isolate was examined by disk diffusion method for its susceptibility to the antimicrobial agents including ampicillin (A, 10 μg), cefazolin (CZ, 30 μg), ceftiraxone (Cro, 30 μg), chloramphenicol (C, 30 μg), streptomycin (S, 10 μg), sulfamethoxazole-trimethoprim (Sxt, 1.25/23.75 μg), and tetracycline (T, 30 μg). In addition, resistance to three fluoroquinolones: flumequine (Ub, 30 μg) of limited spectrum quinolone and enrofloxacin (En, 5 μg) as well as ciprofloxacin (Ci, 5 μg) of broad spectrum quinolone. While single bacterial colony was taken into 5 mL of Mueller-Hinton broth (MHB; Merck, Taiwan) and cultured at 37°C for 8 hrs, bacterial broth was then adjusted to 0.5 Mcfarland and plated on Mueller-Hinton agar (MHA; Merck, Taiwan). Antimicrobial disks (BD Diagnostic systems, USA) were plated onto MHA agar and then incubated at 37°C for 18 hrs. Susceptibility and resistance were determined according to the interpretation criteria to *E. coli* (ATCC No. 25922) established by Clinical Laboratory Standards Institute (CLSI) standard [30]. Multi-drug resistance (MDR) isolate is defined as that isolate resistance to two or more antibiotics belonging to different antibiotic classes.

**Plasmid and genotype analysis**

Plasmid DNA pattern was determined by Kado and Liu method [31] and purified plasmid DNA was subjected to gel electrophoresis with 0.6% SeaKem GTG agarose (Cambrex Bio Science Rockland, Inc, Rockland, ME, USA) at 50 V for 2.5 hrs. Genotypes of all isolates were determined by PFGE analysis with restriction endonuclease *XbaI* digestion. The procedure of PFGE analysis was described earlier [32]. The digested DNA was separated by CHEF Mapper XA system (BioRad, Hercules, California, USA) in 0.5 × TBE at 14°C for 22 h with Auto-Algorithm model of 30-600 kb, 6 V/cm, switching interval 4.0-70.0 sec. The genotypes were defined as 3 band differences between two isolates [33].

**Results**

**Prevalent serogroups and serovars among chicken lines and locations**

Prevalence of *Salmonella* differed between chicken lines (0% for layer vs 0.3% for breeder broiler and 11.3% for broiler) and ages from 10.3% for Chick and 3.8% for NHC of Taiwan broiler chicken (Table 1). 164 *Salmonella* isolates belonged to serogroup C1, B, D, C2-C3, E, and G in the decreasing order and the number of serogroups differed among 3 counties. Further, region-specific serogroups were identified as serogroup G in Chiayi, serogroup D in Tainan, and serogroup C2-C3...
and E in Pintung (Table 1). In Chiayi, age-associated serogroups were found for serogroup C1 Salmonella in Chick group and serogroup B and G in NHC group (Table 1).

164 Salmonella isolates were firstly examined for their genotypes by XbaI-PFGE analysis (Figure 1) and further isolates of each genotype were serotyped by traditional agglutination method. In total, 18 PFGE patterns belonged to 13 serovars (Table 2). Except S. Albany and S. Havana that consisted of multiple genotypes, PFGE genotypes matched exactly with serotypes. 13 serovars were S. Derby, S. Kubacha, S. Mons, and S. Typhimurium (containing S. Typhimurium var. Copenhagen) of serogroup B, S. Choleraesuis (containing non-typable serovar), S. Grampian, S. Hissar, and S. Redba of serogroup C1, S. Albany and S. Blockley of serogroup C2-C3, S. Enteritidis of serogroup D, S. Anatum of serogroup E and S. Havana of serogroup G (Table 2). Predominant serovar in each serogroup was S. Mons, not S. Typhimurium, in serogroup B, S. Choleraesuis from

Table 1 Prevalence of Salmonella serogroups in different layer- and broiler chickens in three Counties

| Serogroup | Countya | Chiayi | Tainan | Pintung | Total isolates |
|-----------|---------|--------|--------|---------|---------------|
|           | Layer   | Breeder| Broiler| NHC     | Chick         |
| B         | 0       | 0      | 1      | 0       | 16            |
| C1        | 0       | 0      | 0      | 0       | 0             |
| C2        | 0       | 0      | 0      | 0       | 0             |
| D         | 0       | 0      | 0      | 0       | 0             |
| E         | 0       | 0      | 0      | 0       | 0             |
| G         | 0       | 0      | 0      | 0       | 0             |
| Total     | 0       | 1      | 17     | 5       | 77            |
| Prevalence| (%)     | 0.0    | 11.3   | 3.8     | 10.3          |

a The number of each serogroup was determined in our laboratory by examination of Salmonella isolated from cloacal samples of chicken in Chiayi County and from surveillance of Tainan and Pintung County.

b NHC: 9-wk-old Native Hybrid Chickens (simulated native chicken) of Taiwan broiler chickens

c Chick: one-day-old NHC chicks.

Figure 1 XbaI-digested PFGE genotypes of each Salmonella serogroups. M: lambda ladder size marker. SC1: non-typable serogroup C1 Salmonella. SC16: S. Redba. C34: S. Derby. SW1: S. Grampian. P15: S. Blockley. P18, P24, and P34: S. Albany. P23: S. Mons. C31: S. Typhimurium var. Copenhagen. SR2: S. Kubacha. P1: S. Derby. P10: S. Typhimurium. C11: S. Enteritidis. P22: S. Anatum. SC9 and SC10: S. Havana. Genotypes I to IV are defined as difference more than 3 bands between two isolates [33].
Table 2 Characterization of *Salmonella* isolates by 4 methods

| Serogroup | Serovar | County | Chicken lines | Resistance type | PFGE genotype | Plasmid type | Total isolates |
|-----------|---------|--------|---------------|-----------------|---------------|--------------|----------------|
| Derby     | Pintung | NHC    | E             | IV              | S             | 1            |
| Kubacha   | Pintung | NHC    | M             | Illa            | 2a            | 2            |
|           | Chiayi  | NHC    | J             | Illa            | 4a            | 1            |
|           | Broiler |        |               |                 | 1             | 1            |
|           | Chiayi  | NHC    | K             | Id              | 1a            | 1            |
|           | Breeder | C      | I             | e               | 2b            | 1            |
| B         | Mons    |        |               |                 |               |              |
|           | Tainan  | NHC    | L             | II              | 4             | 1            |
| Typhimurium var. Copenhagen | Tainan | NHC | M | V | 3a | 2 |
| Choleraesuis | Chiayi | Chick | A | III | 1 | 59 |
|           | Tainan  | NHC    | C1            | IV              | 1             |              |
|           | Grampian|        |               |                 |               |              |
|           | Hissar  | Chiayi | A | I | 5 | 10 |
|           | Redba   | Chiayi | A | II | 5 | 1 |
| C2        | Blockley| Pintung | NHC | E | II | 3 |
|           | Albany  | Pintung | NHC | J | III | 5 |
|           | F       |         |               |                 |               |              |
| D         | Enteritidis | Tainan | NHC | I | 3 | 7 |
|           |         |         |               |                 | 1             | 7            |
| E         | Anatum  | Pintung | NHC | J | I | 1 |
|           |         |         |               |                 | B             | 2            |
| G         | Havana  | Chiayi | A | II | 2 |

*a*Antibiogram of each isolate was determined by the resistance to antimicrobials ampicillin (A), chloramphenicol (C), ciprofloxacin (Ci), ceftriaxone (Cr), cefazolin (Cz), enrofloxacin (En), flumequine (Ub), streptomycin (S), sulfamethoxazole-trimethoprim (Sxt), tetracycline (T). The association of resistance type with antibiogram was the followings: resistance type A for antibiogram S, B for Ub, C for Ubs, D for ST, E for SxtUb, F for CSTUb, G for ASSxtTUb, H for ACSSxtTUb, and M for ACCiEnSSxtTUb.

*b*PFGE genotypes was determined by 3 band differences between two isolates [Figure 1, [32]].

*c*Plasmid was analyzed by Kado and Liu method (30, supplementary Figure 1). Plasmid profile was determined by plasmid size and number (supplementary Table 2).

*d*NT: non-typable

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Chick and S. Grampian from NHC in serogroup C1, and S. Albany in serogroup C2-C3 (Table 2).

**Antimicrobial susceptibility**

All isolates were susceptible to CZ and Cro. In contrast to resistance only to streptomycin for 77 S. Choleraesuis isolates in Chick group and two isolates of serogroup G, all isolates were MDR (Table 3). Serogroup B, C2-C3 and E were highly resistant to A, C, S, Sxt, T and Ub. However, serogroup D was relatively low in resistance to above antimicrobials. Serogroup and serovars isolated from broiler and NHC group differed in resistance to three quinolone antimicrobials. Except serogroups E and G, all serogroups, were nearly 100% resistance to Ub and only serogroups B and C1 were resistant to En and Ci (Table 3). Among 164 isolates, we only found 4 En-resistant S. Mons and 13 En and Ci-resistant isolates including 2 S. Kubacha isolates, 2 S. Typhimurium isolates, and 1 S. Typhimurium var. Copenhagen isolates of serogroup B and 8 S. Grampian isolates of serogroup C1 (Table 2). Importantly, near 40% of isolates from Pintung were resistant to En and Ci. According to resistance to 9 antimicrobials tested, 13 antibiograms differed among serogroups and serovars (Table 2 and 3). Highest drug-resistant types L with antibiogram ACCiEnSxtTUb and M with antibiogram ACCiEnSxtTUb were only found in serogroup B and C1 of NHC group from Pintung mostly and Tainan. *Salmonella* genomic island (SGI) related ACSSuT resistance was found in serogroup B, C2 and E. Resistance to antimicrobials tested varied among 3 counties (Table 3 and Additional file 1: Table S1). Highest resistance was found in isolates from Pintung, followed by Tainan, and Chiayi and lowest Sxt resistance rate was observed in isolates from Tainan.

**Plasmid profile analysis**

Based on plasmid number and size determined by gel electrophoresis and plasmid size marker 50 kb and 90 kb of OU7526, in total 19 plasmid profiles were identified and the plasmid profiles and their number differed among serogroups and serovars (Additional file 2: Table S2; Additional file 3: Figure S1). Among 13 serovars, S. Albany, S. Blockley, S. Havana, and S. Redba as well as few isolates of S. Choleraesuis, S. Enteritidis, and S. Typhimurium lacked plasmid. All other serovars harbored at least one plasmid and differed in plasmid profile.

**Sero var association between chicken and human isolates**

S. Albany, S. Anatum, S. Choleraesuis, S. Derby, S. Enteritidis, and S. Typhimurium were in common for 13 chicken serovars and 66 human serovars and other 7 serovars of chicken isolates were not or barely observed in human (Table 2, 4 and 5). Total serovar number of each serogroup decreased from serogroup C1, B, C2, E to D for human isolates (Table 4). Despite of the presence of 66 serovars, there were only presence of 11 H1 antigens including b, c, d, j, k, r, y, eb, g-complex, and z-complex and 5 H2 antigens including -, z6, lw, 1-complex, and en-complex (Table 4). Common antigens in all serogroups were “T” for H1 antigen: and “-” for H2 antigen. In compared the chicken and human isolates from Taiwan, United Kingdom and United States, the common serovars were S. Typhimurium, S. Enteritidis, S. Anatum, and S. Derby with common antigens of . “g complex; i; z4,z24; and e,h” for H1 antigen and “- and 1 complex” for H2 antigen (Table 5).

**Discussion**

As one of main pathogen to cause foodborne diseases, *Salmonella* has been frequently reported among different animal sources, especially more divergent *Salmonella* serovars found in chickens [34]. With the limited serovars in 164 chicken isolates, serogroups C2, D, E and G were restricted in one county and serogroup B and C1 were found in all three counties (Table 2), suggesting possibly that serogroup B and C1 isolates may

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Table 3 Differences in prevalence of resistance to 9 antimicrobials among serogroups and Counties

| Antibiotics | Serogroup (%) | County (%) |
|-------------|---------------|-----------|
|             | B  | C1 | C2  | D  | E  | G  | Chiayi | Tainan | Pintung |
| A           | 61.5 | 11.4 | 100  | 0  | 100 | 0  | 23.8   | 47.1   | 77.4    |
| C           | 89.7 | 10.2 | 91   | 0  | 100 | 0  | 90.5   | 70.6   | 74.2    |
| Ci          | 12.8 | 9.1  | 0    | 0  | 0   | 0  | 0      | 2.9    | 38.7    |
| En          | 20.5 | 9.1  | 0    | 0  | 0   | 0  | 4.7    | 8.8    | 38.7    |
| S           | 97.4 | 100  | 91   | 55.6| 100 | 100| 100    | 76.5   | 93.5    |
| Sxt         | 94.9 | 12.5 | 91   | 55.6| 100 | 100| 85.7   | 47.1   | 96.8    |
| T           | 94.9 | 12.5 | 91   | 55.6| 100 | 100| 85.7   | 76.5   | 93.5    |

* A for ampicillin, C for chloramphenicol, Ci for ciprofloxacin, En for enrofloxacin, S for streptomycin, Sxt for sulfamethoxazole-trimethoprim, T for tetracycline, and Ub for Ub for flumequine.
be more adapted to chicken. In human isolates, we found that the serovar number in each serogroup were not associated positively with the serogroup prevalence, such as highest serovar number in low prevalent serogroup C1 vs lower serovar number in high prevalent serogroup B and serogroup D (Table 4). These results imply that serogroup C1 may occasionally infect human isolates. Further, serovars are determined by flagellins: H1 and H2 antigens encoded by \textit{fliC} and \textit{fljB}. A son of the most important immunogens, flagellin interacts with the toll-like receptor 5 (TLR5) to activate NFκB pathway and proinflammatory genes to regulate innate and adaptive immune system [35-38]. However, aflagellar serovars \textit{S}. Pullorum and \textit{S}. Gallinarum cause more severe infection than flagellar serovars in chicken because of aflagellar \textit{S}. Typhimurium, \textit{S}. Albany, \textit{S}. Derby, \textit{S}. Anatum and \textit{S}. Havana were common in both hosts (Table 5). However, these serovars shares same antigens: g complex; i; and z4, z24 of H1 antigen and 1 complex and - of H2 antigens (Table 5), implying these antigens may be important for \textit{Salmonella} transmission between chicken and human.

Prevalent serogroups and serovars are related to chicken lines (Table 1)[9,10] and ages [15]. In layer, age-related prevalence was reported earlier [15] and no \textit{Salmonella} was isolated from 1-year-old layers in the present study (Table 1). Such age-associated clearance may be due to stronger antigen-specific T-cell response in older chicken [41] and not related to B-cell response [42]. Age-related serovars were also identified in Taiwan broiler chickens (Table 2). Almost all isolates were \textit{S}. Choleraesuis and non-typable \textit{Salmonella} (possibly monophasic \textit{S}. Choleraesuis) of serogroup C1 in Chick group and \textit{S}. Mons of serogroup B in NHC group (Table 2). As swine-adapted pathogen, \textit{S}. Cholearesuis has seldom reported from chicken. However, \textit{S}. Choleraesuis in 1-day-old chicks may be contaminated from

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**Table 4 The H1 and H2 antigens of 66 Salmonella serovars of human isolates collected from 2003 to 2005**

| H antigen | Serogroup | B | C1 | C2 | D | E | Others |
|-----------|-----------|---|----|----|---|---|--------|
| H1        |           | 11| 19 | 9  | 7 | 8 | 12     |
| b         | ±²        | - | +  | -  | - | + | -      |
| c         | -         | + | -  | +  | - | - | -      |
| d         | +         | - | +  | -  | + | - | +      |
| i         | +         | + | +  | +  | - | + | +      |
| k         | +         | + | +  | -  | - | - | -      |
| H1        | r         | - | -  | -  | - | + | -      |
|          | y         | - | +  | -  | - | - | -      |
|          | e,h       | - | -  | -  | - | + | -      |
| g complex | f,g,l,r/s,f,l,r,m, [p]/g,p | +/+/+/² | -/-/- | -/-/- | -/+/- | -/-/- | -/-/- |
|           | g,m, l/s,g,m, [p],v/g,t | -/-/- | +/-/+ | +/+/- | -/+/- | -/+/- | -/+/- |
| l complex | l,v/l,w/l,z13 | -/-/- | -/-/- | -/+/- | +/+/- | -/-/- | +/+/- |
| z complex | z+/z10/z29/z38 | -/+/-/- | -/+/-/- | -/+/-/- | -/+/-/- | -/+/-/- | -/+/-/- |
| Total antigens | 6 | 7 | 5 | 4 | 5 | 4 | 4     |

- ±² means presence (+) or absence (-) of b antigen.
- +/+/-/² indicates presence (+) of antigens f,g,l,r,s and absence (-) of antigens [f,l,r,m, [p],g,p.
the hatchery, particular from eggshell membrane; in which S. Typhimurium, not S. Choleraesuis, is main serovar [43]. If highly invasive S. Choleraesuis could infect chicks and use the chicken as reservoir, it will lead to a public problem of circulating such high invasive serovar in animals. In broiler, prevalence of Salmonella differed between chicken parts (2.36% for legs and 4.25% for breasts of broiler) [19]. Further, prevalent serovars differ between sampling sources e.g. the S. Anatum and S. Rispen in chicken meat [44] and S. Blockley, S. Hadar and S. Bredeney in the cecal samples (24).

Several methods have been developed to differentiate clinical isolates. In this study, PFGE patterns almost matched serotypes, although S. Albany and S. Havana lacked plasmid (Table 2). These results may imply that recent evolution of Salmonella might be mainly through plasmid acquisition to introduce beneficial genes for host serovar to survival.

Antimicrobial susceptibility of Salmonella can be used to monitor drug abuse in different regions (Table 2) [46] and animal sources [44,47]. Early study reported that Salmonella from chicken, not from human, pig and cattle, was less resistance to A, C, and Sxt [47]. Nevertheless, resistance to T was frequently found in chicken isolates [48]. Since discovery of ACSSuT-resistant region in SGI of S. Typhimurium DT104 [49], variations within SGI and complex integron In104 change the antimicrobial resistance [50]. In this study, our chicken isolates were highly resistant to antimicrobials A, C, S, Sxt, T and Ub (Table 3). These results imply that S. Albany, S. Anatum, S. Grmpian, S. Hissar, S. Kubacha, S. Mons, and S. Typhimurium with resistance types from H to M may be derived from misuse of antimicrobials or due to presence of SGI and/or integron [51]. Mechanism to develop En and Ci resistance is due to mutation in quinolone-resistance determining region or expression of

| Table 5 Serovars of chicken isolates associated with those of human isolates collected from 2003 to 2005 |
|--------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Serogroup B Serovars of chicken isolates in this study | Prevalence (%) of serovar of chicken and human isolates from different area | | | | | | | | | | | | | |
| | H antigen | 2003 | 2004 | 2005 | 2003 | 2004 | 2005 | 2003 | 2004 | 2005 | 2003 | 2004 | 2005 | 2003 | 2004 | 2005 | 2003 | 2004 | 2005 | 2003 | 2004 | 2005 |
| | | USA* | UK* | USA* | USA* | UK* | USA* | USA* | UK* | USA* | USA* | UK* | USA* | USA* | USA* | USA* | USA* | USA* | USA* |
| Serogroup B Derby fg | 0.2 | 0.3 | 0.3 | 2.4 | 0 | 0 | 3.8 | 2.7 | 0.03 | 0.2 | 0.34 | 2.3 | 0.2 | 0.34 | 0.2 | 0.34 | 0.2 | 0.34 | 0.2 | 0.34 |
| Kubacha | i | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mons d | i | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Typhimurium i | 1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serogroup C1 Choleraesuis c | 0 | 0 | 0 | 0.03 | 4.2 | 0 | 0 | 0.05 | 4.3 | 0.03 | 0 | 0.02 | 2.0 | 0 | 0.02 | 2.0 | 0 | 0.02 | 2.0 | 0 | 0.02 |
| Grampian r | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hissar c | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Redba | Z0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serogroup C2-C3 Blockley k | 1.5 | 0 | 0 | 0.18 | 0 | 0 | 0 | 0.23 | 0 | 0.05 | 0 | 0.14 | 0 | 0 | 0.14 | 0 | 0 | 0.14 | 0 | 0 | 0.14 |
| Albany | Z0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serogroup D1 Enteritidis [f,g,m. [p] | 1.7 | 3.8 | 5.2 | 13.1 | 22.7 | 9.8 | 18 | 14.10 | 22.9 | 4.7 | 4.5 | 18.6 | 24.4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serogroup E Anatum e,h | 1.6 | 0.5 | 0.6 | 0.47 | 1.0 | 0 | 0 | 0.7 | 1.1 | 0.64 | 0.6 | 0.54 | 0.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Serogroup G Havana | f,g, [s] | 0.2 | 1.2 | 0.08 | 0 | 0 | 0.6 | 0.7 | 0.089 | 0.1 | 0.27 | 0.8 | 0.07 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total Salmonellae | 2038 | 924 | 37442 | 529 | 164 | 717 | 35661 | 2557 | 3743 | 665 | 36214 | 2228 |

*data from Salmonella Annual Summary for clinical Salmonella isolates from nonhuman and human sources reported to the Disease Control and Prevention (CDC) and the USDA National Veterinary Services Laboratory (NSVL), USA.

*data from Annual Report and Accounts 2008/2009 of Veterinary Laboratory Agency, Department of Environment, Food and Rural Affairs, United Kingdom.

*data from the Disease Control and Prevention (CDC), Taiwan.
efflux pump [52]. Earlier, fluoroquinolone-resistant *Salmonella* was seldom reported in poultry’s isolates worldwide [10,44,47,48]. Until recently, resistance to similar fluoroquinolones: En and Ci has been reported from chicken in Spain [16]. In contrast to same prevalence of resistance to En and Ci in swine and human isolates [32], we found that resistance rate to En was higher than that of Ci (Table 2). However, En and Ci resistant isolates were only found in few serovars of serogroups B and C1 and mainly in Pintung area (Table 3). These results indicate that possibly En was misuse in Pintung county to induce resistance in prevalent serovars.

**Conclusion**

13 chicken serovars were identified and differed in drug resistance and prevalence associated with chicken lines, ages and regions. Five serovars were common between these chicken serovars and 66 human serovars

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**Abbreviations**

A: ampicillin; BHIA: brain heart infusion agar; C: chloramphenicol; CDC: Center for Disease Control; Cip: ciprofloxacin; CFT: Simmon’s citrate agar; Cx: ceftriaxone; C2: cefazolin; En: enrofloxacin; GN: gram-negative broth; LA: lysine iron agar; MDR: multi-drug resistance; MHA: Mueller-Hinton agar; MHB: Mueller-Hinton broth; MIO: mobility-indole-ornithine agar; MH: native hybrid chicken; ORN: Moller’s ornithine decarboxylase medium; PFGE: pulsed-field gel electrophoresis; S: streptomycin; SIM: sulfide-indole-kmotility medium; Sxt: sulfamethoxazole-trimethoprium; T: tetracycline; TSI: triple sugar iron agar; TS: urea agar; VP: Voges-Proskauer medium; XLD: xylose lysine deoxycholate agar.

**Acknowledgements**

This work was funded by grants from Council of Agriculture under grant [97 AS-14.6.1-BQ-B4(9)] and National Science Council (NSC96-2314-B-415-001), Taoyuan, Taiwan; Y-MH and C-PW are professors of Department of Animal Science, National Chiayi University, Chiayi, Taiwan; C-YL is professor of Department of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan; CC is associate professor of Graduate Institute of Veterinary Public Health, School of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan; CC is the chairman of Department of Microbiology and Immunology, National Chiayi University, Chiayi, Taiwan.

**References**

1. al-Nahlhi HM, al-Ogaily ZH, Nasar TJ: Representative *Salmonella* serovars isolated from poultry and poultry environments in Saudi Arabia. Rev Sci Tech 1999, 18:700-709.
2. Berghold C, Kornschober C, Lederer L, Allerberger F: Occurrence of *Salmonella* Enteritidis phage type 29 in Austria: an opportunity to assess the relevance of chicken meat as source of human *Salmonella* infections. EuroSurveill 2004, 9:31-34.
3. Boonmar S, Bangtrakulnonth A, Pornrunangwong S, Marnrim N, Kaneko K, Ogawa M: *Salmonella* in broiler chickens in Thailand with special reference to contamination of retail meat with *Salmonella enteritidis*. J Vet Med Sci 1998, 60:1233-1236.
4. Gast RK, Gurya R, Guard-Boulden J, Holt PS, Moore RW: Colonization of specific regions of the reproductive tract and deposition at different locations inside eggs laid by hens infected with *Salmonella enteritidis* or *Salmonella heidelberg*. Avian Dis 2001, 51:40-44.
5. Gast RK, Gurya R, Guard-Boulden J, Holt PS: In vitro penetration of egg yolks by *Salmonella* Enteritidis and *Salmonella Heidelberg* strains during thirty-six-hour ambient temperature storage. Poul Sci 2007, 86:1431-1435.
6. Phan TT, Khi AT, Ogasawara N, Tam NT, Okatani AT, Akiba M, Hayashidani H: Contamination of *Salmonella* in retail meats and shrinkers in the Mekong Delta, Vietnam. J Food Prot 2005, 68:1077-1080.
7. Yu CY, Chou SJ, Yeh CM, Chao MR, Huang KC, Chang YF, Chiu CS, Well F, Chiu CH, Chu CM: Prevalence and characterization of multidrug-resistant (type ACSSuT) *Salmonella enterica* serovar Typhimurium strains in isolates from four gosling farms and a hatchery farm. J Clin Microbiol 2008, 46:522-526.
8. Yu CY, Chou CH, Chou SJ, Chao MR, Yeh CM, Lo DY, Su YC, Hong YM, Weng BC, Tsai KS, Huang KC: Comparison of the association of age with the infection of *Salmonella enterica* serovar Typhimurium in Pekin ducks and roman geese. Poul Sci 2008, 87:1544-1549.
9. Limwongprane S, Hayashidani H, Okatani AT, Ono K, Hirota C, Kaneko K, Ogawa M: Prevalence and persistence of *Salmonella* in broiler chicken flocks. J Vet Med Sci 1999, 61:255-259.
10. Snow LC, Davies RH, Christiansen KH, Carruge-Mas JJ, Wales AD, O’Connor JL, Cook AJ, Evans SJ: Survey of the prevalence of *Salmonella* species on commercial laying farms in the United Kingdom. Vet Rec 2007, 161:471-476.
11. Hacking WC, Mitchell WR, Carlson HC: Sources of Salmonellae in broiler chickens in Ontario. Can J Comp Med 1978, 42:392-399.

12. Rigby CE, Pettit JR, Baker MF, Bentley AH, Salmons MO, Lior H: Sources of Salmonellae in an uninfected commercially-processed broiler flock. Can J Comp Med 1988, 44:267-274.

13. Rigby CE, Pettit JR, Baker MF, Bentley AH, Salmons MO, Lior H: Flock infection and transport as sources of Salmonellae in broiler chickens and carcasses. Can J Comp Med 1980, 44:328-337.

14. Wales A, Breslin M, Carter B, Sayers R, Davies R: A longitudinal study of environmental Salmonella contamination in caged and free-range layer flocks. Avian Pathol 2007, 36:187-191.

15. Li X, Payne JB, Santos FB, Levine JF, Anderson KE, Sheldon BW: Sources of contamination from faecal high-rise houses and characterization of the Salmonella isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poult Sci 2007, 86:591-597.

16. Capita R, Alonso-Calleja C, Pietro M: Prevalence of Salmonella enterica serovars and genovars from chicken carcasses in slaughterhouses in Spain. J Appl Microbiol 2007, 103:1366-1375.

17. Vaeteewootacharn K, Sutra S, Vaeteewootacharn S, Sithigon D, Jamjane O: Prevalence of Salmonella populations and prevalence in layer flocks from commercial high-rise houses and characterization of the Salmonella isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poult Sci 2007, 86:591-597.

18. Wales A, Breslin M, Carter B, Sayers R, Davies R: A longitudinal study of environmental Salmonella contamination in caged and free-range layer flocks. Avian Pathol 2007, 36:187-191.

19. Li X, Payne JB, Santos FB, Levine JF, Anderson KE, Sheldon BW: Sources of contamination from faecal high-rise houses and characterization of the Salmonella isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poult Sci 2007, 86:591-597.

20. McQuiston JR, Fields PI, Nash JH, Yoshida C, Franklin K, Konczy P, McQuiston JR, Fields PI: Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-κB and proinflammatory gene program activation in intestinal epithelial cells. BMC Microbiol 2004, 4:33.

21. Iqbal M, Philbin VJ, Withanje GSK, Wigley P, Beal RK, Goodchild MJ, Barrow P, McConnell I, Maskell DJ, Young J, Rumstead N, Boyd Y, Adrian L, Smith AL: Identification and functional characterization of chicken Toll-like receptor 5 reveals a fundamental role in the biology of infection with Salmonella enterica Serovar Typhimurium. Infect Immun 2005, 73:2344-2350.

22. Anderson N, Smith BD, Strobe KL, Barrett SJ, Cookson BT, Logan SM, Aderem A: Evasion of Toll-like receptor 5 by flagellated bacteria. Proc Natl Acad Sci USA 2005, 102:9247-9252.

23. McParland D, Cowlin L, Harkins SJ, Cusack B, Thomas GD, Perry MC, Swayne DE: Evaluation of a multiplex PCR assay for the detection of Salmonella enterica serovars Typhimurium, Enteritidis, and Infantis. J Clin Microbiol 2003, 41:2395-2400.

24. McQuiston JR, Fields PI, Nash JH, Taboada EN, Rahn K: Methodologies to towards the development of an oligonucleotide microarray for determination of Salmonella serotypes. J Microbiol Methods 2007, 70:261-271.

25. Cardinale E, Gros-Claude J, Rivoal K, Rose V, Tall F, Mead GC, Salvat G: Epidemiological analysis of Salmonella enterica serovar enterica serovars Hadar, Brancaster and Enteritidis from humans and broiler chickens in Senegal using pulsed-field gel electrophoresis and antibiotic susceptibility. J Microbiol Methods 2005, 99:988-997.

26. Gaul SB, Wedel S, Edrman MM, Harris DJ, Harris JT, Ferris KE, Hoffman J: Use of pulsed-field gel electrophoresis of conserved XbaI fragments for identification of swine Salmonella enterica serotypes. J Clin Microbiol 2007, 45:472-476.

27. Murase T, Senju Y, Maeda T, Tanaka M, Sakae H, Matsutomo Y, Kaneda Y, Ito T, Otsubo K: Monitoring of chicken houses and an attached egg-processing facility in a laying farm for Salmonellosis contamination between 1994 and 1998. J Food Prot 2001, 64:1912-1916.

28. Pieszkus J, Miliuk J, Michalskaiee L, Zagrebneviene G: The distribution of Salmonella serovars in chicken and humans in Lithuania. J Vet Med A Physiol Pathol Clin Med 2006, 53:12-16.

29. van Duijkeren E, Vanvelzen R, Houtveen H, Van Pelt W: Characterization of multiple-antimicrobial-resistant Campylobacter fetus isolates from retail ground meats. J Clin Microbiol 2006, 44:2798-2804.

30. Boyd D, Cloeckaert A, Chaslus-Dancla E, Mulvey MR: Characterization of variant Salmonello genomic island 1 multidrug resistance regions from Salmonella enterica serovar Kentucky. J Antimicrob Chemother 2001, 47:1354-1360.
serovars Typhimurium DT104 and Agona. Antimicrob Agents Chemother 2002, 46:1714-22.

50. Levings RS, Djordjevic SP, Hall RM. SGI2, a relative of Salmonella genomic island SGI1 with an independent origin. Antimicrob Agents Chemother 2008, 52:2529-37.

51. Havlickova H, Hradecka H, Bernardyova I, Rychlik I. Distribution of integrons and SGI1 among antibiotic-resistant Salmonella enterica isolates of animal origin. Vet Microbiol 2009, 33:193-8.

52. Chen S, Cui S, McDermott PF, Zhao S, White DG, Paulsen I, Meng J. Contribution of target gene mutations and efflux to decreased susceptibility of Salmonella enterica serovar Typhimurium to fluoroquinolones and other antimicrobials. Antimicrob Agents Chemother 2007, 51:535-542.

doi:10.1186/1471-2180-10-86
Cite this article as: Chiu et al. Characterization of 13 multi-drug resistant Salmonella serovars from different broiler chickens associated with those of human isolates. BMC Microbiology 2010 10:86.

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