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Farm and environmental distribution of *Campylobacter* and *Salmonella* in broiler flocks

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ABSTRACT

The objective of this study was to determine the prevalence of *Campylobacter* and *Salmonella* in broilers and their distribution in the indoor and outdoor farm environment. Nine hundred samples (400 faecal; 300 indoor environment; 200 outdoor environment), were collected from 10 individual broiler houses on 10 farms. *Campylobacter jejuni* prevalence was significantly higher ($P = 0.003$) in faeces (29.5%; 118/400) than the environment (0.8%; 4/500) in contrast to *Salmonella* Typhimurium from faecal (8.8%; 35/400) and environmental (8.4%; $n = 42/500$) sources ($P = 0.217$). *S.* Typhimurium predominantly exhibited antimicrobial resistance (AR) to streptomycin (46%) and tetracycline (31.5%). *C. jejuni* isolates exhibited AR only to tetracycline (55.5%). The PFGE profile revealed 100% similarity between *S.* Typhimurium isolates from faecal and environmental sources. No relationship was detected between *C. jejuni* isolates. The low prevalence of *Campylobacter* and *Salmonella* in the outdoor environment indicates that it may not be a significant reservoir for transmission of these pathogens on broiler farms.

1. Introduction

Campylobacter and *Salmonella* are among the leading causes of foodborne illness in the United States, and frequently associated with handling or consumption of raw poultry products (Scallan et al., 2011; CDC, 2011). *Salmonella* and *Campylobacter* cause an estimated one million and 800,000 cases, respectively, of foodborne illnesses in the US each year (Scallan et al., 2011). Transmission of both pathogens from poultry to humans is a serious public health concern. Thus, there has been significant focus on preventing the colonization and spread of *Campylobacter* and *Salmonella* in poultry houses. *Campylobacter jejuni* is the most prevalent *Campylobacter* species in poultry, with flock colonization rates typically ranging from 40–90% (Evans and Sayers, 2000; Rasschaert et al., 2007). Positive flocks often have close to 100% *Campylobacter* prevalence as the pathogen quickly spreads throughout the flock (Evans and Sayers, 2000). *Campylobacter* is also routinely isolated from retail chicken breast, with a prevalence of 38.3% in 2010 (NARMS, 2012). *Salmonella* Kentucky, *S.* Enteritidis, and *S.* Typhimurium are commonly reported serovars isolated from poultry (Liljebjelke et al., 2005; van de Giessen et al., 2006; Foley et al., 2011). For *Salmonella*, flock colonization rates can vary greatly, but typically fall within 6–30% as reported previously (Liljebjelke et al., 2005; van de Giessen et al., 2006; Gutierrez et al., 2009). In 2010, the prevalence of *Salmonella* in retail chicken breast was 13.0% (NARMS, 2012).

Antimicrobial susceptibility of *Campylobacter* and *Salmonella* in poultry often varies depending on geographic region and local production practices. According to the 2010 executive report from the National Antimicrobial Resistance Monitoring System (NARMS), *Campylobacter* isolated from chickens often exhibit antimicrobial resistance (AR) to ciprofloxacin, nalidixic acid, and tetracycline (NARMS, 2012). *Salmonella* isolated from chickens more often exhibit resistance to sulfisoxazole (46%) and tetracycline (56%) (NARMS, 2012). The issue of AR development in the poultry industry has been well studied and the focus has been on preventing fomite transmission of resistant bacterial pathogens from poultry houses to the external environment (Bailey et al., 1996; Harbaugh et al., 2006). While vertical transmission has been documented, horizontal transmission is thought to be a main factor influencing the presence of *Campylobacter* in broiler flocks (Ellis-Iversen et al., 2011; Ridley et al., 2011). Accumulation of dust in a poultry house is more common around ventilation fans, which aids in the movement of dust from the inside to the outside environment (Davies, 2005). Both vertical transmission from the breeding hens and horizontal transmission from previous flocks or the environment are thought to be important in the spread of *Salmonella* spp. in and between flocks (Liljebjelke et al., 2005; Foley et al., 2011).

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While *Campylobacter* and *Salmonella* infections are often asymptomatic in broilers, colonization of these pathogens in broilers presents a significant risk to human health through ingestion of contaminated retail meat. Thus, improving our understanding of the on-farm transmission dynamics of *Campylobacter* and *Salmonella* will allow for implementation of better control measures to reduce pathogen prevalence. The aim of this cross-sectional study was to examine the prevalence of *Campylobacter* and *Salmonella* in 10 commercial enclosed broiler houses. The birds, as well as the indoor and outdoor environments, were sampled to determine transmission dynamics of the two pathogens in these flocks. An important objective was to determine the distribution and possible reservoirs of the two pathogens in the indoor and outdoor environments.

2. Materials and methods

2.1. Sample collection from flock and environment

Faecal and environmental samples were collected from 10 commercial broiler houses in North Carolina between October 2010 and March 2011. A single house was sampled at one of 10 farms visited. The farms were operated by a single, large-scale broiler production company in North Carolina with average flock sizes of approximately 20,000 birds per house. Birds were raised on the house floor with reused litter and had no access to the outdoors. The farms used coccidiostats (salinomycin, narasin, nicarb) and antibiotics (bacitracin methylene disalicylate, 3-nitro) for prophylaxis and treatment of birds. At each farm, 40 pooled fresh faecal samples and 50 environmental samples were collected. Fresh faecal samples (10 g) were collected using a clean, gloved hand directly from the house floor. The faecal samples were later divided and processed separately for the isolation of *Campylobacter* and *Salmonella*. Two sets of environmental samples (one set for each pathogen) were collected; including 10 samples each of outdoor environmental samples (outside swab and grass) and indoor environmental samples (feed, litter, and inside swab). Ten grams of feed, litter and grass samples were collected aseptically into sterile Whirl-Pak bags (Nasco, WI, USA). The inside and outside environmental swab samples were collected by wiping a sterile, moist swab 10 times per side along the interior or exterior sides of the house. Four of each indoor and outdoor environmental sample were specifically collected from areas around or on the ventilation fans. This was done to determine whether ventilation fans were playing a role in transmission of pathogens between the inside and outside of the house. Samples were immediately transported on ice to the laboratory for processing.

2.2. Campylobacter isolation and identification

A loopful (approximately 1 g) of faecal material was streaked directly onto Campy-Cefex selective agar plates (Brucella Agar (BBL, Becton Dickinson, USA) with 1% ferrous sulfate, 0.5% sodium bisulfite, 1% pyruvic acid). These samples were incubated under microaerophilic conditions (CO_2: 10%, O_2: 5%, N_2: 85%) at 42 °C for 48 h. Presumptive colonies were then selected and streaked onto Mueller Hinton (MH, Difco, Becton Dickinson, USA) agar plates and incubated under microaerophilic conditions at 42 °C for 48 h to obtain a pure culture. Presumptive cultures were biochemically confirmed using catalase (3% H_2O_2) and oxidase (tetramethul-*p*-phenylenediamine) tests (Difco, Becton Dickinson, USA). An initial enrichment step was used to isolate *Campylobacter* from environmental samples. Ninety millilitter of Bolton Broth (Oxoid, UK) was added for selective enrichment of feed, litter, and grass samples while 30 ml of Bolton Broth was added to each inside and outside swab. These samples where then mixed thoroughly and incubated under microaerophilic conditions at 42 °C for 48 h. Next, one loopful (approximately 10 μl) of the Bolton Broth suspension was streaked onto Campy-Cefex agar and processed in the same manner as faecal samples. The DNeasy Blood and Tissue Kit (Qiagen, Germany) was used to purify the DNA following manufacturer's protocol. A multiplex PCR was used to speciate *Campylobacter* as previously described (Cloak and Fratamico, 2002). Confirmed isolates were then stored in Brucella Broth containing 20% glycerol at −80 °C for future analysis.

2.3. Salmonella isolation and identification

Ninety millilitter of buffered peptone water (BPW, Difco, Becton Dickinson, USA) was added to specimen cups containing 10 g of either fresh faecal or environment samples (grass, litter, feed). Thirty millilitter of BPW was added to the bags containing inside and outside swabs, mixed thoroughly, and incubated at 37 °C for 24 h. Next, 100 μl of suspension was transferred to 9.9 ml of Rappaport–Vassiliadis (RV, Difco, Becton Dickinson, USA) broth and incubated at 42 °C for 24 h. After incubation, a loopful of RV broth suspension was streaked onto XLT4 selective agar plates (Remel, Thermo Fisher Scientific, USA) and incubated at 37 °C for 24 h. A single black colony per plate was picked and confirmed by biochemical tests, including triple sugar iron (Remel, Thermo Fisher Scientific, USA) and urea agar slants (Oxoid, UK). The isolates were stored on lactose broth agar slants (Difco, Becton Dickinson, USA) at room temperature and in Brucella Broth containing 20% glycerol at −80 °C for future analysis. The DNeasy Blood and Tissue Kit (Qiagen, Germany) was used following manufacturer's protocol to purify the DNA. The purified DNA was used as the template in two multiplex PCRs to serotype *Salmonella* isolates as described previously (Alvarez et al., 2004).

2.4. Antimicrobial susceptibility testing

Campylobacter and *Salmonella* isolates were tested for antimicrobial susceptibility by broth micro dilution method using the Sensititre-semiautomated system (Trek Diagnostic System, Inc., OH, USA). The minimum inhibitory concentration (MIC) was determined using the CVM1AGNF plate for *Salmonella* and the CAMPY (Trek Diagnostic System, Inc., Cleveland, OH) plate for *Campylobacter* isolates against 15 and nine antimicrobials, respectively. The CVM1AGNF plate includes amikacin (AMI), ampicillin (AMP), amoxicillin/clavulanic acid (AUG), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), sulfisoxazole (FIS), cefoxitin (FOX), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), trimethoprim/sulfamethoxazole (SXT), tetracycline (TET), and ceftiofur (TIO). The CAMPY plate includes azithromycin (AZI), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), florfenicol (FFN), gentamicin (GEN), nalidixic acid (NAL), telithromycin (TEL) and tetracycline (TET). The strains *Escherichia coli* ATCC25922 and *C. jejuni* ATCC33560 were used as quality controls. The protocol and breakpoints were determined according to Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI, 2010).

2.5. Characterization of antimicrobial resistance (AR) determinants

Antimicrobial resistant *Salmonella* isolates were screened for the presence of class 1 Integron and corresponding resistance genes based on their AR phenotypes. The primers used for detection of different AR genes were as follows: ESBL genes-bla_{TEM} and bla_{PSE} (Carlson et al., 1999), bla_{CMY-2} (Zhao et al., 2001) genes encoding tetracycline resistance *tet*(*A*), *tet*(*B*) and class I integrons (Ng et al., 1999), aminoglycosides-*aad A1/A2* and *strA/B* (Madsen

et al., 2000), chloramphenicol-*cml* (Briggs and Fratamico, 1999), kanamycin-*aphAI* (Frana et al., 2001), and sulfisoxazole-*sul2* (Aarestrup et al., 2003). All resistant *Campylobacter* isolates were tested for the presence of the *tet*(O) gene, using primers DMT1-F and DMT2-R following the protocol described previously (Gibreel et al., 2004).

2.6. Pulsed field gel electrophoresis (PFGE) analysis

Clonal relationships of *Salmonella* and *Campylobacter* were determined by PFGE using the PulseNet protocol (Ribot et al., 2001, 2006). Briefly, 400 µl of overnight culture cells were lysed and intact genomic DNA was digested in agarose-embedded plugs with *Xba*I and *Sma*I restriction enzymes for *Salmonella* and *Campylobacter*, respectively. The restriction fragments were separated by electrophoresis in 0.5X TBE buffer, 1% ultrapure agarose (Seakam Gold Agarose, Maine, USA) for 18 h at 14 °C in a pulsed field gel electrophoresis system (CHEFF DR III, BioRad, USA) using pulsed times of 2.2–63.8 s for *Salmonella* and 6.75–38.35 s for *Campylobacter*. The *Xba*I digested *S.* Braenderup H9812 were used as the reference DNA marker. Gels were stained with ethidium bromide, followed by washing with nanopure water and photographed under UV light.

2.7. Statistical analysis

Prevalence estimates from different samples were compared using the Fisher's exact two-tailed test using statistical software (SigmaPlot, CA, USA). Significance was defined at $P \leqslant 0.05$. Clonal relationships among these isolates were analyzed using Bionumerics version 6.1 (Applied Maths, USA). The banding patterns were compared using the Dice coefficient and unweighted pair group method with arithmetic mean (UPGMA) with 1.5% optimization and position tolerance.

3. Results

3.1. Campylobacter and Salmonella prevalence

Nine hundred samples (faecal $n = 400$; environmental $n = 500$) were collected from 10 commercial broiler houses and tested for the presence of *Campylobacter* and *Salmonella.* One hundred and twenty-two *Campylobacter* isolates were recovered from the samples. *Campylobacter* was isolated from 29.5% ($n = 118$) of faecal samples, with 40% of houses testing positive. *Campylobacter* prevalence was significantly higher in faecal material (29.5%) than in the combined indoor and outdoor environmental samples (0.8%; $n = 4$; $P = 0.003$). *Campylobacter* was rarely isolated from the surrounding farm environment. Only two houses tested positive for the environmental samples including two inside swabs, a single outside swab, and a single grass sample. All litter and feed samples collected in the study tested negative for *Campylobacter*. It is important to highlight that all environmental samples in three of the four houses with positive faecal samples tested negative. Additionally, there was one house with a positive swab inside and swab outside sample where all faecal samples were negative. Multiplex PCR was used to determine speciation of the 122 *Campylobacter* isolates. Three isolates were not culturable despite multiple attempts. All 119 isolates were *C. jejuni* and were further characterized at phenotypic and genotypic levels.

Seventy-six *Salmonella* were isolated from the samples collected. These included *Salmonella* isolated from 8.8% ($n = 35$) of faecal samples, with 70% of houses testing positive. In contrast to *Campylobacter*, *Salmonella* was isolated most frequently from litter at 28%, followed by swab inside (7%), feed (3%), outside swab (2%), and grass (1%). The environmental samples positive for *Salmonella* originated from five houses, two of which had negative faecal samples. There was no significant difference in *Salmonella* prevalence between faecal (8.8%) and the combined environmental samples (8.4%; $P = 0.217$). All the *Salmonella* isolates were identified as serotype *Salmonella* Typhimurium. Overall, there was no significant difference between *C. jejuni* and *S.* Typhimurium prevalence in faecal samples ($P = 0.874$). However, *S.* Typhimurium prevalence in the combined environment was significantly higher than that of *C. jejuni* ($P < 0.001$). Co-occurrence with both pathogens was detected in faecal samples from three houses (30%), but no association was detected between the prevalence of *C. jejuni* and *S.* Typhimurium ($P = 0.138$).

3.2. Antimicrobial susceptibility testing

All the *C. jejuni* ($n = 119$) and *S.* Typhimurium isolates ($n = 76$) were tested for antimicrobial susceptibility to a panel of antimicrobials by the broth micro dilution method. The *C. jejuni* isolates were resistant only to tetracycline (55.5%; $n = 66$). Of the 76 *S.* Typhimurium isolates tested, 28.9% ($n = 22$) were pansusceptible, while the remainder showed wide spectrum AR. The highest frequency of AR was exhibited to streptomycin (46%; $n = 35$) followed by tetracycline (31.5%; $n = 24$), ampicillin, amoxicillin/clavulanic acid, ceftriaxone, ceftiofur, cefoxitin and sulfisoxazole (25% each; $n = 19$), kanamycin (13.1%; $n = 10$), and gentamicin (9.2%; $n = 7$). All isolates were susceptible to amikacin, chloramphenicol, trimethoprim/sulfamethoxazole and quinolones. Multidrug resistant (MDR; resistance to $\geqslant$3antimicrobials) *S.* Typhimurium was detected from isolates from both faecal (17.1%; $n = 6$) and environmental samples (48.8%; $n = 20$). The common MDR patterns identified are listed in Table 1.

3.3. Molecular characterization of antimicrobial resistance determinants

All the tetracycline resistant *C. jejuni* isolates ($n = 119$) were found to carry the *tet*(*O*) gene. The *S.* Typhimurium isolates were tested for 11 different AR genes encoding resistance to different antimicrobials and the results are shown in Table 1. AR to ampicillin was encoded by the bla_{TEM} gene (52.6%; $n = 10$) while the isolates resistant to β-lactams, including cephalosporins, carried the bla_{CMY-2} gene (100%; $n = 19$). The streptomycin resistant isolates carried either *strA* (74.2%; $n = 26$) or the *aad A1* (20%; $n = 7$) gene. AR to tetracycline was harbored by the *tet*(*A*) (62.5%; $n = 15$) and *tet*(*B*) (37.5%; $n = 9$) genes. All isolates resistant to kanamycin and sufisoxazole were encoded by *aphAI* and *sul2* genes, respectively. Six (23%; $n = 6$) MDR *S.* Typhimurium isolates tested positive for the presence of a 1 kb class I Integron.

3.4. Pulsed field gel electrophoresis (PFGE)

C. jejuni ($n = 119$) and *S.* Typhimurium ($n = 76$) isolates were typed by PFGE using *Sma*I and *Xba*I restriction enzymes, respectively. Three *C. jejuni* isolates were not culturable despite multiple attempts and were not included in PFGE analysis. The majority of the *C. jejuni* isolates with 7–8 bands were clustered in four major clusters ($n = 117$), except for two isolates with unique profiles (Fig. 1). The *C. jejuni* dendrogram showed 100% genotypic similarity among the isolates from faecal and environmental samples, both from within houses and among different houses. The majority of isolates belonged to cluster 2 ($n = 70$), containing isolates from Houses 4, 9 and 10. Houses 4 and 10 contained a single genotype (cluster 2), with the same genotype clustered into two different PFGE profiles for House 9. Two distinct genotypes were detected in House 1, split between clusters 1 ($n = 24$) and 4 ($n = 12$) with a

Table 1
Common MDR patterns identified among *Salmonella* isolates from poultry and the environment.

Faecal		Environment	
Antimicrobial resistance patterns[a]	(n = 35)	(n = 41)	Resistance genes identified
FIS–GEN–STR	8.5 (3)[b]	0	*sul2*, *aadA1*
FIS–GEN–STR–TET	0	9.7 (4)	*sul2*, *aadA2*, *tet(A)*
AMP–AUG–AXO–FOX–STR–TIO	5.7 (2)	12.1 (5)	bla_{CMY-2}, *strA*
AMP–AUG–AXO–FOX–FIS–TIO–TET	2.8 (1)	0	bla_{CMY-2}, *sul2*, *tet(A)*
AMP–AUG–AXO–FOX–FIS–STR–TIO–TET	0	2.4 (1)	bla_{CMY-2}, *sul2*, *strA/B*, *tet(B)*
AMP–AUG–AXO–FOX–FIS–KAN–TIO–TET	0	24.3 (10)	bla_{TEM}, bla_{CMY-2}, *sul 2*, *aphA*, *tet(A)*

[a] Antimicrobials tested: Ampicillin (AMP), Amoxicillin/Clavulanic acid (AUG), Ceftriaxone (AXO), Cefoxitin (FOX), Ceftiofur (TIO) Sulfisoxazole (FIS), Gentamicin (GEN), Kanamycin (KAN), Streptomycin (STR), Tetracycline (TET).
[b] Percent (Number).

single isolate showing a unique PFGE profile. An outdoor swab isolate from House 3 shared 100% genotypic similarity among faecal isolates from House 1 (cluster 1) and an indoor swab had a unique PFGE profile.

S. Typhimurium typing by PFGE produced on average 10–16 bands and isolates were grouped into nine clusters representing 71 isolates (Fig. 2). The remaining five *Salmonella* isolates had unique profiles. *S.* Typhimurium PFGE cluster 1 (Fig. 2) showed 100% genotypic similarity among faecal and indoor environmental isolates including feed, litter and swab inside, originating from four houses. Similarly, clusters 2, 3 and 6 consisted of isolates showing 100% genotypic similarity among faecal, indoor and outdoor environment from not only within the same house but also with isolates from different houses. All MDR isolates in cluster 3 were indoor environmental samples from House 7 and 8 and exhibited resistance to either seven (AMP–AUG–AXO–FOX–FIS–TIO–TET) or eight (AMP–AUG–AXO–FOX–FIS–KAN–TIO–TET) antimicrobials (Fig. 2). The majority of the pansusceptible isolates were grouped in clusters 4, 5 and 6 with a single isolate each exhibiting resistance to streptomycin in clusters 4 and 6. All the isolates that showed resistance to only tetracycline (TET) were clustered together in a single group (cluster 9).

4. Discussion

C. jejuni was isolated from faecal samples in four of the 10 houses sampled. Within positive houses, prevalence was typically very high in faecal samples. This was in agreement with previous studies that have determined that once *Campylobacter* enters a flock, it spreads quickly and colonizes the entire flock (Evans and Sayers, 2000; Newell and Fearnley, 2003). *S.* Typhimurium was comparatively isolated from more broiler houses, but at a lower overall prevalence. The house prevalence detected in this study was high relative to what has been reported in the literature (Liljebjelke et al., 2005; van de Giessen et al., 2006; Gutierrez et al., 2009). A possible explanation may be that all of the houses were managed by the same company with similar production practices. In addition, the *Salmonella* status of the previous flock and inefficient cleaning and disinfection between each flock has been considered to be an additional risk factor for higher prevalence (Rose et al., 2000; Gradel et al., 2005).

C. jejuni was not frequently isolated from indoor or outdoor environments and all litter and feed samples tested negative. Only two houses had environmental samples that tested positive, including a single grass and outside swab sample. There were two *C. jejuni*-positive fan samples, which were collected from the same house. The lack of detection of *Campylobacter* in litter and feed in this study has been reported in previous studies that have found little evidence indicating that these sample areas were a major reservoir for *Campylobacter* (Nesbit et al., 2001; Newell et al., 2011). Additionally, investigators found that *Campylobacter* prevalence in reused and dry litter, such as the litter used by the farms in this study, was lower than in new litter (Berndtson et al., 1996; Chinivasagam et al., 2010). This may be due in part to environmental conditions that were unfavorable for bacterial growth. It is possible that vertical transmission from the hatchery was the main source of colonization for this system. However, this cannot be verified as the hatchery was not sampled in this study.

All *Salmonella* isolates were serotype *S.* Typhimurium. This was unexpected as previous work has typically detected multiple serotypes, even within the same house (Liljebjelke et al., 2005; Marin et al., 2011). However, identification of this serotype has important public health implications. According to the NARMS (2010) report, the most frequently associated serotype among non typhoidal *Salmonella* in chicken retail meat was *S.* Typhimurium (87.8%) (NARMS, 2012). In 2010, 13% of reported cases of human salmonellosis were caused by *S.* Typhimurium (CDC, 2011). Similar to *Campylobacter*, *Salmonella* was infrequently isolated from the indoor and outdoor environments, with the exception of indoor litter samples. The relatively high prevalence of *Salmonella* in the litter has been previously reported (Bailey et al., 2001; Liljebjelke et al., 2005). *Salmonella* positive litter samples detected in our study may have important public health implications. A recent study reported a positive correlation between prevalence of *Salmonella* in litter samples and *Salmonella* isolation from post-chill broiler carcasses (Volkova et al., 2010).

The *C. jejuni* isolates in this study exhibited AR only to tetracycline. Previous reports have documented increasing frequency of AR to macrolides, quinolones, fluoroquinolones and tetracyclines in *Campylobacter* originating from poultry (Chen et al., 2010; Wirz et al., 2010; NARMS, 2012). However, the broiler houses in this study were not given any of the above antimicrobials, potentially explaining the lack of AR in isolates from the houses. *S.* Typhimurium isolates exhibited resistance to a wide spectrum of antimicrobials including streptomycin and tetracycline (Table 1). These findings were relatively higher than previous reports of AR in chickens (Siemon et al., 2007; Alali et al., 2010; NARMS, 2012). Twenty-six *Salmonella* isolates were MDR, which was in agreement with a previous report by Siemon et al. (2007). In this study, salinomycin and bacitracin were used in the feed as coccidiostat and antibiotic, respectively. Previous studies have reported a correlation between use of feed supplemented with salinomycin and bacitracin with the development of MDR with higher frequency of resistance to ceftiofur, gentamicin, streptomycin, and ampicillin in *Enterobacteriaceae* (George et al., 1982; Diarra et al., 2007). AR to the β-lactam class of antimicrobials, including third generation cephalosporins such as ceftriaxone and ceftiofur, and MDR patterns in commercial poultry and indoor environment isolates is causing public health concern, since these antimicrobials are used to treat human salmonellosis.

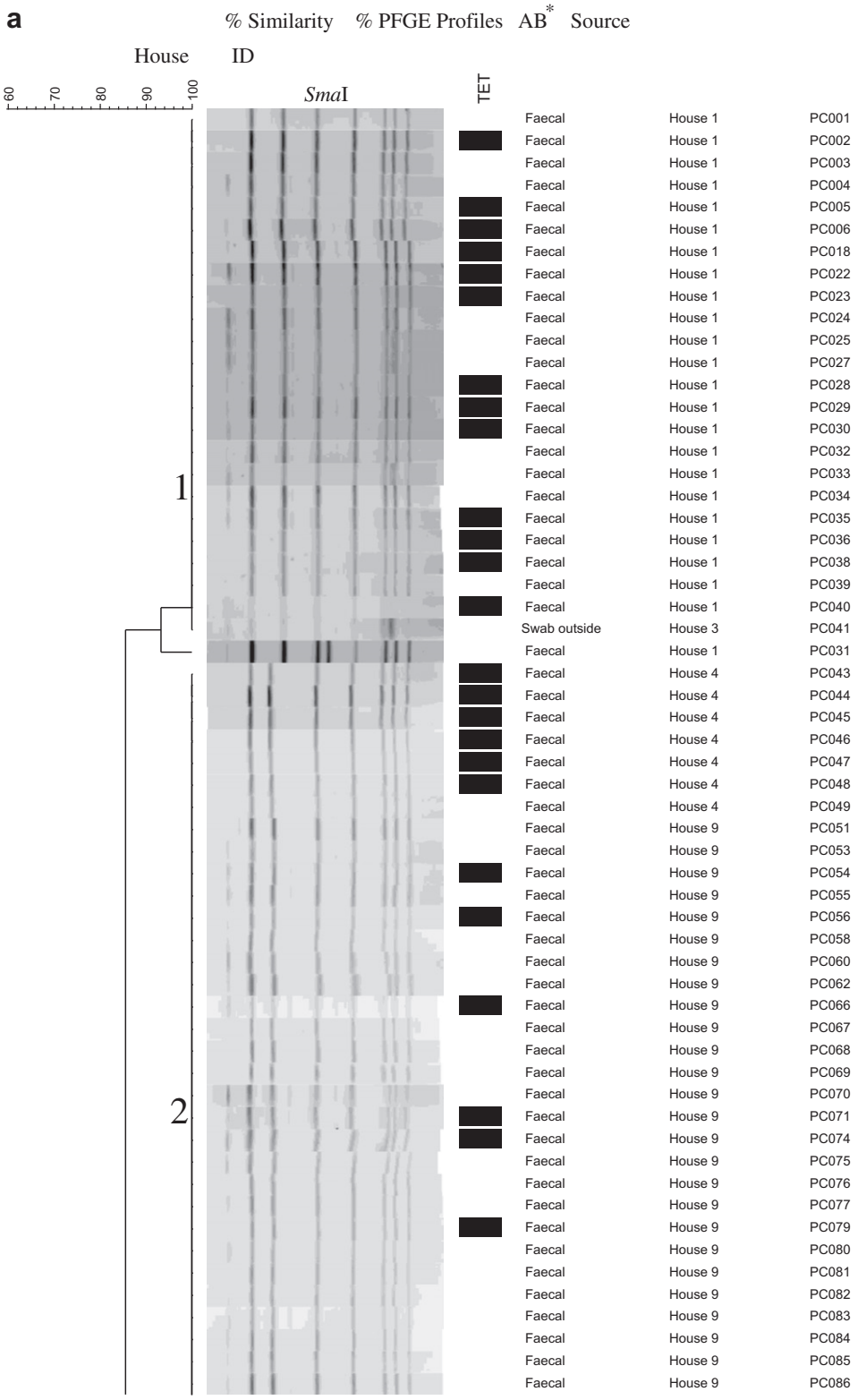

Fig. 1. PFGE profiles of *Campylobacter* isolates from faecal and environment.*Antimicrobials (AB): Tetracycline (TET).

The antimicrobial resistant *Campylobacter* and *Salmonella* isolates were characterized at the molecular level to determine the genes responsible for coding resistance to different antimicrobials. All tetracycline resistant *Campylobacter* isolates carried the *tet*(*O*) gene, which has been most frequently associated with tetracycline resistance (Avrain et al., 2004; Gibreel et al., 2004). Tetracycline resistant *S.* Typhimurium isolates harbored *tet*(*A*) and *tet*(*B*) genes and have been shown to be the predominant alleles reported previously (Diarrassouba et al., 2007; Chuanchuen and Padungtod, 2009). All ampicillin resistant isolates were encoded by the bla_{TEM} gene, in accordance with previous reports that the penicillin class of AR was mediated by the TEM β-lactamase enzymes (Peirano

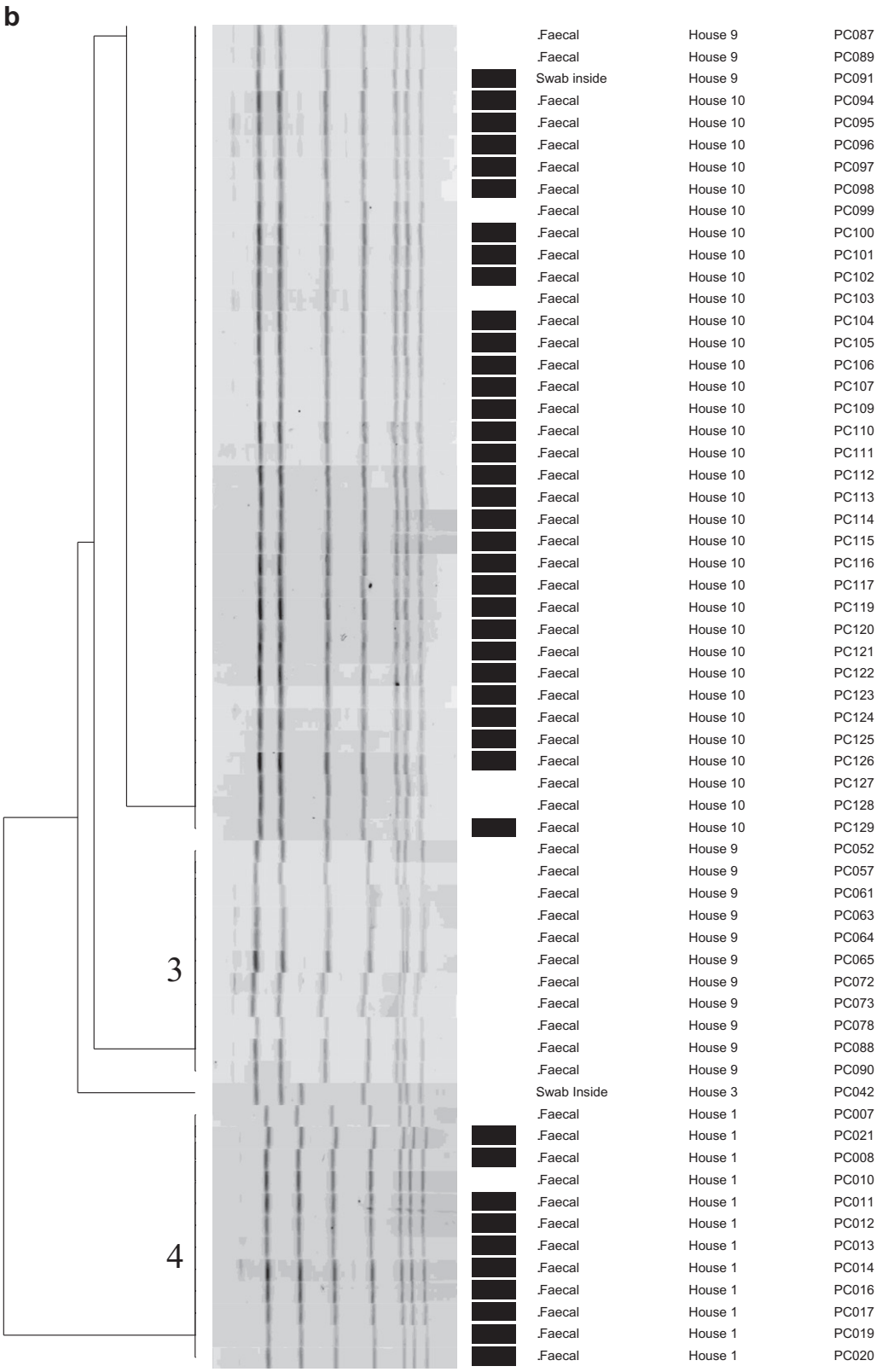

Fig. 1 (*continued*)

et al., 2006; Hur et al., 2011). A previous report on *E. coli* and *Salmonella* isolated from broiler farms stated that resistance to β-lactams, including cephalosporins, was commonly due to plasmid mediated β-lactamase resistant gene bla_{CMY-2} similar to what we found in our *S.* Typhimurium isolates (Diarrassouba et al., 2007). The presence of ESBL producing bla_{TEM} and plasmid-mediated AmpC enzyme bla_{CMY-2} in the majority of indoor environmental isolates highlights the role of the environment in the rapid dissemination of resistance to cephalosporins, the front line of antimicrobials for human salmonellosis treatment. We detected class I

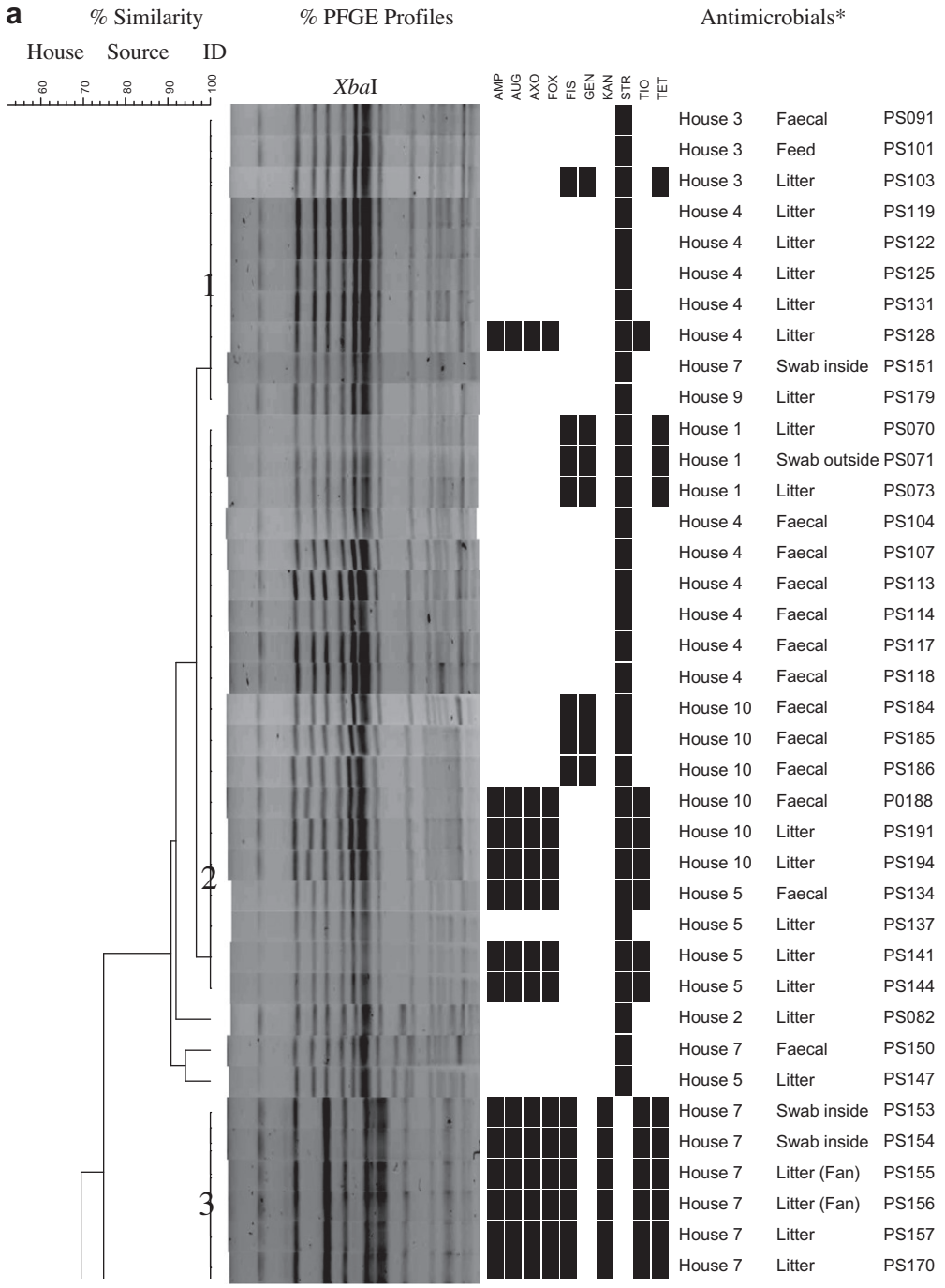

Fig. 2. PFGE profiles of *Salmonella* isolates from faecal and environment.*Ampicillin (AMP), Amoxicillin/Clavulanic acid (AUG), Ceftriaxone (AXO), Cefoxitin (FOX), Ceftiofur (TIO) Sulfisoxazole (FIS), Gentamicin (GEN), Kanamycin (KAN), Streptomycin (STR), Tetracycline (TET).

integrons (1 kb) in 23% of the MDR isolates. Class I Integrons play an important role in the dissemination of AR among different *Salmonella* strains in humans and animals (Gebreyes and Altier, 2002; Peirano et al., 2006).

The *Campylobacter* and *Salmonella* isolates from broiler faecal and environmental samples were typed by PFGE to examine genotypic relationships. The *C. jejuni* isolates from the five positive houses revealed four major PFGE clusters with two additional unique profiles. Three of these *C. jejuni* positive houses contained two or more genotypes. This was in agreement with previous studies that have shown between 40–77% of houses contain two or more genotypes (Höök et al., 2005; Bull et al., 2006; Messens et al., 2009). Interestingly, the isolate recovered from the outside swab from House 3 had a different PFGE profile than the indoor litter sample from that same house (Fig. 2). While there were only two indoor and one outdoor environmental *C. jejuni* isolates for genotyping, the difference in PFGE profile types provided further evidence that *Campylobacter* was not being transmitted between the indoor and outdoor farm environments in this study, as has been reported earlier (Nesbit et al., 2001).

The *S.* Typhimurium isolates revealed genetic diversity both within and between the houses. This was highlighted by the detection of multiple genotypes in seven of the nine positive houses and four of the nine clusters consisting of isolates from multiple

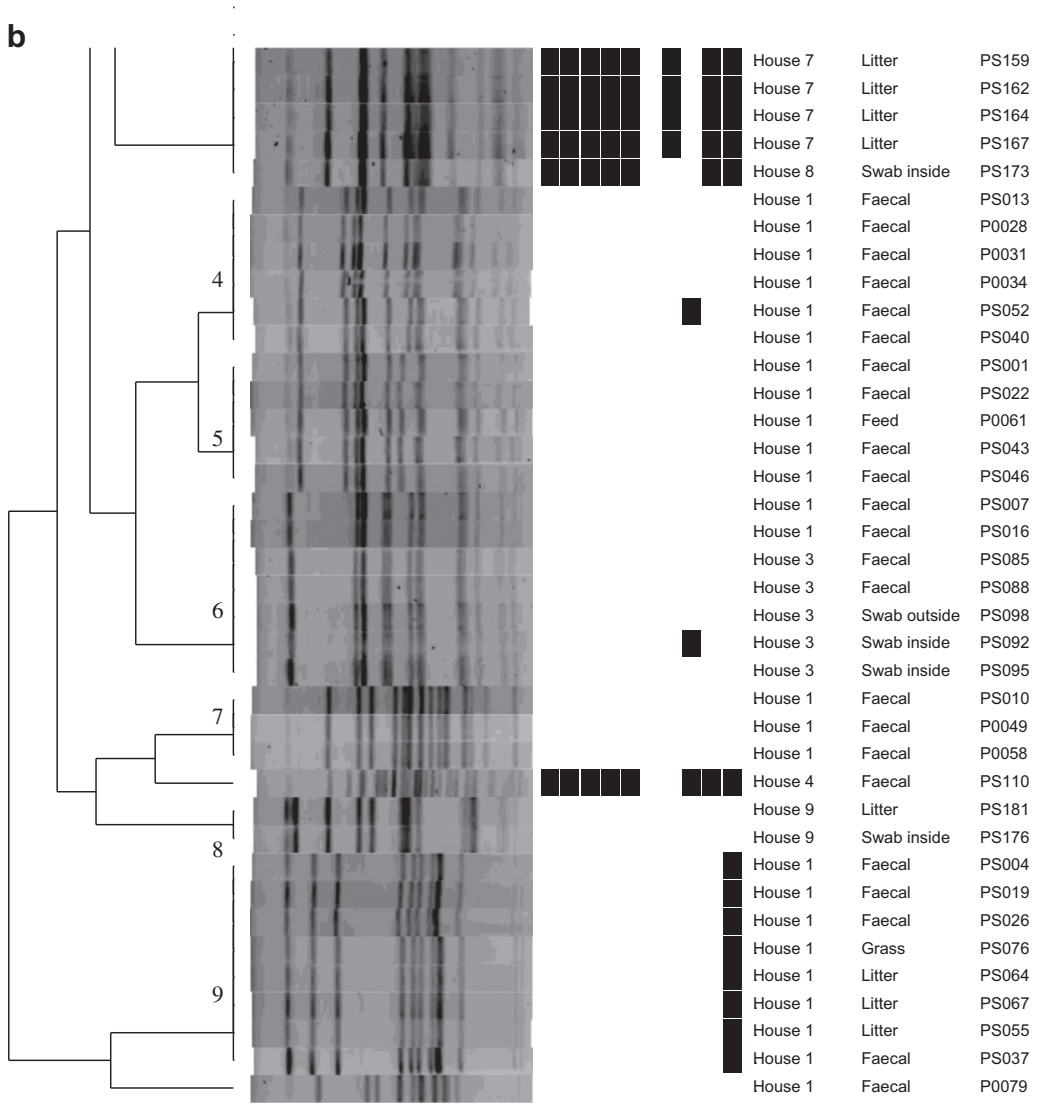

Fig. 2 (*continued*)

houses. All houses were operated by the same large-scale company, so it is therefore not surprising that the same *S.* Typhimurium PFGE strain was detected in different houses. Another possible explanation is that vertical transmission from a common hatchery resulted in the same PFGE strains being detected in different houses. The genetic diversity among the same *Salmonella* serotype detected in this study has been previously reported in the literature (Liebana et al., 2001; Sander et al., 2001). The high prevalence of *S.* Typhimurium in the litter and the common PFGE profiles with faecal samples indicates that the litter might be an important reservoir for *Salmonella* in broiler houses.

5. Conclusions

The low prevalence of *Campylobacter* and *Salmonella* in the outdoor environment suggests that transmission of these pathogens between the indoor and outdoor farm environments does not occur. However, the high prevalence of *S.* Typhimurium in the litter and the common PFGE profiles with faecal samples indicates that the litter might be an important reservoir for *Salmonella* in broiler flocks. The prevalence of antimicrobial resistant *Campylobacter* and *Salmonella* in broilers and their environment has important implications from a food safety perspective since a decrease in farm pathogen load might reduce carcass contamination at slaughter.

6. Disclosure statement

No competing financial interested exist.

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