Food Research International 45 (2012) 947–952

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia

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ARTICLE INFO

Article history:
Received 30 November 2010
Received in revised form 22 February 2011
Accepted 28 February 2011

Keywords:
Antibiotic resistance
Ducks
Prevalence
Salmonella serovars

ABSTRACT

An investigation was carried out to determine the prevalence and antibiotic resistance of *Salmonella* serovars in ducks, their rearing and processing environments in Penang, Malaysia. A total of 531 samples collected from wet markets and duck farms, were examined from August 2009 to October 2010. The overall prevalence of *Salmonella* serovars was 23.5% (125/531). The 125 *Salmonella* isolates belong to 10 different serovars namely Typhimirium (29.6%), Enteritidis (12.0%), Gallinarum (2.4%), Braenderup (12.0%), Albany (11.2%), Hadar (20.8%), Derby (6.4%), Weltevreden (1.6%), Newbrunswick (3.4%) and London (0.8%). *Salmonella* serovars also showed various resistance patterns against 13 different antibiotics. All the serovars were resistant to erythromycin but susceptible to cephalothin, gentamicin and ceftriaxone. Plasmids were detected in 91 (72.8%) of the isolates with sizes ranging from 1.4 to 23.1 Kbp. Our findings provide baseline information on the distribution of *Salmonella* serovars in ducks, their rearing and processing environments, and indicate that ducks should be considered as an important source of food-borne pathogens.

1. Introduction

Salmonella is a major cause of food-borne illness in humans. Farm animals and foods of animal origin are important sources of human *Salmonella* infections. *Salmonella* Typhimurium and Enteritidis are the most frequently reported serovars associated with human food-borne illnesses (Suresh, Hatha, Sreenivasan, Sangeetha, & Lashmanaperumalsamy, 2006). Mead et al. (1999) estimated that, non-typhoidal *Salmonella* species are the second largest cause of food-borne illnesses after *Campylobacter* species. *Salmonella* species can cause systemic infections especially in children and immuno-compromised individuals, while healthy individuals suffer from symptoms such fever, diarrhoea, nausea, abdominal pain, vomiting and occasionally septicaemia (Coburn, Grass, & Finlay, 2007; Willford, Manley, Rebelein, & Goodridge, 2007; Gonzales-Barron, Redmonda, & Butler, in press). In recent years human *Salmonella* infections associated with animal-derived pet treats including pigs' ears have been reported in the United States and Canada (Morbidity & Mortality Weekly Report, 2006, 2008), while in Ireland, Adley, Dillon, Morris, Delappe, and Cormican (2011) isolated *Salmonellae* belonging to eight different serotypes from pig ear treats. Efficient, specific and rapid methods for isolating and detecting *Salmonellae* are important for clinical and reporting purposes (Adzitey & Huda, 2011). Such rapid and specific methods based on DNA and transfer-messenger RNA (tmRNA) have been applied to detect and to serotype *Salmonellae* (Wang, Shi, Alam, Geng, & Li, 2008; McGuinness, Barrya, & O'Grady, in press; Prendergast et al., in press).

Salmonella is also a pathogen of significant importance in worldwide animal production and the emergence of antibiotic-resistant strains, due to indiscriminate use of antibiotics in animal feeds as growth promoters and therapeutic agents is a further threat to human and animal health (Forshell & Wierup, 2006). *Salmonella* species are becoming increasingly resistant to antibiotics, making it more difficult to treat patients with severe infections. This makes *Salmonella* serovars that are resistant to multiple antibiotics a continuous and an important subject area of research, and a major concern for food safety. For instance, Willford et al. (2007) tested 21 strains of *Salmonella enterica* serovar Newport and found 20 to be resistant to amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, tetracycline, and sulfamethoxazole. Among these 20 isolates some were also resistant to gentamycin, kanamycin and trimethoprim-sulfamethoxazole. One isolate exhibited complete resistance to all the antibiotics tested. Several studies have also shown that *Salmonellae* exhibit multi-drug resistant patterns (Suresh et al., 2006; Singh, Yadav, Singh, & Bharti, 2010; Yildirim et al., 2011; Adley et al., 2011), which may be chromosomal or plasmid mediated. Mutation in gyrase and topoisomerase genes have also been reported to be associated with fluoroquinolone resistance in multi-drug resistant *Salmonella* isolates recovered from retail meats (Yang et al., 2011).

Duck farming for meat and eggs has been practised for several years. Despite this, little attention has been paid to the association between ducks and food-borne pathogens. Duck meat and eggs are important sources of nutrients that are comparable to those of chickens and hen

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doi:10.1016/j.foodres.2011.02.051

eggs, and are consumed worldwide. It has been suggested that duck meat is of better nutritional quality than chicken due to its lower fat and higher protein content (Anonymous, 2010a). According to FAO, (2009), Malaysia is the third largest producer of duck meat (111,000,000 kg) after China (2,328,796,000 kg) and France (234,360,000 kg). Duck production is an integral part of Malaysia agriculture economy, and producers and processors are encouraged to increase the production for export purposes. In Malaysia, prevalence of *Salmonella* in raw and cooked foods, broiler chickens and vegetables have previously been reported (Arumugaswamy, Rusul, Abdul Hamid, & Cheah, 1995; Rusul, Khair, Cheah, & Son, 1996; Noorzaleha et al., 2003). Information on the prevalence of *Salmonella* serovars in ducks is limited worldwide and in Malaysia there are no published reports available on the prevalence of this pathogen or other food-borne pathogens in ducks.

The present study was carried out to determine the prevalence of *Salmonella* serovars in ducks, their rearing and processing environments. *Salmonella* serovars isolated were also examined for antibiotic resistance and presence of plasmids.

2. Materials and methods

2.1. Sample collection

In this study, a total of five hundred and thirty one ducks and duck related samples were collected aseptically from wet markets, and commercial duck farms, during the period of August 2009 to October 2010 in Penang, Malaysia. Samples obtained from two wet markets were intestinal contents, wash water (water use for washing carcasses after dressing), carcass rinses, floor swabs, and table swabs. Samples obtained from four different farms were faecal samples, cloacal swabs, soil samples, feed samples, drinking water, pond water and egg shell swabs. The samples collected were placed in polystyrene box containing ice and analyzed immediately on reaching the laboratory.

2.2. Isolation and identification of Salmonella

Isolation and identification of *Salmonella* from ducks, their rearing and processing envivronments were done by a modified method of Wallace and Hammack (2007). Swabs were moistened with 0.1% Buffered Peptone Water (Oxoid) just before for swabbing. All swab samples were pre-enriched in 10 ml buffered peptone water (BPW, Oxoid). Ten grams or 10 ml of feed, soil, drinking and pond water samples were pre-enriched in 90 ml BPW. Thirty to forty grams of either faeces or intestinal contents were homogenized for 2 min and 1 g was pre-enriched in 9 ml of BPW. Carcass was placed in sterile plastic bag containing 500 ml of BPW and was mixed by shaking to obtain carcass rinse. The carcass rinses and wash water samples were pelleted by centrifuging (Kubota 6400) at $4472 \times g$ for 15 min at 4 °C. The pellet was resuspended in 9 ml of BPW and pre-enriched. Samples for pre-enrichments were incubated at 37 °C for 24 h. After pre-enrichment 0.1 ml portions were transferred to 10 ml Rappaport Vassiliadis (RV) and Selenite Cystine (SC) broths incubated at 42 and 37 °C, respectively, for 24 h after then 0.1 ml of the culture was spread-plated on Xylose Lysine Deoxycholate (XLD) and Rambach (RA) agars and incubated at 37 °C for 24–48 h. Presumptive *Salmonella* colonies were picked, purified, Gram stained and subjected to the following biochemical tests; triple sugar iron, lysine iron agar, urease and indole production. *Salmonella* isolates were confirmed by Latex Agglutination Kit for *Salmonella* (Oxoid, UK). All media used were purchased from Merck, Germany unless otherwise stated. *Salmonella* isolates were serotyped at the Veterinary Research Institute, Ipoh, Perak, Malaysia according to the Kauffmann and White Scheme (Grimont & Weill, 2007).

2.3. Antibiotic sensitivity of Salmonella serovars

The disk diffusion method described by (Bauer, Kirby, Sherris, & Turk, 1966) was used to determine the antibiotic resistance of 125 *Salmonella* isolates against 13 antimicrobial agents: ampicillin (Amp) 10 μg; chloramphenicol (C) 30 μg; nalidixic acid (Na) 30 μg; streptomycin (S) 10 μg; tetracycline (Te) 30 μg; ceftriaxone (Cro) 30 μg; cephalothin (Kf) 30 μg; erythromycin (E) 15 μg; suphamethoxazole/trimethoprim (Sxt) 22 μg; gentamicin (g) 10 μg; ciprofloxacin (Cf) 10 μg; cefotaxime (Ctx) 30 μg; and norfloxacin (Nor) 10 μg; purchased from Oxoid, UK. Pure cultures were grown overnight in Tryptic Soy Broth (TSB) (Merck, Germany) at 37 °C and the concentration adjusted using sterile TSB until a 0.5 McFarland turbidity was attained. One hundred microliters of the culture was then swabbed onto Mueller Hinton agar (Oxoid, UK) using a sterile cotton swab. Antimicrobial disks were placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37 °C for 16 to 18 h and the results were interpreted as sensitive, intermediate, or resistance according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). *Escherichia coli* were used as control in the antimicrobial susceptibility test.

The Multiple Antibiotic Resistance (MAR) index was calculated and interpreted according to (Krumperman, 1983) using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested. Isolates classified as intermediate on the basis of inhibition zone were considered as sensitive for the MAR index (Singh et al., 2010).

2.4. Plasmid size determination

Single colony of pure *Salmonella* culture was inoculated into 5 ml Luria-Bertani and incubated in orbital shaker (with vigorous shaking) at 37 °C for 16 to 18 h. Cell density was adjusted between 1.6 and 1.9 using spectrophotometer at 600 nm. The overnight culture (1.5 ml) was centrifuged for 5 min at $1000 \times g$ to obtain pellets. Pellets were dried and subjected to plasmid DNA extraction and purification using Promega Wizard® plus Minipreps DNA Purification System by following the manufacturer's instructions (Anonymous, 2010b). In brief, the pellets were suspended in 750 μl (250 μl × 3) cell resuspension solution and lysed in 250 μl cell lysis solution. Ten microliters of alkaline protease was added and then neutralized using 350 μl neutralization solution. The suspension was column washed using 1 ml column wash solution. Afterwards, plasmids where eluted in 40 μl nuclease-free water. Purified plasmids extracted were stored at −20 °C for further analysis. Plasmids were later loaded on 0.7% agarose gel and separated using horizontal gel electrophoresis system (ELITE 300). Plasmid DNA bands were visualized using UV transilluminator (UV TEC Gel Imaging System). Lambda DNA/HindIII marker was used as the molecular weight marker and plasmid size was determined using UVI TEC UVIBand.

2.5. Statistical analysis

The data obtained was analyzed using Chi-Square test for goodness of fit to determine whether significant variations occurred among *Salmonella* serotypes obtained from different samples as adapted by Suresh, Hatha, Harsha, and Lakshmanaperumalsamy (2011). Chi-Square (χ^2) was defined as: $\chi^2 = (o-e)^2/e$ where o is the observed data, e is the expected data and the results obtained were interpreted using Chi-Square distribution table at 5% significant level (Fisher & Yates, 1963).

3. Results and discussion

3.1. Prevalence and distribution of Salmonella species in ducks, duck rearing and processing environments

The presence of *Salmonella* serovars in ducks, duck rearing and processing environments is presented in Table 1. Variation in the prevalence of *Salmonellae* among different categories of samples was found to be significantly different from each other ($P<0.05$). One hundred and twenty five out of five hundred and thirty one (125/531)

Table 1
Distribution of *Salmonella* species in ducks, duck rearing and processing environments.

Duck samples tested	Number of samples tested	Number of positive samples	% prevalence
Faecal sample	105	41	39.0
Pond water	16	5	31.3
Intestinal content	100	28	28.0
Wash water	30	8	26.7
Soil sample	60	14	23.3
Cloacal swab	75	15	20.0
Floor swab	15	2	13.3
Transport crate swab	15	2	13.3
Drinking water	30	3	10.0
Feed sample	30	3	10.0
Carcass rinse	30	3	10.0
Table swab	15	1	6.7
Egg shell swab	10	0	0.0
Overall	531	125	23.5

samples were positive for 125 isolates of *Salmonella*. The 125 *Salmonella* isolates belonged to 10 different serovars. The predominant serovars were *S.* Typhimurium (37/125), *S.* Hadar (26/125), *S.* Enteritidis (15/125), *S.* Braenderup (15/125) and *S.* Albany (14/125). Other serovars isolated were *S.* Derby (8/125), *S.* Newbrunswick (4/125), *S.* Gallinarum (3/125), *S.* Welteverden (2/125) and *S.* London (1/125). Most of the *Salmonell*a serovars (Table 2) were isolated from faecal samples (41/105), intestinal contents (28/100), cloacal swabs (15/75) or soil samples (14/60).

From our results, it is evident that ducks can be reservoirs for *Salmonella* species. Results in Table 1 show that 28/100 (28.0%) of intestinal contents and 15/75 (20.0%) of cloacal swabs obtained from ducks at wet markets and farms were positive for *Salmonella*. The prevalence of *Salmonellae* in duck intestines and cloaca swabs were not significantly different ($P=0.20$) from each other. Thirty-nine percent (41/105) of the faecal samples examined were positive for *Salmonella*, while 6.7% (1/15) of table swab samples were positive for *Salmonella* and differed significantly ($P<0.05$) from each other. *Salmonellae* were also isolated from both rearing and processing environments as samples obtained from duck farms (soil, drinking water and soil samples) were positive for *Salmonella*. Samples obtained from the processing environment (table swabs, floor/crate swabs and wash water) were also positive for *Salmonella*. Healthy ducks like other avian species harbour *Salmonella* species in their gastrointestinal tract and subsequently shed during defecation. *Salmonella* species can survive well in faeces, soil, pond water, drinking water, feed, transport crates, egg shell, processing floor, cutting table and wash water. Survival of *Salmonella* species in soil, floors, cutting boards, transport crates and perhaps faeces suggest that the pathogen can persist in duck farms and slaughtering areas, and infect subsequent flocks and carcasses. These findings are in agreement with that of Pan et al. (2010) and Tran et al. (2004).

Pan et al. (2010) examined 285 duck faecal samples which were collected between 2008 and 2009 for *Salmonell*a species in China. They reported that 5 samples (5.3%) were positive for *Salmonella*. Tran et al. (2004) reported that 31/357 (8.7%) faecal/intestinal samples obtained from ducks reared in the Mekong delta, were positive for *Salmonella*. A year later, Tran et al. (2005) reported that the incidence of *Salmonella* in retail duck meat samples in the same village was 22.3%. Tsai and Hsiang (2004) reported that in Taiwan, 4.6% (91/2000) of ducks and 20.0% (20/100) of the flocks examined were positive for *Salmonella*, respectively. In a specialty poultry market in California, USA, McCrea et al. (2006) studied the incidence of *Salmonella* at various stages of duck processing. They observed that the incidence of *Salmonella* on the farm, post transport, post picking of carcass and post-waxing was $3.3\%\pm1.3$, $3.3\%,\pm1.3$ $6.1\%\pm1.8$, and $11.3\%\pm2.0$ respectively.

In the present study, *S.* Gallinarum was only present in the intestines, *S.* Weltevreden in wash water and *S.* London in feed sample. The probable source of *S.* Weltevreden and *S.* London is not clear but these serovars may have originated from the ducks themselves, rearing or processing environment. *Salmonella* London may have been present in the feed from the feed mill. *Salmonella* Gallinarum is a host adapted *Salmonella* serovar known to cause high morbidity and mortality in chickens (Singh et al., 2010) and can cause similar problem in ducks. In Connecticut, Maryland and Pennsylvania, human *Salmonella* infections were linked to *S.* Hadar from pet ducklings (Morbidity & Mortality Weekly Report, 1992). In Italy, *S.* Hadar outbreak was responsible for the death of a young girl (Bisbini, Leoni, & Nanetti, 2000). *Salmonella* Weltevreden was implicated in food poisoning outbreak involving 24 students in Mangalore, India (Antony, Dias, Shetty, & Rekha, 2009). *Salmonella* London was responsible for outbreaks in Gangwon Province linked to infant formula (Park et al., 2004). Kim et al. (2003) investigated enteritis outbreak in infants caused by *S.* London and concluded that the organism originated from a common contaminated source. Isolation of similar serovars from different samples in the same environment coupled with farming and processing practises suggest that cross-contamination might have taken place; and for *Salmonella* serovars to be present in samples such as wash water, cutting tables, drinking water, pond water and floor swabs, it is obvious cross-contamination is unavoidable.

In this study, *S.* Typhimurium was the predominant serovar which is in agreement with findings of McCrea et al. (2006) who reported that *S.* Typhimurium was the predominant *Salmonella* serovar isolated from ducks in a specialty poultry market in California, USA. Saitanu, Jerngklinchan, and Koowatananukul (1994) also observed that *S.* Typhimurium (5.5%) was the most prevalent serotype in duck eggs in Thailand. In contrast, Tsai and Hsiang (2004) reported that *S.* Potsdam (31.9%) and *S.* Dusseldorf (18.7%) were the predominant serovars in ducks in Taiwan. Other serovars such as *S.* Montevideo, *S.* Newport, *S.* Assinine, *S.*

Table 2
Prevalence of *Salmonella* serovars in ducks, duck rearing and processing environments.

	S. Typhimurium	*S.* Enteritidis	*S.* Gallinarum	*S.* Braenderup	*S.* Albany	*S.* Hadar	*S.* Derby	*S.* Weltevreden	*S.* London	*S.* Newbrunswick
Faecal sample	10	9	0	4	5	0	3	0	0	0
Pond water	0	0	0	0	0	0	1	0	0	1
Intestinal content	13	6	3	5	1	0	0	0	0	0
Wash water	7	0	0	1	0	8	0	2	0	0
Soil sample	4	0	0	1	2	4	0	0	0	0
Cloacal swab	0	0	0	4	0	10	3	0	0	1
Floor swab	2	0	0	0	0	0	0	0	0	0
Transport crate swab	0	0	0	0	2	0	0	0	0	0
Drinking water	0	0	0	0	0	3	0	0	0	2
Feed sample	0	0	0	0	1	1	1	0	1	0
Carcass rinse	0	0	0	0	3	0	0	0	0	0
Table swab	1	0	0	0	0	0	0	0	0	0
Egg shell swabs	0	0	0	0	0	0	0	0	0	0
Total no.	37	15	3	15	14	26	8	2	1	4
Overall (%)	29.6	12	2.4	12	11.2	20.8	6.4	1.6	0.8	3.2

Table 3
Percentage *Salmonella* serovars resistant to different antibiotics.

Antibiotic	*S.* Typhimurium	*S.* Enteritidis	*S.* Gallinarum	*S.* Braenderup	*S.* Albany	*S.* Hadar	*S.* Derby	*S.* Weltevreden	*S.* London	*S.* Newbrunswick
Ampicillin	24.3% (9/37)[a]	60.0% (9/15)	66.7% (2/3)	6.7% (1/15)	42.9% (6/14)	0.0% (0/26)	37.5% (3/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Chloramphenicol	24.3% (9/37)	13.3% (2/15)	33.3% (1/3)	86.7% (13/15)	92.9% (13/14)	15.4% (4/26)	62.5% (5/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Nalidixic acid	81.1% (30/37)	60.0% (9/15)	66.7% (2/3)	86.7% (13/15)	57.1% (8/14)	100% (26/26)	37.5% (3/8)	50.0% (1/2)	0.0% (0/1)	0.0% (0/4)
Streptomycin	2.7% (1/37)	6.7% (1/15)	0.0% (0/3)	26.7% (4/15)	50.0% (7/14)	73.1% (19/26)	12.5% (1/8)	0.0% (0/2)	0.0% (0/1)	100% (4/4)
Tetracycline	78.4% (29/37)	60.0% (9/15)	66.7% (2/3)	86.7% (13/15)	57.1% (8/14)	80.8% (21/26)	0.0% (0/8)	100% (2/2)	100% (1/1)	100% (4/4)
Ceftriaxone	0.0% (0/37)	0.0% (0/15)	0.0% (0/3)	0.0% (0/15)	0.0% (0/14)	0.0% (0/26)	0.0% (0/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Cephalothin	0.0% (0/37)	0.0% (0/15)	0.0% (0/3)	20.0% (3/15)	21.4% (3/14)	0.0% (0/26)	0.0% (0/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Erythromycin	100% (37/37)	100% (15/15)	100% (3/3)	100% (15/15)	100% (14/14)	100% (26/26)	100% (8/8)	100% (2/2)	100% (1/1)	100% (4/4)
Suphamethoxazole/trimethoprim	27.0% (10/37)	13.3% (2/15)	33.3% (1/3)	86.7% (13/15)	85.7% (12/14)	15.4% (4/26)	37.5% (3/8)	0.0% (0/2)	100% (1/1)	25.0% (1/4)
Gentamicin	0.0% (0/37)	0.0% (0/15)	0.0% (0/3)	0.0% (0/15)	0.0% (0/14)	0.0% (0/26)	0.0% (0/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Ciprofloxacin	0.0% (0/37)	0.0% (0/15)	0.0% (0/3)	0.0% (0/15)	0.0% (0/14)	0.0% (0/26)	0.0% (0/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Cefotaxime	0.0% (0/37)	0.0% (0/15)	0.0% (0/3)	0.0% (0/15)	7.1% (1/14)	0.0% (0/26)	0.0% (0/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Norfloxacin	2.7% (1/37)	6.7% (1/15)	0.0% (0/3)	0.0% (0/15)	0.0% (0/14)	0.0% (0/26)	0.0% (0/8)	0.0% (0/2)	0.0% (0/1)	0.0% (0/4)
Overall	26.4% (127/481)	24.6% (48/195)	28.2% (11/39)	38.5% (75/195)	39.6% (72/182)	29.6% (100/338)	22.1% (23/104)	19.2% (5/26)	23.1% (3/13)	25.0% (13/52)

[a] No. of isolates resistant to a particular antibiotic.

Indiana, *S.* Senftenberg, *S.* Heidelberg, *S.* Schwarzengrund, *S.* Cerro, *S.* Tennessee, *S.* Amsterdam, *S.* Agona and *S.* Infantis (Saitanu et al., 1994; Chansiriporchai, Ramasoota, Bangtrakulnonth, Sasipreeyajan, & Svenson, 2000; Tsai & Hsiang, 2004; McCrea et al., 2006), have been reported in ducks and duck eggs. In chickens it is well established that *S.* Enteritidis is the most predominant *Salmonella* serovar followed by *S.* Typhimirium (Suresh et al., 2006; Suresh et al., 2011).

3.2. Antimicrobial resistance and plasmid analysis of the Salmonella serovars

Results in Table 3 show that all *Salmonella* serovars examined were susceptible to ciprofloxacin, ceftriaxone, cefotaxime, norfloxacin and gentamicin except for one strain of *S.* Albany, which were resistant to cefotaxime. All of the isolates were resistant to erythromycin. A large percentage of *Salmonella* serovars were also resistant to tetracycline (57–100%) and nalidixic acid (37.5–81.1%). All strains of *S.* Welterveden, *S.* London and *S.* Newbrunswick serovars which were found infrequently (ranging from 1 to 4 times) were susceptible to most of the antibiotics except tetracycline (all three serovars), erythromycin (all three serovars), streptomycin (only *S.* Newbrunswick), nalidixic acid (only one *S.* Welterveden) and suphamethoxazole-trimethprim (one strain each of *S.* Welterveden and *S.* London).

Pan et al. (2010) observed that *Salmonella* species isolated from faecal samples of domestic animals (chickens, ducks, geese and pigs) were resistant to nalidixic acid (48.8%), tetracycline (46.9%), sulfafurazole (45.7%), ampicillin (43.2%), streptomycin (38.3%) and trimethoprim/sulfamethoxazole (33.3%). They also reported that *Salmonella* isolates from ducks showed the least resistance to the antibiotics tested.

The antimicrobial resistance profile, MAR index and plasmid size of the *Salmonella* serovars are presented in Table 4. All the isolates were resistant to at least one antibiotic. One hundred and twenty five *Salmonella* isolates belonging to 10 different serovars exhibited 29 different antibiogram patterns. *Salmonella* Typhimurium exhibited 12 different resistant patterns to the antibiotics examined. Furthermore 8, 7, 5, 5, 4, 2, 2 and 1, different resistant patterns were shown by *S.* Albany, *S.* Enteritidis, *S.* Braenderup, *S.* Derby, *S.* Hadar, *S.* Newbrunswick, *S.* Weltevreden and *S.* London, respectively. The majority of *S.* Typhimurium (20) and Braenderup (9) isolates showed a resistant pattern of TeNaE. This resistant pattern was also observed in *S.* Enteritidis (5), *S.* Weltevreden (1) and *S.* Gallinarum (1) isolates.

One *S.* Enteritidis strain isolated from faeces was resistant to 8 antibiotics (TeNaAmpSxtCNorES) and had the highest MAR index of 0.62. One isolate each of *S.* Braenderup, *S.* Albany and *S.* Typhimirium was resistant to 7 antibiotics with a high MAR indices of 0.54. Ten and twelve isolates were resistant to 6 and 5 antibiotics with MAR index of 0.46 and 0.38, respectively. Eight antibiotic resistant patterns (TeNaES, TeNaE, TeNaAmpSxtCE, NaSxtCE, AmpE, TeNaAmpE, TeE, and NaE) were shared by 2 to 4 different serovars. The emergence of *Salmonella* serovars with high MAR index suggest that these serovars have originated from environments where antimicrobials are often used as therapeutic or as growth promoters in animal feeds (Krumperman, 1983; Singh et al., 2010). Furthermore, multiple drug resistant *Salmonella* isolates have been suggested to be more virulent than non-multiple drug resistant *Salmonella* isolates (Fluit, 2005; Foley & Lynne, 2008). *Salmonella* serovars resistant to one or more antibiotics have been reported by many investigators (Tsai & Hsiang, 2004; Foley & Lynne, 2008; Pan et al., 2010).

Plasmids were not detected in 27.2% (34/125) of the isolates, while 50 isolates (40%) harboured one plasmid, 32 (25.6%) harboured two plasmids, 7 (5.6%) harboured three plasmids and 2 (1.6%) harboured four plasmids. A large plasmid (23.1 Kbp) was detected in two strains of *S.* Braenderup, two strains of *S.* Enteritidis and one strain of *S.* Hadar. Thirty five isolates harboured plasmids with sizes ranging from 20 to 22.9 Kbp. *Salmonella* isolates that did not harbour plasmids showed 19 different resistant patterns which includes majority of the antibiotics examined except ceftriaxone, gentamicin, ciprofloxacin, cefotaxime and norfloxacin.

Table 4
Antibiotic resistance profile, multiple antibiotic resistance index and plasmid size (range) of individual *Salmonella* serovars.

Name of serovar	Antibiotic resistant profile	No. of isolates	MAR index	Range(s) of plasmid size
S. Albany	TeSxtCES	6	0.38	ND-19.9
	NaAmpSxtCE	2	0.38	8.3–12.4
	NaAmpSxtCKfE	2	0.46	ND
	NaSxtCE	1	0.31	ND
	TeNaAmpSxtCE	1	0.46	7.9
	TeNaES	1	0.31	ND
	NaAmpSxtCtxCKfE	1	0.54	15.6
S. Braenderup	TeNaE	9	0.23	6.6–23.1
	KfE	2	0.15	ND-6.8
	TeNaES	2	0.31	ND-20.9
	TeNaSxtCES	1	0.46	ND
	TeNaAmpSxtCES	1	0.54	16.4–23.1
S. Derby	NaSxtCE	3	0.31	10.6–20.8
	E	2	0.08	ND
	AmpCES	1	0.31	ND
	AmpCE	1	0.23	ND
	AmpE	1	0.15	ND
S. Enteritidis	TeNaE	5	0.23	2.9–23.1
	AmpE	5	0.15	ND-18.0
	TeAmpE	1	0.23	ND
	NaAmpE	1	0.23	ND
	TeNaAmpSxtCE	1	0.46	1.4
	TeNaAmpSxtCNorES	1	0.62	16.4–23.1
	TeNaAmpE	1	0.31	8.7
S. Gallinarum	TeNaE	1	0.23	17.1–21.5
	AmpE	1	0.15	12.9
	TeNaAmpSxtCE	1	0.46	14
S. Hadar	TeNaES	19	0.31	ND-23.1
	NaSxtCE	4	0.31	ND-21.6
	TeNaE	2	0.23	ND
	NaE	1	0.15	21.1
S. London	TeSxtE	1	0.23	20.8
S. Newbrunswick	TeES	3	0.23	17.0–22.1
	TeSxtES	1	0.31	21.7
S. Typhimurium	TeNaE	20	0.23	ND-22.7
	TeNaAmpSxtCE	4	0.46	ND-15.6
	E	3	0.08	9.4–18.0
	NaAmpSxtCE	2	0.38	ND
	TeNaSxtCNorES	1	0.54	3.9
	TeAmpSxtCE	1	0.38	ND
	TeNaSxtCE	1	0.38	ND
	TeNaAmpE	1	0.31	22.1
	NaSxtE	1	0.23	ND
	AmpE	1	0.15	17.7
	TeE	1	0.15	12.2–19.8
	NaE	1	0.15	6.5–19.5
S. Weltevreden	TeE	1	0.15	19.7
	TeNaE	1	0.23	20.1

Key: Ampicillin (Amp) 10 μg; Chloramphenicol (C) 30 μg; Nalidixic acid (Na) 30 μg; Streptomycin (S) 10 μg; Tetracycline (Te) 30 μg; Ceftriaxone (Cro) 30 μg; Cephalothin (Kf) 30 μg; Erythromycin (E) 15 μg; Suphamethoxazole/Trimethoprim (Sxt) 22 μg; Gentamicin (Cn) 10 μg; Ciprofloxacin (Cf) 10 μg; Cefotaxime (Ctx) 30 μg; Norfloxacin (Nor) 10 μg; ND (no plasmid detected).

Isolates harbouring the largest plasmid size of 23.1 Kbp were resistant to tetracycline, nalidixic acid, erythromycin, ampicillin, suphamethoxazole/trimethoprim, chloramphenicol, streptomycin, and norfloxacin, and showed 5 resistant patterns. The smallest plasmid detected (1.4 Kbp) in one of the *S.* Enteritidis isolates was resistant to tetracycline, nalidixic acid, suphamethoxazole/trimethoprim, ampicillin, chloramphenicol and erythromycin. Similarly *Salmonella* isolates with a plasmid size(s) of 7.9 Kbp (one strain of *S.* Albany), 14.0 Kbp (one strain of *S.* Gallinarum), 3.9, 8.8, and 15.6 Kbp (three strains of *S.* Typhimirium) and one *S.* Typhimirium isolate without plasmid were resistant to the same antibiotics. Probably certain plasmid sizes may be responsible for resistance to particular antibiotics. White et al. (2001) found that four *S.* Typhimurium DT104 and one DT104b isolate possessed a 1.0 Kb integron containing *aadA* and 1.2 Kb containing β-lactamase bla_{PSE-1} gene which confers resistance to ampicillin. They also found that a 1.0 Kb and 1.2 Kb integrons in *S.* Agona contained the *aadA1* gene which confers resistance to streptomycin. The 13.078 bp of *S.* Typhimurium isolate contained four genes *aadA2*, *sul1 tetA* and bla_{CARB-2} which encode resistant to streptomycin, sulphonamides, tetraclycline and ampicillin, respectively (Briggs & Fratamico, 1999). Kwon et al. (2002) in their work reported that the 1.0, 1.6, and 2.0 Kbp amplicons in *S.* Gallinarum contained one (*addA1a*), two (*aadB–aadA1b*) and three cassettes (*dhfrXII–orf–aadA2*) respectively, providing resistances against aminoglycosidase (*aadA1a*, *aadA1b*, *aadB*, and *aadA2*) and trimethoprim (*dhfrXII*).

Nonetheless since some of the isolates were resistant to one or more antibiotics and yet did not harbour any plasmids, the antibiotic resistance might be chromosomally mediated or mediated by other mobile elements such as transponons. The finding of both plasmid and non-plasmid mediated antibiotic resistant isolates is consistent with other studies (Rodrigue et al., 1992; Ansary, Haneef, Torres, & Yadav, 2006).

4. Conclusion

Our work indicated that the occurrence of *Salmonella* species in ducks, duck rearing and processing environments was relatively high. The prevalence rate ranged from 0.0 to 39.0%. Ten different serovars with an overall prevalence ranging from 0.8 to 29.6% were obtained. These serovars showed different antibiotic resistance percentages

(19.2–39.6%), antibiotic resistant profiles (resistant to 1–8 antibiotics), MAR index (0.08–0.62) and plasmid size (1.4–23.1 Kbp) with a tendency for serovars from the same source to show similar characteristics. The relatively high, varying occurrence and multi-drug resistant *Salmonella* serovars in ducks at the production and processing levels necessitates the need to implement interventions to minimise cross-contaminations at all stages in handling live ducks, duck meats, and processing equipments. Therefore measures to reduce *Salmonella* colonization, transmission and contamination needs to be improved in the study area. This involves strict adherence to biosecurity measures and to increased implementation of hazard analysis and critical control point (HACCP) to help curb the spread of *Salmonella* in ducks, their rearing and processing environments.

Acknowledgments

This research was supported by the Postgraduate Research Grant Scheme of the Universiti Sains Malaysia (1001/PTEK1ND/843007). The authors are also grateful to IPS-USM for providing a fellowship to the first author.

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