

Research Note

Prevalence and Antimicrobial Resistance of *Salmonella* Isolates from Chicken Carcasses in Retail Markets in Yangon, MyanmarAUNG ZAW MOE,^{1,2,*} PETER PAULSEN,³ DUANGPORN PICHPOL,⁴ REINHARD FRIES,⁵ HERLINDE IRSIGLER,⁶ MAXIMILIAN P. O. BAUMANN,^{6,7} AND KYAW NAING OO²

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ABSTRACT

A cross-sectional investigation was conducted concerning prevalence, antimicrobial resistance, multidrug resistance patterns, and serovar diversity of *Salmonella* in chicken meat sold at retail in Yangon, Myanmar. The 141 chicken meat samples were collected at 141 retail markets in the Yangon Region, Myanmar, 1 November 2014 to 31 March 2015. Information on hygienic practices (potential risk factors) was retrieved via checklists. *Salmonella* was isolated and identified according to International Organization for Standardization methods (ISO 6579:2002) with minor modifications. Twelve antimicrobial agents belonging to eight pharmacological groups were used for antimicrobial susceptibility testing (disk diffusion method). *Salmonella* was recovered from 138 (97.9%) of the 141 samples. The isolates were most frequently resistant to trimethoprim-sulfamethoxazole (70.3% of isolates), tetracycline (54.3%), streptomycin (49.3%), and ampicillin (47.1%). Resistance was also found to chloramphenicol (29.7%), amoxicillin-clavulanic acid (17.4%), ciprofloxacin (9.4%), tobramycin (8.7%), gentamicin (8%), cefazolin (7.2%), lincomycin-spectinomycin (5.8%), and norfloxacin (0.7%). Among the 138 *Salmonella* isolates, 72 (52.2%) were resistant to three or more antimicrobial agents. Twenty-four serovars were identified among the 138 *Salmonella*-positive samples; serovars Albany, Kentucky, Braenderup, and Indiana were found in 38, 11, 10, and 8% of samples, respectively. None of the potential risk factors were significantly related to *Salmonella* contamination of chicken carcasses. This study provides new information regarding prevalence and antimicrobial resistance and *Salmonella* serovar diversity in retail markets in Yangon, Myanmar.

Key words: Antimicrobial susceptibility; Market checklists; Risk factors; *Salmonella* prevalence; Serovar diversity

Salmonella is a member of the family *Enterobacteriaceae*. In humans, these pathogenic bacteria can cause enteric fever (*Salmonella* Typhi or *Salmonella* Paratyphi) and acute gastroenteritis (12). Although more than 2,500 serovars of *Salmonella* have been identified, the majority of human *Salmonella* infections are caused by *Salmonella enterica* subsp. group I (18).

Salmonella is most frequently detected in poultry meat and less often in pig or bovine meat (7). Thus, contaminated raw poultry products are a significant source of human salmonellosis (33). This issue is important for food safety in Myanmar because the demand for poultry meat is expected to double in the next 3 to 5 years (16). The total poultry population of Myanmar was 264.6 million birds in fiscal year 2014 to 2015, mainly chicken (91%). The Yangon region has the largest poultry inventory, with 42 million

birds. In the same fiscal year (2014 to 2015), chicken meat production was estimated at 164,000 tons in Yangon and 1,264,000 tons in all of Myanmar (20).

The burden of disease caused by *Salmonella* is substantial. An estimated 93.8 million cases of gastroenteritis caused by *Salmonella* occur globally each year, with 155,000 deaths. Nearly 80.3 million cases of *Salmonella* gastroenteritis are foodborne (19). In the United States, *Salmonella* is estimated to cause more than 1.2 million illnesses each year, with more than 23,000 hospitalizations and 450 deaths and \$3.3 billion in total medical expenditures and lost productivity each year (3, 24). Foodborne campylobacteriosis occurs more frequently in numerous regions (23), and *Campylobacter* can also be found in poultry (14, 22); there are fewer fatalities compared with salmonellosis.

Salmonella infections that result in temporary gastroenteritis usually do not require antimicrobial treatment, in contrast to invasive infections (4). Traditionally, ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole have

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been used to treat such severe cases of salmonellosis. However, the increasing number of *Salmonella* strains resistant to antimicrobial agents has led to a decrease in the efficacy of these treatments (2). Fluoroquinolones and broad-spectrum cephalosporins are currently the preferred drugs for treatment of human salmonellosis cases because of the low number of *Salmonella* isolates that are resistant to these drugs (8).

The objective of this study was to determine the prevalence of antimicrobial resistance and the *Salmonella* serovar diversity in chicken at retail markets in Yangon, Myanmar. Another aim was to identify conditions and practices in markets that may constitute risk factors for *Salmonella* contamination of chicken carcasses.

MATERIALS AND METHODS

Sample collection. A cross-sectional study was conducted to assess the prevalence and antimicrobial resistance of *Salmonella* in chicken meat from all retail markets in Yangon, Myanmar. The 141 retail markets were listed as being in the Yangon city area (32). Each market was visited between 1 November 2014 and 31 March 2015. At each market, one shop was randomly selected from a paper list of random numbers (simple random sampling). According to the usual practice in such retail shops, the staff cut the chicken breast from the carcass upon request, and the sample was taken directly from the cutting board and put in a sterile polyethylene bag. For every sample, fresh sterile gloves were used to place the sample into the bag to avoid cross-contamination. The bag was then sealed and shipped in a cooler with ice to the laboratory within 8 h. Additional information on the hygiene practices in that retail shop was retrieved by using a checklist.

Isolation and identification of *Salmonella*. Samples were analyzed at the Diagnostic Laboratory of the Livestock Breeding and Veterinary Department in Yangon; *Salmonella* was isolated and identified according to International Organization for Standardization method ISO 6579:2002 ("Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of *Salmonella* spp.," ISO, Geneva, Switzerland) with minor modifications.

A 25-g sample of skin from the chicken breast was transferred to a sterile stomacher bag (400 mL; Sterilin, Newport, UK) with 225 mL of buffered peptone water (Merck, Darmstadt, Germany). Sample surfaces were rinsed by vigorously shaking the bag by hand about 200 times. The rinse liquid was then incubated at $37 \pm 1^\circ\text{C}$ for 18 ± 2 h, 0.1 mL of this preenrichment broth culture was transferred into a tube containing 10 mL of Rappaport-Vassiliadis-soya broth (RVS; Merck), and this culture was incubated at $1.5 \pm 1^\circ\text{C}$ for 24 ± 3 h. In parallel, 1 mL of the same preenrichment broth was transferred into a tube containing 10 mL of Muller Kauffmann tetrathionate novobiocin broth (MKTTn; Merck) and incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 h.

The RVS and MKTTn cultures were then streaked onto xylose lysine Tergitol 4 agar (XLT4; Merck) and brilliant green phenol red lactose sucrose agar (BPLS; Merck) plates, incubated in an inverted position at $37 \pm 1^\circ\text{C}$ for 24 ± 3 h, and then checked for typical *Salmonella* colonies.

Biochemical and serological confirmation. For confirmation of *Salmonella* identification, five typical colonies were chosen from each XLT4 and BPLS plate for biochemical confirmation on triple sugar iron agar (Merck), urea agar (Conda, Madrid, Spain),

and motility indole lysine medium (Difco, Bordeaux, France) after incubation at $37 \pm 1^\circ\text{C}$ for 24 ± 3 h. These confirmed isolates were streaked on nutrient agar (NA; Oxoid, Basingstoke, UK) and incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 h, and colonies were confirmed by slide agglutination with *Salmonella* O polyvalent antisera (Serotest, S&A Reagents, Bangkok, Thailand). All isolates positive for *Salmonella* after biochemical and serological confirmation were transferred into half-strength NA in 1.5-mL Eppendorf tubes and stored at 4°C .

Antimicrobial susceptibility test. Antimicrobial susceptibility testing (ampicillin, amoxicillin-clavulanic acid, cefazolin, chloramphenicol, ciprofloxacin, gentamicin, lincomycin-spectinomycin, norfloxacin, streptomycin, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole) was done according to Clinical and Laboratory Standards Institute (CLSI) methods (5) at the Veterinary Public Health Centre for Asia Pacific (Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand) in April 2015. Interpretation of results followed CLSI methods (6). *Escherichia coli* ATCC 25922 was used as a reference strain.

Serotyping. Serotyping of *Salmonella* isolates was performed at the Meat Hygiene Section of the Institute of Food Safety and Food Hygiene (Department of Veterinary Medicine, Freie Universität, Berlin, Germany) by slide agglutination according to the Kauffmann-White scheme (10) with commercial antisera (Sifin, Berlin, Germany).

Data collection for market checklist. Information on hygienic measures and technical practices at markets was retrieved through interviews and observations based on a checklist (Table 1) on the day of the visit.

Data analysis. The resistance pattern of *Salmonella* isolates was analyzed by R-commander software, version 3.2.0 (<http://socserv.mcmaster.ca/jfox/Misc/Rcmdr/>). Answers to questions on the checklist were used to test for risk factors for *Salmonella* contamination. *Salmonella* status of samples was cross-tabulated with the answers to each question or item in the checklist, and odds ratios with 95% confidence intervals (CIs) were calculated. The significance of the associations was tested with a chi-square or Fisher's exact test in R-commander 3.2.0. Results were considered significant at $P < 0.05$.

RESULTS

Prevalence and antimicrobial resistance of *Salmonella*. The prevalence of *Salmonella* in chicken meat samples from the 141 retail markets in Yangon was 97.9% (95% CI, 93.9 to 99.3%). For these *Salmonella* isolates, resistance to trimethoprim-sulfamethoxazole was most common (70.3% of isolates), followed by resistance to tetracycline (54.3%), streptomycin (49.3%), ampicillin (47.1%), chloramphenicol (29.7%), amoxicillin-clavulanic acid (17.4%), ciprofloxacin (9.4%), tobramycin (8.7%), gentamicin (8%), cefazolin (7.2%), lincomycin-spectinomycin (5.8%), and norfloxacin (0.7%) (Table 2).

Multidrug resistance in *Salmonella*. Among the 138 *Salmonella* isolates, 72 (52.2%) were considered multidrug resistant (i.e., resistant to three or more antimicrobial agents), and 39 patterns of multidrug resistance were found.

TABLE 1. Prevalence of *Salmonella* in chicken meat obtained from 141 retail markets and answers to questions regarding store structure, hygienic practices, and conditions, Yangon, Myanmar, November 2014 to March 2015

Question ^a	Salmonella prevalence (no. of samples/ no. of answers)	
	Positive	Negative
General items		
Chicken slaughtered in market	24/25	114/116
Shop located within a specific meat-selling zone of market	91/94	47/47
Shop located within 5 m of sewage	109/111	30/30
Meat and offal		
Nonedibles discarded by feeding to stray animals	5/6	133/135
Nonedibles discarded to garbage	24/24	114/117
Nonedibles discarded by selling for animal feed	109/111	29/30
Meat stored close or next to internal organs	133/136	5/5
Only chicken meat sold	113/115	25/26
Staff		
Seller wears apron	11/12	127/129
Seller's hands cleaned with soap	46/46	92/95
Seller's hands cleaned with water	57/60	81/81
Food contact surfaces and cleaning		
Wooden cutting boards	135/138	3/3
Contact surface of table made of wood	89/91	49/50
Contact surface of table cement and/or tile	37/37	101/104
Contact surface of table steel	10/11	128/130
Contact surface of table bamboo	2/2	136/139
Table higher than 60 cm	102/105	36/36
Table covered by tablecloth	81/82	57/59
Use of single wipe cloth for chicken, hands, and equipment	109/110	29/31

^a Questions with 100% positive or 100% negative answers are not included.

TABLE 2. Top five major antimicrobial resistance patterns of 138 *Salmonella* isolates from chicken carcasses from 141 retail markets, Yangon, Myanmar, November 2014 to March 2015

Resistance pattern ^a	No. of isolates	% of isolates	95% CI (%)
SXT, TE, S, AMP, C, AMC	7	5.1	2.5–10.1
SXT, TE, S, AMP, C	7	5.1	2.5–10.1
SXT, TE, S	6	4.3	2.0–9.2
SXT, TE, S, AMP	4	2.9	1.1–7.2
SXT, TE, S, AMP, C, TOB, CN	4	2.9	1.1–7.2

^a SXT, trimethoprim-sulfamethoxazole; TE, tetracycline; S, streptomycin; AMP, ampicillin; C, chloramphenicol; AMC, amoxicillin-clavulanic acid; TOB, tobramycin; CN, gentamycin.

TABLE 3. *Salmonella* serovars isolated from raw chicken meat from 141 retail markets, Yangon, Myanmar, November 2014 to March 2015^a

Salmonella serovar(s)	No. of samples	% of samples
Albany	53	38.4
Kentucky	15	10.9
Braenderup	14	10.1
Indiana	11	8.0
Virchow	5	3.6
Brunei	5	3.6
Weltevreden	4	2.9
Derby, Typhimurium, Enteritidis, Wagania	3 each	2.2 each
Diogoye, Bareilly, Lexington, Stanley, Agona, Hindmarsh	2 each	1.4 each
Cerro, Yoruba, Mbandaka, Newport, Stuttgart, Paris, Apeyeme	1 each	0.7 each

^a A total of 138 *Salmonella* isolates were recovered.

Only seven isolates (three of *Salmonella* Braenderup, two of *Salmonella* Enteritidis, and one each of *Salmonella* Albany and *Salmonella* Brunei) were susceptible to all 12 antimicrobial agents. One isolate (*Salmonella* Indiana) was resistant to 10 antimicrobial agents among the eight groups of antimicrobial agents (Table 2).

Salmonella serovars. Twenty-four serovars were identified among the 138 *Salmonella* isolates. The most commonly identified serovars were *Salmonella* Albany (53 isolates; 38%) followed by *Salmonella* Kentucky (15 isolates; 11%), *Salmonella* Braenderup (14 isolates; 10%), and *Salmonella* Indiana (11 isolates; 8%) (Table 3).

Risk factors. Overall, no significant associations between risky or unhygienic practices in the shops (Table 1) and the presence of *Salmonella* on chicken meat were found. Items for which responses were 100% negative or 100% positive were not included in Table 1. All shops were retail shops, and none had an established pest control system. Meat was transported to the shops and stored there at ambient temperature, and meat was handed over to consumers without any cover or wrapping. No water and towels were available in the shops to allow cleaning of workers' hands. Protective gloves were not used.

DISCUSSION

Prevalence of *Salmonella*. The prevalence of *Salmonella* in chicken meat from retail markets in Yangon, Myanmar, was clearly higher than that recently reported in four studies in China and Vietnam, with 43.3, 52.2, 45.9, and 48.7% *Salmonella*-positive chicken carcasses (25, 26, 28, 31). In a previous study in Myanmar, the proportion of *Salmonella*-positive chicken samples was 4% in five A-grade markets in the Yangon region in 2005 (13) but was 72.5 and 90% in chicken samples in the Mandalay region in 2009 (11, 21). Similar results (68%) were reported for beef samples in Mandalay in 2012 (27). However, different types

of meat samples and isolation methods complicate comparisons. Although sampling a whole carcass (25, 26, 28, 31) may be more sensitive for recovering *Salmonella* than sampling only one piece of meat with the skin on (as done in our study), the recovery of *Salmonella* from 97.9% of samples indicates that either *Salmonella* levels per carcass were high or that contamination of meat occurred during portioning by the staff (25).

Antimicrobial resistance. Resistance to trimethoprim-sulfamethoxazole was found in 70.3% of the isolates of this study. This finding corresponds closely to the 74.4% resistance to cotrimoxazole reported previously (21) but was three times higher than the cotrimoxazole resistance reported among *Salmonella* isolates from beef (27), with 24.4% in Myanmar, and about double (34.6%) that reported in a study in Vietnam (26). Resistance to tetracycline was found in 54.3% of the isolates, which was about half the prevalence reported in previous studies in Myanmar (100%) (13, 21, 27). Because of these high resistance rates reported for 2005 to 2009, the use of tetracyclines has been reduced in poultry production, which may account for the decline in resistance we observed in Myanmar. The percentage of resistant isolates was comparable to that reported for *Salmonella* on chicken meat in Vietnam (59.1%) (26) but was significantly lower than that recently reported in China (71.1%) (28). *Salmonella* resistance to ciprofloxacin was low, i.e., 9.4% in this study, similar to the 7.3% reported in 2012 (27), but was considerable lower than the rates reported in a 2009 study (87.2%) (21) in Myanmar and less than half that found in a previous study in China (22.5%) (28). Regarding resistance to quinolones, which are the principal antimicrobial agents used in the treatment of human salmonellosis, our study revealed the prevalence of resistance of 9.4% to ciprofloxacin and 0.7% to norfloxacin, much lower than the prevalences reported in the European Union (11 to 100%) (8), China (12.1%) (29), and Thailand (27.27%) (1). Reduced susceptibility to ciprofloxacin among *Salmonella* clinical isolates have been reported for the Philippines (15% of isolates resistant), Singapore (25% resistant), and Thailand (46.2% resistant) (17).

Regarding multidrug resistance, the percentage of the resistant isolates that were multidrug resistant (i.e., 52.2%) was quite comparable to that reported for Vietnam (57%) (26) but lower than that reported for China (77%) (28). When compared with European Union data, our results rank in an intermediate position; the percentages of multidrug-resistant *Salmonella* isolates were sometimes lower, e.g., in France (2.4%), Spain (11.5%), and Germany (14.6%), but sometimes higher, e.g., in Hungary (78.7%) and Slovenia (93.3%) (8).

This study was the first comprehensive and detailed examination of *Salmonella* serovars in Myanmar. Among 138 *Salmonella* isolates, 24 *Salmonella* serovars were identified. The four most frequently identified *Salmonella* serovars were Albany, Kentucky, Braenderup, and Indiana, accounting for 38, 11, 10, and 8% of isolates, respectively. *Salmonella* Albany was the most frequently observed serovar in the present study, at a prevalence similar to that

reported in Vietnam (34.1%) (26), whereas *Salmonella* Typhimurium and *Salmonella* Enteritidis were predominant serovars in many other studies (9, 15, 25, 28, 30).

In previous work from 2005 in Myanmar (13), only a few isolates were tested, and *Salmonella* serovars Enteritidis, Newport, Pullorum, and Senftenberg accounted for 2, 1, 1, and 1 of the 6 isolates, respectively. In contrast, in our study, *Salmonella* Enteritidis and *Salmonella* Newport accounted for merely 2 and 1% of isolates; *Salmonella* Pullorum and *Salmonella* Senftenberg were not detected.

Risk factors. In this study, none of the practices evaluated in retail shops were identified as significant risk factors for the presence of *Salmonella*, despite the large number of items checked (Table 1). The hygienic conditions observed are not unexpected for “wet” markets with a quick turnover of raw meats, with lack of refrigeration and shortcomings in sanitation facilitating growth and persistence of foodborne pathogens. These conditions may explain the high proportion of *Salmonella*-positive samples. Cleaning of utensils with potable water, covering meat, and other hygienic practices seem to be associated with lower *Salmonella* prevalence in chicken at retail (wet) markets (31).

Further studies should help determine whether contamination of chicken meat in these markets is due to the nearly ubiquitous presence of this pathogen in these locations or whether chicken meat was contaminated before it arrived at the markets. Because poultry was slaughtered in only 18% (25 of 141) of the markets studied, the majority of chicken carcasses must have come from slaughter facilities outside the markets. Thus, a high prevalence of *Salmonella* carriage in the live poultry chain and of contamination events at the slaughter facilities or the retail shops might have contributed to the outcomes reported here.

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