RESEARCH NOTE

SALMONELLA PREVALENCE, ANTIBIOGRAMS AND SEROTYPES FROM CHICKEN MEAT AND EGG IN KHON KAEN CITY, THAILAND

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Abstract. Multidrug resistant (MDR) *Salmonella* is increasing globally. In this study, *Salmonella* prevalence in chicken meat (n = 123) and chicken egg (n = 404) from fresh produce markets and outlets in Khon Kaen city, northeastern Thailand, was 44% and 2% (egg shell not yolk), respectively. Of 66 *Salmonella* isolates, all were resistant to at least one of 20 drugs from 8 different groups of antibiotics, with the majority (23%) to 1 drug, followed by 2 drugs (23%) and 3 drugs (18%). The most prevalent serotype of drug-resistant bacteria was *S*. Enteritidis (15%), followed by *S*. Albany (15%) and *S*. Agona (5%). One *S*. Albany and one *S*. Kentucky isolate were resistant to nine drugs. These data should be of value in controlling *Salmonella* in the poultry industry in northeastern Thailand.

Keywords: *Salmonella*, antibiogram, chicken meat, egg, prevalence, Thailand

INTRODUCTION

Salmonella is one of the leading causes of foodborne illnesses of public health concern worldwide. It is present in food of animal origin, such as chicken and pigs, and to a lesser extent in some other species and its products (Vindigni *et al*, 2007). Some other food products, such as beef, chicken and duck eggs, unpasteurized milk and milk products are also implicated as carriers for *Salmonella* (EFSA, 2004; Saengthongpinit *et al*, 2014). Human becomes infected via consumption of the contaminated aforementioned food. A study in four northeastern provinces of Thailand reported an overall prevalence of *Salmonella* in eggs of 0.63%, a low rate (Neunchat *et al*, 2015). Likewise, a study in the northeastern region of Thailand indicated *Salmonella* presence in farm environment as well as in slaughterhouse

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setting, carcasses, cages, paper cartons, chopping boards, conveyer belts, water, and certain animal products (Noppon *et al*, 2013a,b). In Thailand, intensive animal production still relies on antimicrobial usage to promote growth and well-being of the animals, resulting in an increasing incidence of drug resistance, including among *Salmonella* isolates (Angkittitrakul *et al*, 2005).

Drug-resistant *Salmonella* has become a crucial threat to humans. The world is now approaching a post-antibiotic era where antimicrobials can no longer be used as effective as before. During the past 30 years there has emerged worldwide multidrug-resistance phenotypes of *Salmonella*, such as in *S*. Enteritidis, *S*. Newport and *S*. Typhimurium (Hur *et al*, 2012). Of special concern is the emergence of resistance to quinolones, fluoroquinolones and extended-spectrum cephalosporins (Hur *et al*, 2012). Yearly there are increasing numbers of multi-drug resistance (MDR) bacteria (Chan, 2011). It is crucial that every sector be prepared to tackle the situation. Severe *Salmonella* infection often requires antibiotic therapy to aid in the elimination of the infecting agent (Hur *et al*, 2012). A study in northeastern Thailand indicated 95.9% of *Salmonella* isolates were MDR (Sinwat *et al*, 2015) and 84.0% in southern Thailand (Lertworapreecha *et al*, 2013).

Hence, the present study investigated the current prevalence, antimicrobial resistance profiles and serotypes among *Salmonella* isolated from raw chicken meat and egg samples from various outlets in Khon Kaen, northeastern Thailand.

MATERIALS AND METHODS

Sample collection

Frozen and chilled chicken meat samples and eggs were collected from fresh produce markets ($n = 123$) and other outlets ($n = 404$) in Khon Kaen, northeastern Thailand during August - November, 2015. Samples were transported to the Department of Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Khon Kaen University within 3 hours at 4°C and immediately processed on arrival.

Salmonella isolation and identification

Salmonella was isolated and identified using the procedure of ISO 6579:2002/AMD 1:2007 (ISO, 2007; Mainar-Jaime *et al*, 2013). In brief, five typical colonies were collected from each positive sample and confirmed by biochemical tests, then pure isolates were stored in 0.1% buffered peptone water (BPW; Oxoid, Basingstoke, Hampshire, UK) plus 20% glycerol as stock cultures at -80ºC until further needed.

Determination of *Salmonella* serotypes

Salmonella serotype identification was conducted at the reference laboratory, Medical Science Center 7 Khon Kaen, Ministry of Public Health, Thailand using slide agglutination test based on the Kaufman-White scheme commercially available antisera (S&A Reagents Lab, Bangkok, Thailand) (Popoff and LeMinor, 1992; Popoff *et al*, 2004; Guibourdenche *et al*, 2010).

Antimicrobial susceptibility testing

A disc diffusion method was employed for antibiotic resistance testing among *Salmonella* chicken meat and egg isolates according to CLSI (2010-2015) and EUCAST (2016), using 8 different groups of drugs: penicillin [penicillin G, (P, 10 μg), amoxicillin+clavulanic acid (AMC, 30 μg) and tazobactam+piperacillin (TZP, 110 μg)], cephalosporin [cephalexin (CL, 30 μg), cefoxitin (FOX, 30 μg), cefoperazone (CFP,

75 μg), ceftriaxone (CRO, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), and cefepime (FEP, 30 μg)], quinolone [ciprofloxacin (CIP, 5 μg), levofloxacin (LEV, 5 μg), nalidixic acid (NA, 30 μg), and norfloxacin (NOR, 10 μg)], sulfonamide [sulphamethoxazole+trimethoprim (SXT, 25 μg)], tetracycline [tetracycline (TET, 30 μg)], aminoglycoside [gentamicin (GEN, 10 μg)], macrolide [azithromycin (AZM, 15 μg)], and others [chloramphenicol (C, 30 μg) and polymyxin B (PB, 300 U)]. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as reference bacteria. Resistance to ≥3 drugs is considered as MDR *Salmonella*.

RESULTS

The overall prevalence of *Salmonella* isolated from chicken meat and egg samples was 12% (63/527), with a higher occurrence (54/123; 44%) in chicken meat compared to eggs (shell not yolk) (9/404; 2%). The highest frequency (23%) of antimicrobial resistance was to P, followed by P/TET, P/NA, P/AZM, P/GEN, and P/SXT (23%) and P/TET/AMC, P/SXT/TET, P/SXT/NA, P/TET/GEN, and P/AZM/NA (18%) (Table 1). The antibiograms revealed 54% of *Salmonella* isolates could be categorized as MDR, with highest to 9 antimicrobials (2 isolates). No isolate was sensitive to all 20 antimicrobials tested. The most prevalent antimicrobial-resistant serotype (from 19 different serotypes identified) was *S.* Enteritidis (15%), followed by *S.* Albany (15%) and *S.* Agona (5%).

DISCUSSION

Severe *Salmonella* infection requires antibiotic therapy to eliminate the causative agent. A problem for many decades is the development of antimicrobial resistance. Of special concern is the emergence of resistance to quinolones, fluoroquinolones and extended-spectrum cephalosporins, such as ceftiofur and ceftriaxone (Hur *et al*, 2012). In the current study, 23% of *Salmonella* were resistant to just one type of drug *ie*, penicillin G, implying that this drug can no longer be used for treatment of salmonellosis. A previous study conducted in northeastern region of Thailand reported MDR *Salmonella* prevalence of 95.9% sampled from pork, chicken meat, and humans (Sinwat *et al*, 2015, 2016), a much higher value than the present study. This may be attributable to the fact that newer classes of antimicrobials and more groups of drugs were used in the current study.

The majority of *S.* Enteritidis (8 isolates) had a 2R antibiogram, and 2 were 3R. In addition, *S.* Albany and *S.* Kentucky were of the 9R category, suggesting that these two serotypes are potential sources of MDR genes for other salmonellae in the future. There has been an increasing concern over the past 30 years regarding the worldwide emergence of MDR phenotypes among such *Salmonella* serotypes as *S.* Enteritidis, *S.* Newport and *S.* Typhimurium. The current study indicated that not only the three aforementioned serotypes that have been of prime concern, *S.* Albany and *S.* Kentucky need to be added to the list. Among the seven drugs in the cephalosporin group, three *Salmonella* serotypes, namely, *S.* Albany, *S.* Anatum and *S.* Kentucky were resistance to CAZ (3rd generation cephalosporins). Nevertheless, no *Salmonella* serotype was found resistant to the 4th generation cephalosporin FEP. In addition, *S.* Kedougou was resistant to three 3rd generation cephalosporins, namely, CFP, CRO and CTX, indicating that use of 3rd generation cephalosporins will have to be closely

Table 1
Antibiogram and serotype of *Salmonella* isolates (n = 66) from chicken meat and egg samples from Khon Kaen city, northeastern Thailand, August - November, 2015.

Resistance profile	Drug (number of isolates)	Percent	Serotype (number of isolates)
1R	P (15)	23	Saintpaul (1), Typhimurium (1), Mbandaka (2), Agona (4), Weltevreden (1), Salmonella ent 8,20; (1), Stanley (1), Braenderup (2), Emek (2)
2R	P/TET (1), NA/P (11), P/AZM (1), GEN/P (1), SXT/P (1)	23	Rissen (1), Enteritidis (8), Albany (1), Virchow (3), Corvallis (1), Braenderup (1)
3R	AMC/TET/P (2), TET/SXT/P (4), NA/SXT/P (4), GEN/TET/P (1), NA/AZM/P (1)	18	Kedougou (2), Enteritidis (2), Agona (4), Albany (3), Give (1)
4R	AMC/TET/SXT/P (1), NA/C/SXT/P (3), C/TET/SXT/P (2), NA/AZM/SXT/P (2)	12	Rissen (2), Molade (1), Albany (2), Virchow (1), Mbandaka (1), Anatum (1)
5R	NOR/CIP/NA/TET/P (2), NOR/CIP/NA/LEV/P (1), NA/C/AZM/SXT/P (1)	6	Kentucky (3), Albany (1)
6R	NOR/CIP/NA/CFP/TET/P (3), NA/C/AZM/SXT/CL/P (1)	6	Emek (2), Kentucky (1), Albany (1)
7R	CAZ/AMC/C/GEN/TET/SXT/P (1), CFP/AMC/C/AZM/TET/SXT/P (1), NOR/CIP/NA/CFP/TET/LEV/P (3), CTX/CRO/CFP/TZP/AMC/TET/P (1)	9	Anatum (2), Kentucky (2), Albany (1), Kedougou (1)
9R	NA/CAZ/CRO/CFP/AMC/C/SXT/CL/P (1), NOR/CIP/NA/CAZ/CFP/TZP/TET/LEV/P (1)	3	Albany (1), Kentucky (1)

nR, resistant to n antimicrobial(s); P, penicillin G (10 μg); AMC, amoxicillin + clavulanic acid (30 μg); TZP, tazobactam + piperacillin (110 μg); CL, cephalexin (30 μg); FOX, cefoxitin (30 μg); CFP, cefoperazone (75 μg); CRO, ceftriaxone (30 μg); CTX, cefotaxime (30 μg); CAZ, ceftazidime (30 μg); FEP, cefepime (30 μg); CIP, ciprofloxacin (5 μg); LEV, levofloxacin (5 μg); NA, nalidixic acid (30 μg); NOR, norfloxacin (10 μg); SXT, sulphamethoxazole + trimethoprim (25 μg); TET, tetracycline (30 μg); GEN, gentamicin (10 μg); AZM, azithromycin (15 μg); C, chloramphenicol (30 μg); PB, polymyxin B (300 U).

monitored. Other *Salmonella* serotypes were resistant to at least one antimicrobial in the test cephalosporin group.

At present, the occurrence of *Salmonella* isolates resistant to these antibiotics has increased (White *et al*, 2001; Jones *et al*, 2002; Van *et al*, 2003; Angkittitrakul *et al*, 2005; Cailhol *et al*, 2006; Lauderdale *et al*, 2006; Cui *et al*, 2008; Pan *et al*, 2009; Xia *et al*, 2009; Yang *et al*, 2010; Economou and Gousia, 2015). Therefore, continuing monitoring programs of *Salmonella* resistance and also its prevalence in the food supply chain is necessary due to the public health implications of the potential spread of antimicrobial-resistant bacteria. Furthermore, a holistic animal management approach, such as pertinent and stringent control of antibiotic agents in the poultry and livestock productions, early clinical and microbiological diagnosis, proper treatment, and implementation of strict high sanitary standards in the food industry are also required if the burden of *Salmonella* infection in humans to be significantly decreased.

In summary, a survey of chicken meat and eggs in fresh produce markets and outlets in Khon Kaen city shows that the prevalence of MDR *Salmonella* contamination was high (54.5%), and two serotypes were resistant to up to nine antimicrobials. Emerging resistance to third generation cephalosporins should be closely monitored of prime concern, in particular among *S.* Kedougou.

ACKNOWLEDGEMENTS

Partial funding of the study came from the incubation project of Khon Kaen University, fiscal year 2011 (round 4) and a grant of the Faculty of Veterinary Medicine, Khon Kaen University.

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