

# SEROTYPES AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA ENTERICA* ISOLATED FROM PORK, CHICKEN MEAT AND LETTUCE, BANGKOK AND CENTRAL THAILAND

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**Abstract.** Food of animal origins, particularly pork and chicken meat, has long been recognized as major sources of human salmonellosis. There have been recent reports of human salmonellosis outbreaks due to consumption of leafy green vegetables such as lettuce. In this study, 120 (40 pork, 40 chicken meat and 40 lettuce) samples were randomly collected from retail markets in Bangkok and central Thailand during June to August 2015 for *Salmonella* serotype identification and antimicrobial susceptibility testing. *Salmonella* was found in 82%, 62% and 20% of pork, chicken meat and lettuce samples, respectively. The top 5 most common *Salmonella* serotypes were Panama (15%), Schwarzengrund (12%), Rissen, Anatum, and Stanley (11% each), Albany (9%), and Indiana (8%). A high percentage of *Salmonella* isolated from food of animal origin were resistant to multiple antimicrobial drugs, including ampicillin, chloramphenicol, nalidixic acid, sulfamethoxazole-trimethoprim, and tetracycline. From antibiogram pattern analysis, the most common serotypes constituted isolates that were multidrug resistant. The study indicates that *Salmonella* was still present in various kinds of food and that certain serotypes have become predominant, a phenomenon not previously reported in Thailand.

**Keywords:** *Salmonella* serotype, antimicrobial resistance, chicken meat, lettuce, pork meat, Thailand

## INTRODUCTION

*Salmonella enterica* is one of the most common foodborne pathogens, leading to millions of cases of gastroenteritis, and thousands of hospitalized patients and

even deaths worldwide each year (Pui *et al*, 2011; Hur *et al*, 2012). In Thailand, *Salmonella* is widespread in all parts of the country (Bangtrakulnonth *et al*, 2004), with the important sources of infection being animal intestinal tracts, particularly swine and chicken, and subsequent shedding of the bacteria into feces (Angkititrakul *et al*, 2005). This provides an unavoidable mechanism for *Salmonella* contamination in human food supply chain. Although foods of animal origin are the major sources of *Salmonella* contamination, re-

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cent outbreaks also have been attributed to consumption of leafy green vegetables, mainly lettuce (Sivapalasingam *et al*, 2004; Falkenstein, 2009; Quiroz-Santiago *et al*, 2009). This is probably due to the use of animal manure as fertilizer in such vegetable cultivation.

Due to the extensive use of antimicrobials in livestock and poultry for growth promotion and prevention of animal diseases, an increasing number of antimicrobial resistant *Salmonella* in humans and animals has been reported, and this has become a serious global public health problem (Yang *et al*, 2010; Hur *et al*, 2012). Furthermore, the high prevalence of multi-drug resistant (MDR) *Salmonella*, particularly to clinically effective drugs, such as fluoroquinolones and third-generation cephalosporins, is a cause of major concern. Isolated MDR *Salmonella* strains have been found to be of serotypes important to public health, such as Agona, Anatum, Cholerasuis, Derby, Kentucky, Schwarzengrund, and Typhimurium (Hur *et al*, 2012). These drug-resistant strains can spread from animal reservoirs via the food chain supply to humans, causing infection resulting in treatment failure or high mortality.

In order to ensure the effectiveness of *Salmonella* outbreak control programs, there is a need for continuous surveillance and monitoring of *Salmonella* contamination and antimicrobial susceptibility in human foods. Hence, this study investigated the prevalence, distribution and antimicrobial resistance of *Salmonella* serotypes isolated from pork, chicken meat and lettuce in Bangkok and central Thailand.

## MATERIALS AND METHODS

### Food samples

Forty samples each of pork, chicken meat and lettuce were randomly pur-

chased from retail markets in Bangkok and central Thailand during June to August 2015. Each sample was packed in individual sterile plastic bag, stored in a cool box and transported to the laboratory on the same day of collection for isolation and identification of *Salmonella*.

### *Salmonella* isolation and identification

*Salmonella* isolation technique followed that of the Bacteriological Analytical Manual (FDA, 2009). Twenty-five gram each of pork and chicken meat and 50 g of lettuce were homogenized with 225 ml of buffered peptone water (BPW) (Lab M, Lancashire, UK) supplemented with novobiocin selective supplement (SR0161E, Oxoid, Hamshire, UK) in a BagMixer (Interscience, St Nom la Bretèche, France) for 2 minutes and incubated at 37°C for 24 hours. One loopful of sample was stabbed into modified semi-solid Rappaport-Vassiliadis (MSRV) medium (Oxoid) and then streaked onto xylose lysine desoxycholate (XLD) agar (Oxoid). Plates were incubated overnight at 42°C (MSRV medium) or 37°C (XLD medium). Typical colony of *Salmonella* was tested with triple sugar iron (TSI) agar, lysine iron agar (LIA) and other confirmatory biochemical tests (motility, indole production, citrate, and urease). This study was approved by the Biosafety Committee, Thammasat University (approval no. 018/2015).

### *Salmonella* serotyping

All *Salmonella* isolates were placed in nutrient agar tubes and sent to the WHO National *Salmonella* and *Shigella* Center in Bangkok for serological testing. Serotyping of *Salmonella* isolates was performed using slide agglutination with O and H *Salmonella* antisera (S&A Reagent Laboratory LMT, Bangkok, Thailand) according to the Kauffman-White scheme (Grimont and Weill, 2007).

### Antimicrobial susceptibility assay

Antimicrobial susceptibility assay was performed using the disk diffusion method of the Bauer-Kirby technique according to the Clinical and Laboratory Standards Institute protocols (CLSI, 2013). Seven antimicrobials (Oxoid) were used: ampicillin (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), norfloxacin (10 µg), sulfamethoxazole-trimethoprim (25 µg), and tetracycline (30 µg). Inhibition zones were measured and interpreted using CLSI guideline. *Escherichia coli* ATCC 25922 was used as the control strain.

## RESULTS

### Prevalence and distribution of serotypes in food samples

The prevalence of *Salmonella* contamination in pork, chicken meat and lettuce samples was 82% (33/40), 62% (25/40) and 20% (8/40), respectively. Among the 19 serotypes identified, the 5 most common serotypes were Panama (15%), Schwarzengrund (12%), Rissen, Anatum, and Stanley (11% each), Albany (9%), and Indiana (8%) (Table 1). Panama serotype was the most common in pork (24%) and lettuce (25%), while in chicken Schwarzengrund serotype was the most common (24%).

### Antimicrobial susceptibility of *Salmonella* isolates

The majority of *Salmonella* isolates obtained from pork were resistant to ampicillin (97%), tetracycline (91%) and sulfamethoxazole-trimethoprim (55%) (Table 2). Those isolated from chicken meat mainly were resistant to nalidixic acid (76%), followed by ampicillin (60%) and then chloramphenicol (48%). Lower numbers of *Salmonella* isolated from lettuce were resistant to antimicrobial drugs:

25% with resistance to nalidixic acid and ampicillin. A number of *Salmonella* serotypes isolated from pork and chicken meat exhibited intermediate resistance to nalidixic acid (Table 2). Antimicrobial resistance to cefotaxime (third generation cephalosporin) was observed in *S. Rissen* isolated from pork and to norfloxacin (fluoroquinolone) in *S. Albany* isolated from chicken meat. Of the 39 multi-drug resistant (MDR) *Salmonella* isolates 76% (25/33), 48% (12/25) and 25% (2/8) were from pork, chicken meat and lettuce, respectively (Table 3). Among the 5 most common serotypes, Schwarzengrund and Anatum serotypes were MDR strains in all isolates, followed by Panama (80%), Rissen (71%), Albany (67%), Stanley (28%), and Indiana (20%).

## DISCUSSION

This study revealed that the retail pork had the highest *Salmonella* contamination, followed by chicken meat and then lettuce. High contamination in food of animal origin implied inadequate quality control of hygienic practice in food supply chain, particularly in the carcass production process. Traditional slaughterhouse has been recognized as a significant source of *Salmonella* contamination of chicken carcass and pork product in Thailand due to the absence of temperature control in any of the steps of meat processing (Pardungtod *et al*, 2008).

*Salmonella* serotyping analysis in this study was at variance with the previous data reported in Thailand, which indicated that Weltevreden and Enteritidis serotypes are the most common *Salmonella* isolated from humans and food of animal origin (Bangtrakulnonth *et al*, 2004; Sirichote *et al*, 2010). However, Malicharn and Vanaprasertsak (2011) and Inatsu *et al*

Table 1  
Prevalence and distribution of *Salmonella* serotypes from food.

No.	Serotype	Number of isolates (%)			
		Pork (n = 40)	Chicken meat (n = 40)	Lettuce (n = 40)	Total (n = 120)
1	S. Panama	8 (24)		2 (25)	10 (15)
2	S. Schwarzengrund	1 (3)	6 (24)	1 (12)	8 (12)
3	S. Rissen	6 (18)		1 (12)	7 (11)
4	S. Anatum	7 (21)			7 (11)
5	S. Stanley	5 (15)	1 (4)	1 (12)	7 (11)
6	S. Albany	1 (3)	5 (20)		6 (9)
7	S. Indiana		5 (20)		5 (8)
8	S. Braenderup	1 (3)	2 (8)		3 (4)
9	S. Kentucky		2 (8)		2 (3)
10	S. 4, 5, 12: i:-	2 (6)			2 (3)
11	S. Derby	1 (3)			1 (1)
12	S. Bareilly	1 (3)			1 (1)
13	S. Give		1 (4)		1 (1)
14	S. Agona		1 (4)		1 (1)
15	S. Chester		1 (4)		1 (1)
16	S. Saintpaul		1 (4)		1 (1)
17	S. Hvitvingfoss			1 (12)	1 (1)
18	S. Weltevreden			1 (12)	1 (1)
19	S. Bangkok			1 (12)	1 (1)
	Total no. (% prevalence)	33 (82)	25 (62)	8 (20)	66 (55)

(2013) recently have reported a low frequency or none of both serotypes from various kinds of food in Thailand. The most predominant serotypes found in our study were included among the 10 or 25 most common serotypes isolated from humans and other sources reported in these two studies.

Among 19 identified *Salmonella* serotypes, Panama serotype was the most predominant in pork, whereas in chicken it was Schwarzengrund serotype, and in lettuce, except Panama there were no difference in the frequencies of identified serotypes. *S. Panama* seems to be increasing in prevalence in human infection in Thailand, and has been the cause of a number of outbreaks in

many countries (Soto *et al*, 2001; Bangtrakulnonth *et al*, 2004). Importantly, several reports have indicated that *S. Panama* has the ability to cause extra-intestinal infections in humans, in particular bacteremia and meningitis, contributing to a high mortality rate (Hendriksen *et al*, 2009; Sirichote *et al*, 2010). To date, although no specific reservoir of *S. Panama* has been identified, our results suggest that pork might be the significant source of this serotype. It is well accepted that *S. Schwarzengrund* is found commonly in chicken (Poppe *et al*, 1991; Limawongpranee *et al*, 1999). Bangtrakulnonth *et al* (2004) have shown evidence of increasing proportion of this serotype among chicken in Thailand.

Table 2  
Frequency of antimicrobial resistance of *Salmonella* serotypes isolated from food.

Serotype	Number of antimicrobial resistant <i>Salmonella</i> isolates																							
	AMP			C			CTX			SXT			TE			NA (I) <sup>a</sup>			NOR					
	P	C	L	P	C	L	P	C	L	P	C	L	P	C	L	P	C	L	P	C	L			
Panama	8	-	0	8	-	0	-	0	0	8	-	0	8	-	0	0	0	0	0	0	0	0	0	0
Schwarzengrund	1	6	1	6	1	0	0	0	0	0	1	0	0	1	0	1	0	1	6	1	0	0	0	0
Rissen	6	-	1	2	-	0	-	0	3	-	0	3	-	1	6	-	1	1	-	0	0	-	0	0
Anatum	7	-	-	4	-	-	-	-	6	-	-	7	-	-	7	-	-	3	-	-	0	-	-	-
Stanley	5	0	0	0	0	0	0	0	0	0	0	5	0	0	5	0	0	2	0	1	0	0	0	0
Albany	1	5	-	1	3	-	0	0	1	2	-	1	3	-	1	4	-	1	4	-	0	2	-	-
Indiana	-	0	-	-	1	-	-	0	-	0	-	5	-	5	-	4	-	-	4	-	-	-	0	-
Braenderup	0	0	-	0	0	-	0	0	0	0	0	1	1	1	0	1	2	-	2	-	0	0	0	-
Kentucky	-	2	-	-	2	-	-	0	-	0	-	0	-	0	-	0	-	1	1	-	-	-	0	-
S. 4, 5, 12: i:-	2	-	-	0	-	-	-	-	-	0	-	2	-	-	1	-	-	1	1	-	-	-	0	-
Derby	1	-	-	1	-	-	-	-	-	0	-	1	-	-	0	-	-	0	-	-	-	-	0	-
Bareilly	1	-	-	0	-	-	-	-	0	-	0	0	-	0	1	-	-	0	-	-	-	-	0	-
Give	-	0	-	-	0	-	-	0	-	0	-	0	-	0	0	-	1	-	1	-	-	-	0	-
Agona	-	1	-	-	0	-	-	0	-	1	-	1	-	1	-	-	1	-	1	-	-	-	0	-
Chester	-	0	-	-	0	-	-	0	-	0	-	0	-	0	-	-	0	-	0	-	-	-	0	-
Saintpaul	-	1	-	-	0	-	-	0	-	0	-	0	-	0	-	-	0	-	0	-	-	-	0	-
Hvittingfoss	-	-	0	-	-	0	-	-	0	-	0	-	-	0	-	-	-	0	-	-	-	-	-	0
Weltevreden	-	-	0	-	-	0	-	-	0	-	0	-	-	0	-	-	-	0	-	-	-	-	-	0
Bangkok	-	-	0	-	-	0	-	-	0	-	0	-	-	0	-	-	-	0	-	-	-	-	-	0
Total number (%)	32	15	2	17	12	1	3	0	0	18	55	4	16	1	13	30	91	11	44	1	13	33	19	76
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<sup>a</sup>Exhibited intermediate resistance. AMP, ampicillin; C, chloramphenicol; CTX, cefotaxime; NA, nalidixic acid; NOR, norfloxacin; SXT, sulfamethoxazole-trimethoprim; TE, tetracycline. P, pork (n = 33); C, chicken (n = 25); L, lettuce (n = 8).

Table 3  
Antimicrobial resistance patterns of *Salmonella* serotypes isolated from food.

Serotype (number of isolates)	Antibiogram pattern	Number of resistant <i>Salmonella</i> isolates				Total number of MDR isolates (%)
		Pork (n = 33)	Chicken meat (n = 25)	Lettuce (n = 8)		
Panama (10)	AMP SXT TE C	7	-	0	8 (80)	
	AMP SXT TE C NA <sup>1</sup>	1	-	0		
	AMP C NA	1	5	1	8 (100)	
	AMP C NA SXT	0	1	0		
Rissen (7)	AMP TE	2	-	0	5 (71)	
	AMP TE SXT	1	-	1		
	AMP TE NA	1	-	0		
	AMP TE SXT C NA <sup>1</sup>	1	-	0		
Anatum (7)	AMP TE SXT CTX C NA <sup>1</sup>	1	-	0		
	AMP TE SXT	2	-	-	7 (100)	
	AMP TE NA C	1	-	-		
	AMP TE SXT C	1	-	-		
Stanley (7)	AMP TE SXT NA	1	-	-		
	AMP TE SXT NA C	1	-	-		
	AMP TE SXT C NA <sup>1</sup>	1	-	-		
	NA	0	0	1	2 (28)	
Albany (6)	AMP TE	2	0	0		
	AMP TE NA	2	0	0		
	AMP TE NA <sup>1</sup>	1	0	0		
	AMP	0	1	-	4 (67)	
Indiana (5)	AMP NA	0	1	-		
	AMP TE C NA	0	1	-		
	AMP SXT TE C NA	1	0	-		
	AMP SXT TE C NA NOR	0	2	-	1 (20)	
Braenderup (3) Kentucky (2)	TE NA	-	3	-		
	TE NA I	-	1	-		
	TE C NA	1	1	-		
	NA	1	2	-	0 (0)	
S. 4, 5, 12: i:- (2)	AMP C NA	-	1	-	1 (50)	
	AMP C NA <sup>1</sup>	-	1	-		
	AMP TE NA	1	-	-	1 (50)	
	AMP TE NA <sup>1</sup>	1	-	-		
Derby (1) Bareilly (1) Give (1) Agona (1) Saintpaul (1)	AMP TE C	1	-	-	1 (100)	
	AMP NA	1	-	-	0 (0)	
	NA	-	1	-	0 (0)	
	AMP E SXT TE NA	-	1	-	1 (100)	
Total of MDR isolates (%)	25 (76)	12 (48)	2 (25)	39 (59)		

<sup>1</sup>Exhibited intermediate resistance. AMP, ampicillin; C, chloramphenicol; CTX, cefotaxime; NA, nalidixic acid; NOR, norfloxacin; SXT, sulfamethoxazole-trimethoprim; TE, tetracycline.

The other most common *Salmonella* serotypes isolated from pork were Anatum, Rissen and Stanley. This finding concurred with previous studies that these serotypes are associated with pigs (Vindigni and Srijan, 2007; Sirichote *et al*, 2010; Lertworapreecha *et al*, 2013). In chicken, Albany and Indiana were the other most common serotypes. Lertworapreecha *et al* (2013) have reported that *S. Albany* is the most predominant in chicken in southern Thailand. To the best of our knowledge, Indiana has never been reported as a common serotype isolated from various sources in Thailand, but Yang *et al* (2010) have reported that Indiana is the most predominant serotype isolated from chicken in Shaanxi, China. This implies that cross-contamination of this serotype might have taken place recently between the countries.

Results of antimicrobial susceptibility testing were consistent with previous findings that *Salmonella* isolated from food of animal origin exhibits high resistance to various antimicrobial drugs including ampicillin, chloramphenicol, nalidixic acid, sulfamethoxazole-trimethoprim, and tetracycline (Thong and Modattessi, 2011; Lertworapreecha *et al*, 2013). Resistance to nalidixic acid may contribute to treatment failure or delay response in fluoroquinolone-treated patients with salmonellosis (CLSI, 2013). Almost all *Salmonella* isolates, however, were still susceptible to clinically important drugs prescribed in life-threatening cases, namely, fluoroquinolones and third-generation cephalosporins, except those isolates of *S. Albany* and *S. Rissen* that are resistant to these antibiotics (Sirichote *et al*, 2010).

Antibiogram pattern analysis revealed a high proportion of MDR strains

from isolates, particularly from pork and chicken meat. Schwarzengrund, Anatum, Panama, Rissen, and Albany were the predominant MDR serotypes observed, similar to previous reports (Aarestrup *et al*, 2007; Sirichote *et al*, 2010). Our study indicated that there is still extensive use or misuse of antimicrobial agents as feed additives in livestock production in Thailand, which has contributed to the appearance and dissemination of antibacterial-resistant especially MDR *Salmonella* strains, which can be transmitted to humans via the consumption of contaminated food of animal origin.

Although lettuce did not have a high prevalence of *Salmonella* contamination in this study, it must still be of concern and be included in surveillance programs. There is a high possibility of cross-contamination of *Salmonella* serotype from animal origin to vegetables as manure fertilizers from animal feces are in use. This can also lead to contamination of the local water supply (Giaccone *et al*, 2012).

In conclusion, this study demonstrates that food of animal origin was still the significance source of *Salmonella* contamination, but the most common serotypes, Weltevreden and Enteritidis, as reported in earlier studies, was present at very low frequency and not found, respectively. It implies that the serotypes found in this study might represent the currently prevailing serotypes widespread in (central) Thailand. Furthermore, almost all isolates were resistant to multiple antimicrobial drugs, a situation of utmost concern. The Thai public health authorities should take action to prevent and control the spread of *Salmonella* by enforcing stricter policies in food safety and the use of antimicrobials as feed additives in livestock production.

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