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Antimicrobial Resistance in *Salmonella enterica* Isolated From Pork, Chicken, and Vegetables in Southern Thailand

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ABSTRACT

Background: *Salmonella* is a common causative agent of food-borne illness in humans. Infection by this pathogen is usually due to ingestion of contaminated insufficiently cooked foods. Despite the fact that several studies of the prevalence and antimicrobial resistance of *Salmonella* have been conducted in Thailand, there is limited data available concerning the more rural areas of the country.

Objectives: This study aimed to investigate the prevalence and antimicrobial resistance profiles of *Salmonella* in meat and vegetable samples taken from Phatthalung Province, Thailand.

Materials and Methods: Pork and chicken meat and fresh vegetable samples were randomly selected from retail markets in Phatthalung Province. *Salmonella* isolation and identification were performed on the same day of sample collection. Serovar typing was performed by slide agglutination according to The Kauffman and White scheme. Antimicrobial susceptibility testing was performed by disc diffusion method, and antimicrobial susceptibility patterns were analyzed by the WHONET 5 program.

Results: The prevalence of *Salmonella* in retail pork, chicken meat, and fresh vegetables were 82% (34/41), 67.5% (27/40), and 46% (37/80), respectively. The *Salmonella* isolated from pork, and vegetables were most resistant to tetracycline (77 and 33%) while the *Salmonella* isolated from chicken meat was most resistant to streptomycin (92%). Thirty-one samples (68%) isolated from pork and thirty-two samples (84%) isolated from chicken meat were of MDR strains. whereas only 7 samples (29%) isolated from vegetables exhibited resistance to two or more antimicrobial drugs.

Conclusions: These results show that retail meat and vegetables can serve as a reservoir of multiple antimicrobial resistant *Salmonella* and can probably be a potential route of transmission of these pathogens into human population.

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▸ *Implication for health policy/practice/research/medical education:*

This study provides useful information on surveillance of antimicrobial resistance in *Salmonella* and monitoring for preventing in food-borne disease at Phatthalung, Southern of Thailand. We have observed that retail meat (pork and chicken meat) and fresh vegetables can serve as a source for MDR strains of *Salmonella* that may affect to consumer health.

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1. Background

Salmonella is a common causative agent of food-borne illnesses in humans and a growing worldwide public health problem (1, 2). In Thailand, epidemiological reports indicate that *Salmonella* is encountered as the most common cause of diarrhea, which is widespread in all parts of the country (3). *Salmonella* is able to colonize in animal intestinal tracts, mainly swine and chicken, and shed in the feces (4-6). Therefore, *Salmonella* contamination in pork or chicken is unavoidable in the human food supply chain. Additionally, probably due to the use of manure fertilizer in their cultivation, *Salmonella* contamination can also be found in various types of vegetables (7). The illness is usually transmitted through food contaminated by feces. Furthermore, the resistance of *Salmonella* to a range of antimicrobial agents has become a serious global concern in public health.

An increasing number of antimicrobial resistant *Salmonella* has been reported in both developed and developing countries. Recently, a study in Denmark reported that the frequency of quinolone-resistant *S. Enteritidis* has increased from 0.8% in 1995 to 8.5% in 2000 (8). According to an investigation in England and Wales from 2000 to 2004, antimicrobial resistant *S. Enteritidis* has increased from 19% in 2000 to 35% in 2004, and antimicrobial resistant *S. Typhimurium* has increased from 82% in 2000 to 90% in 2002 (9). Moreover, a consequence of multidrug resistant (MDR) Salmonella infection has been previously reported, in which patients who were infected by MDR strain of *S. typhimurium* had an approximately 10 times higher mortality rate than general population (10). To ensure adequate consumer protection, especially in urban areas, several methods for decreasing contamination have been implemented in animal production and slaughtering system. In the countryside of Thailand, most animal and vegetable production is supplied for local consumption and therefore, the issue of food safety and *Salmonella* contamination in food, animal products, and vegetables may receive insufficient attention in these areas.

2. Objectives

To reduce contamination and illness caused by *Salmonella* infection, the prevalence of *Salmonella* contamination and antimicrobial susceptibility data in meat and vegetable products must be monitored. Hence, the aims of this study were to investigate the prevalence and antimicrobial resistance profiles of *Salmonella* found in contaminated pork, chicken meat, and vegetables in Phatthalung Province, Southern Thailand.

3. Materials and Methods

3.1. Samples

Forty-one samples of pork, 40 samples of chicken meat, and 80 samples of fresh vegetables were randomly selected from retail markets in Phatthalung Province during the period of October to December 2010. Samples were kept in separate sterile plastic bags, stored in cool boxes, and transported to laboratory on the same day of sample collection for isolation and identification of *Salmonella*.

3.2. Salmonella Isolation and Identification

Approximately 25 Grams of surface area of pork, chicken meat, and fresh vegetables were cut into small pieces by sterile scissors before being added to a stomach bag containing 225 mL of buffered peptone water (BPW) and incubated at 37 °C for 24 hours. Subsequently, 100 μL and 5 mL of pre-enriched cultures were transferred to Modified Semi-solid Rappaport-Vassiliadis (MSRV) medium (Criterion, U.S.A) and Rappaport Vassiliadis (RV) broth, respectively and incubated at 42 °C for 24 hours. Then, one loopful from each of the enriched broths were streaked onto Xylose Lysine Desoxycholate (XLD) agar (Himedia, India) plates and incubated at 37 °C for 18 to 24 hours. At least 5 single typical colonies of *Salmonella* were randomly picked up and stabbed into Triple Sugar Iron (TSI) agar (Criterion, U.S.A) and Lysine Indole Motile (LIM) medium (Himedia, India) and incubated at 37 °C for 18 to 24 hours. Typical characteristics of *Salmonella* exhibited on TSI agar were selected to subculture on Tryptic Soy (TSA) agar (Himedia, India). Suspected *Salmonella* colonies were confirmed by biochemical reactions (motility, indole production, lysine decarboxylase and carbohydrate fermentation) and slide agglutination with *Salmonella* O antigen antiserum. Final confirmation test was obtained through *Salmonella* serotyping performed by slide agglutination with O and H *Salmonella* antisera (S&A reagent, Thailand).

3.3. Antimicrobial Susceptibility Testing

All isolates were tested for 10 antimicrobial drugs (oxoids); tetracycline (30 μg), ciprofloxacin (5μg), chloramphenicol (30 μg), ampicillin (10 μg), norfloxacin (10 μg), nalidixic acid (30 μg), streptomycin (10 μg), trimethoprim/sulfamethoxazole (25 μg), cephalothin (30 μg), and gentamicin (10 μg). Susceptibility testing was performed according to recommendations of Clinical and Laboratory Standards Institute (CLSI) (11). To standardize bacterial suspension (in 0.8% NaCl), the density of suspension was adjusted to 0.5 McFarland and spread over the entire surface of Mueller Hinton agar (MHA) plates using a sterile cotton swab. Antimicrobial discs were placed on the agar surface followed by incubation of the plates at 37 °C for 24 hours. Inhibition zones were measured by Venire Caliper and interpreted accordingly by CLSI recommendations. The results were analyzed by WHONET 5 program.

4. Results

4.1. Prevalence of Salmonella Serotypes in Samples

Of 161 samples, 41 samples were prepared from retail pork, 40 samples from chicken meat, and 80 samples from fresh vegetables. The prevalence of *Salmonella* contamination in retail pork, chicken meat, and fresh vegetables were 82% (34/41), 67.5% (27/40), and 46% (37/80), respectively. Among all positive samples, 45 isolates, 38 isolates, and 29 isolates of *Salmonella* were segregated from pork, chicken meat, and vegetables, respectively. The most predominant serotypes isolated from pork, chicken meat, and fresh vegetables were *S.* Rissen (28.8%), *S. albany* (44.7%), and *S. Typhimurium* (33.3%), respectively. Frequency of isolated serotypes from pork, chicken meat, and vegetables is presented in *Table 1*.

Table 1. *Salmonella* serotypes Isolated From Pork, Chicken Meat, and Vegetable Samples at Phatthalung Province, Thailand.

	Pork, No. (%)	Chicken Meat, No. (%)	Vegetables, No. (%)
Rissen	13 (28.8)	3 (7.8)	
Anatum	3 (6.6)		
Weltevreden	12 (26.6)	1 (2.6)	6 (20)
Typhimurium	2 (4.4)	7 (18.4)	10 (33.3)
Give	4 (8.8)	5 (13.15)	
Dirby	1 (2.2)		
Kentucky	1 (2.2)	1 (2.6)	
Bredeney	9 (20)		
Albany		17 (44.7)	
Hvittingfoss		1 (2.6)	7 (23.3)
Kalamu		3 (7.8)	
Eastbourne			3 (10)
Paratyphi B			2 (6.6)
Kotu			1 (3.3)
Total	45	38	29

4.2. Antimicrobial Susceptibility Testing and Multi Drug Resistance Profiles

Antimicrobial susceptibility testing was performed for all isolates. The results revealed that *Salmonella* isolated from pork were mostly resistant to tetracycline (77%), streptomycin (71%), and ampicillin (51%). For *Salmonella* isolated from chicken samples, highest resistance was observed against streptomycin (92%) followed by nalidixic acid (76%), ampicillin (68%), and chloramphenicol (68%). Despite of meat samples, fewer antimicrobial resistant *Salmonella* were observed in vegetable samples, mostly resistant to tetracycline (33%) followed by ampicillin (20%) and streptomycin (20%). All antimicrobial resistance profiles in *Salmonella* isolated from each sample are presented in *Table 2*.

Table 2. Antimicrobial Resistance of *Salmonella* serotypes Isolated From Pork, Chicken Meat, and Vegetables

	Antimicrobial Resistance of *Salmonella* Serovars Isolated From Pork, Chicken Meat and Vegetables																													
	TCY			CHL			CEP			AMP			CIP			NAL			STR			NOR			GEN			SXT		
	P	C	V	P	C	V	P	C	V	P	C	V	P	C	V	P	C	V	P	C	V	P	C	V	P	C	V	P	C	V
Rissen	6	3	0	0	3	0	1	0	0	1	3	0	0	0	0	0	3	0	6	3	1	0	0	0	0	0	0	6	0	0
Anatum	3	-	-	0	-	-	1	-	-	1	-	-	0	-	-	0	-	-	1	-	-	0	-	-	0	-	-	0	-	-
Weltevreden	11	1	1	3	1	0	5	0	0	9	1	1	0	0	0	0	1	0	10	1	0	0	0	0	0	0	0	1	0	1
Typhimurium	1	4	2	0	4	0	0	1	0	2	4	0	0	0	0	0	6	1	2	7	2	0	0	0	0	0	0	0	0	2
Give	4	2	-	1	1	-	0	0	-	3	1	-	0	0	-	0	3	-	3	3	-	0	0	-	0	0	-	0	1	-
Dirby	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	1	-	-	0	-	-	0	-	-	0	-	-
Kentucky	1	0	-	0	1	-	0	0	-	1	1	-	0	0	-	0	1	-	1	1	-	0	0	-	0	0	-	0	0	-
Bredeney	9	-	-	2	-	-	6	-	-	6	-	-	0	-	-	0	-	-	8	-	-	0	-	-	0	-	-	1	-	-
Albany	-	11	-	-	12	-	-	1	-	-	13	-	-	0	-	-	11	-	-	16	-	-	1	-	-	0	-	-	1	-
Hvitingfoss	-	1	4	-	1	0	-	0	2	-	1	3	-	0	2	-	1	1	-	1	3	-	0	0	-	0	0	-	0	0
Kalamu	-	1	-	-	3	-	-	0	-	-	2	-	-	0	-	-	3	-	-	3	-	-	0	-	-	0	-	-	0	-
Eastboume	-	-	3	-	-	0	-	-	0	-	-	2	-	-	1	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0
Paratyphi B	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0

Kotu	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0
Total (%)	35 (77)	23 (60)	10 (33)	5 (11)	26 (68)	0 (0)	13 (28)	2 (5)	2 (6)	23 (51)	26 (68)	6 (20)	0 (0)	0 (0)	3 (10)	0 (0)	29 (76)	2 (6)	32 (71)	35 (92)	6 (20)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	8 (17)	2 (5)	3 (10)

Abbreviations: TCY, Tetracycline; CHL, Chloranphenicol; CEP, Cephalothin; AMP, Ampicillin; CIP, Ciprofloxacin; NAL, Nalidixic acid; STR, Streptomycin; NOR, Norfloxacin; GEN, Gentamicin; SXT, Trimethoprim/sulfamethoxazole; P, Pork (N= 45); C, C hicken meat (N=38), V = Vegetables (N=30)

Table 3. Multiple Antimicrobial Resistances of *Salmonella* Isolated From Pork, Chicken Meat, and Vegetables.

Pork Isolates			Chicken Isolates			Vegetables isolates		
Serovar	**Antibiogram**	**Number**	**Serovar**	**Antibiogram**	**Number**	**Serovar**	**Antibiogram**	**Number**
Anatum								
	TCY CEP AMP STR	1	Albany	TCY STR	1	Eastbourne	TCY CIP	1
Bredeny								
	TCY AMP STR	1		NAL STR	1		TCY AMP	2
	TCY CEP STR	1		AMP NAL STR	2	Hvittingfoss	AMP CIP STR	1
	TCY CHL CEP	1		CHL NAL STR NOR	1		CEP AMP NAL STR	1
	TCY CHL AMP STR	1		CHL CEP AMP NAL STR	1		CEP AMP CIP STR	1
	TCY CEP AMP STR	4		TCY CHL AMP STR	5	Typhimurium	TCY NAL	1
Give								
	TCY AMP STR	3		TCY CHL AMP NAL STR	5			
Kentucky								
	TCY AMP STR	1	Give	AMP NAL STR	1			
Rissen								
	TCY STR	5		TCY NAL STR	2			
	TCY STR AMP	1	Hvittingfoss	TCY CHL AMP NAL STR	1			
Typhimurium								
	TCY AMP STR	1	kalamu	CHL AMP NAL STR	2			
Weltevreden								
	TCY STR	1		TCY CHL NAL STR	1			
	TCY CEP	1	Kentucky	CHL AMP NAL STR	1			
	TCY AMP STR	3	Rissen	TCY CHL AMP NAL STR	3			
	TCY CEP AMP STR	2	Typhimurium	NAL STR	3			
	TCY CHL AMP STR	1		TCY CHL AMP NALSTR	3			
	TCY CHL CEP AMP STR	1		TCY CHL CEP AMP STR	1			
	TCY CEP AMP NAL STR	1	Weltevreden	TCY CHL AMP NAL STR	1			
	TCY CHL AMP CIP NAL STR	1						
Total		31 (68%)	Total		32 (84%)	Total		7 (29%)

Abbreviations: TCY, Tetracycline; CHL, Chloranphenicol; CEP, Cephalothin; AMP, Ampicillin; CIP, Ciprofloxacin; NAL, Nalidixic acid; STR, Streptomycin; NOR, Norfloxacin; GEN, Gentamicin; SXT, Trimethoprim/sulfamethoxazole; P, Pork (N= 45); C, C hicken meat (N=38), V = Vegetables (N=30)

In addition, all *Salmonella* isolated were analyzed for multiple drugs resistance profiles, and it was revealed that in thirty-one pork samples (68%) several isolates were of MDR strains, mostly *S. weltevreden*. Thirty-two samples (84%) isolated from chicken were of the MDR strains, mostly *S. albany*. However, only 7 samples (29%) isolated from vegetables exhibited resistance to two or more antimicrobial drugs. Distribution of serotypes and multiple antimicrobial resistance patterns pertaining to pork, chicken meat, and vegetables are presented in *Table 3*.

5. Discussion

The problems attributed to *Salmonella* infection have increased significantly, in terms of both incidence and severity. Furthermore, an increase of antimicrobial resistance in this pathogen makes the treatment of infection more difficult that probably results in death. Therefore, epidemiological information and monitoring systems are necessary to control *Salmonella* infection in public health sector. In this study, we observed prevalence and antimicrobial susceptibility patterns of *Salmonella* isolated from pork, chicken meat, and vegetables. The study showed that the retail pork was highly contaminated by *Salmonella*, followed by chicken meat and fresh vegetables. These products, according to many reports, are the resources of *Salmonella* contamination (1, 2, 12, 13).

Serotyping of *Salmonella* isolated from pork indicated that *S.* Rissen was the most predominant serotype followed by *S. weltevreden*, whereas *S. albany* and *S. typhimurium* were the most predominant serotyping found in chicken meat and vegetables, respectively. Our results are inconsistent with previous findings in Thailand which indicated that *S. albany* and *S. give* were predominantly found in swine and chicken farms, respectively (14). The inconsistency in observed serotypes may be attributed to different areas studied and sample types used in this investigation. Although serotyping were not the same as in other studies, those that were observed were included within 25 most common serotyping isolated from human and other sources in Thailand (15).

High contamination of *Salmonella* implies that hygienic performance in carcass production processes , especially in slaughters of Phattalung Province area, probably are insufficiently attended. Antimicrobial susceptibility testing revealed high resistance rates against several antimicrobial drugs used in both medical and agricultural fields. *Salmonella* isolated from pork samples were commonly resistant to tetracycline, ampicillin, and streptomycin, as was in those isolated from chicken, which were additionally resistant to nalidixic acid. High resistance to those antimicrobial drugs were consistent to previous observations from various countries (16-18), which implies that the wide consumption of such antimicrobials as feed additives in livestocks contributes to emergence and dissemination of resistance in *Salmonella*. In addition, it had also been reported the sub-therapeutic doses of antimicrobial drugs in animal husbandry as a responsible factor in emergence and maintenance of multiple antimicrobial resistant pathogenic bacteria (19-21).

According to several reports, our results demonstrated that the fluoroquinolone groups such as ciprofloxacin and norfloxacin are still the most effective drugs to treat *Salmonella* infection (22-24). In recent years, evidence for decreasing susceptibility to fluoroquinolones in *Salmonella* has been reported. Increasing resistance to fluoroquinolones is growing as an issue receiving special attention, since fluoroquinolones are effective drugs against *Salmonella* in clinical performance and are usually considered as treatment of choice in life threatening cases (25). Our study found that *Salmonella* isolated from fresh vegetables were different from meat samples in both serotype distributions and antimicrobial resistance patterns. This imply that contamination in vegetable samples may originate from environmental sources that are different from animal contamination. However, this study indicated that retail meat and animals can serve as a source for MDR strains of *Salmonella* that may transfer to vegetables. Association or cross contamination between meat and vegetable samples is subject to further evaluations.

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Authors' Contribution

None declared.

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