EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN *SALMONELLA* ISOLATED FROM PORK, CHICKEN MEAT AND HUMANS IN THAILAND

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Abstract. Forty samples each of pork and chicken meat were collected from local retail markets in Khon Kaen, northeast Thailand, for *Salmonella* isolation and identification during 2003. Fifty-four isolates of *Salmonella* obtained from diarrheal patients admitted at a hospital located in the same town were serotyped. All isolates were also tested for antimicrobial sensitivity against amoxicillin (Amx), chloramphenicol (Chl), norfloxacin (Nor), ciprofloxacin (Cip), gentamicin (Gm), sulfamethoxazole and Trimethoprim (Sxt), Tetracycline (Tet), Streptomycin (Str) and sulfamethoxazole (Sulfa). The results showed that 26 (65%) pork samples and 30 (75%) chicken meat samples were contaminated with *Salmonella*. The most prevalent serovar in pork was *S.* Rissen (61.5%), followed by *S*. Stanley and *S*. Lexington (11.5%). In chicken meat, the most prevalent serovar was *S.* Anatum (33.3%), followed by *S.* Rissen (16.7%). Among isolates from human patients, *S*. Rissen (20.4%) and *S.* Stanley (18.5%) were the most frequently identified serovars. All the isolates were resistant to Str and Sulfa. None were resistant to Nor and Cip. Resistance to Amx, Chl, Gm, Sxt, and Tet in pork was 15.4, 15.4, 3.9, 15.4 and 88.5%, respectively. The resistance to those antimicrobial agents in chicken meat was 30.0, 26.7, 6.7, 20 and 100%, respectively, and in human patients was 27.8, 20.4, 5.6, 31.5 and 92.6%, respectively. Statistical analysis found no difference in the rate of resistance to Amx, Chl, Gm, and Sxt among the different sources of the *Salmonella* isolates (p>0.05 for each antimicrobial agent). Our results indicate that antimicrobial resistant *Salmonella* strains were widely spread among pork and chicken meat, and in humans.

INTRODUCTION

Food-borne disease caused by *Salmonella* spp is an important public health problem in Thailand. A high incidence of *Salmonella* contamination has been found in many species, such as pigs, cattle, poultry and humans. *Salmonella* is transmitted to humans mainly via contaminated foods, especially from animal products. Salmonellosis patients show clinical signs of diarrhea, cramps, nausea, vomiting, and may have bacteremia in severe cases. A reported isolation rate of *Salmonella* in children with acute dysentery was 18% (Bodhidatta *et al*, 2002). The serovar reported most in human patients in Thailand was *S*. Weltevreden, followed by *S*. Enteritidis (National Institute of Health, 2001). A survey of *Salmonella* in frozen chicken meat and ready to eat Thai food during 1993-1996 showed that *S.* Enteritidis and *S*. Anatum, respectively, were most frequently isolated (Boonmar *et al*, 1998a). From the report of the Salmonella and Shigella Center of Thailand, there has been a similar increasing trend in the incidence of human salmonellosis caused by *S*. Enteritidis and the isolation of the bacteria in chicken meat (Sakai and Chalermchaikit, 1996).

Previously, sulfamethoxazole-trimethoprim was the drug of choice for the treatment of diarrhea. However, Salmonella isolated from salmonellosis patients has recently been found to have

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increasing resistance to this type of antimicrobial therapy. The resistance caused failure of regular antimicrobial therapy and has increased the severity of infection (Angulo *et al*, 2004). Patients infected with antimicrobial resistant strains were more likely to be hospitalized (Martin *et al*, 2004). Norfloxacin is used now due to high resistance to sulfamethoxazole-trimethoprim. Excessive use and over the counter purchase of antimicrobial agents in Thailand is common in both humans and farm animals. The use of antimicrobial agents as a feed additive in farm animals is not always done under veterinarian supervision. This causes a rapid increase in both the animal's resistance to certain bacteria and in the level of antimicrobial residues in animal products. Improved epidemiological data on the prevalence of salmonella and on the levels of antimicrobial resistance in both humans and animals are needed in order to find a mechanism to control the increasing incidence of antimicrobial resistance. The objectives of this study were to isolate and identify *Salmonella* serovars in pork, and chicken meat, and in humans and to test these isolates for antimicrobial sensitivities. In addition, serotype and resistance patterns across the different species were examined to identify any similarities.

MATERIALS AND METHODS

Sample collection

Between January and December 2003, forty samples of pork and forty samples of chicken meat were collected from retail markets in Khon Kaen, a province in northeast Thailand, for salmonella isolation and identification. The isolates were then tested for antimicrobial sensitivity. Fifty-four isolates of *Salmonella* obtained from patients admitted at Srinakarin Hospital, also located in Khon Kaen, during 2003 for the treatment of diarrhea, were serotyped and tested for antimicrobial sensitivities.

Bacterial isolation and identification

Twenty-five grams of meat samples were enriched in 250 ml of triptic soy broth (TSB; Merck, Darmstadt, Germany) at 37ºC for 24 hours. The suspension was placed on modified semisolid Rappaport medium (MSRV; Merck) at 3 peripheral spots with 2 loops each, and then incubated at 42ºC for 24 hours. Positive colonies were streaked on xylose-lysine-desocholate agar (XLD; Merck) and Hektoen enteric agar (HE; Merck) and incubated at 37ºC. The plates were inspected after 24 hours and positive colonies were placed on triple sugar iron agar (TSI; Merck) and motility indole-lysine agar (MIL; Merck). Colonies, either from XLD or HE, which tested positive on both TSI and MIL were identified as *Salmonella*.

The slide agglutination test with O-antigen (Biotechnical; Bangkok, Thailand) was used to group the *Salmonella* isolates. The isolates were then submitted to the laboratory at the Department of Medical Science, Ministry of Public Health for further identification of the serovars.

Antimicrobial susceptibility test

The isolates were tested for antimicrobial sensitivity against amoxicillin 10 µg (Amx), chloramphenicol 30 µg (Chl), norfloxacin 10 µg (Nor), ciprofloxacin 5 µg (Cip), gentamicin 10 µg (Gm), sulfamethoxazole and trimethoprim 25 µg (Sxt), tetracycline 30 µg (Tet), streptomycin 10 µg (Str) and sulfamethoxazole 25 µg (Sulfa) by disk diffusion test (Oxoid; Hampshire, England) according to the National Committee for Clinical Laboratory Standards directions (NCCLS, 1999).

Statistical analysis

Differences in the resistance rates among isolates from the three sample sources were analyzed by chi-squared analysis using EpiInfo 6.04d (CDC, 2001). Only data about resistance to Amx, Chl, Gm, and Sxt were used in the analysis due to the distribution of the data. The analysis was done separately for each antimicrobial agent.

RESULTS

The bacterial culture showed that 26 (65%) pork samples and 30 (75%) chicken meat samples were contaminated with *Salmonella* (Table 1). The most frequently identified serovars in pork were *S*. Rissen (61.5%), followed by *S*. Stanley and *S*. Lexington (11.5%). The most prevalent serovar in chicken meat was *S*. Anatum (33.3%), followed by *S*. Rissen (16.7%). *S*. Rissen (20.4%) and *S*. Stanley (18.5%) were the two most prevalent serovars in isolates from

Table 1
Results of *Salmonella* isolation from pork and chicken meat.

Sample	n	Number positive (%)	Group	Serovar (number, %)
Pork	40	26 (65)	B	*S.* Stanley (3, 11.5)
			C	*S.* Rissen (16, 61.5)
			D	*S.* Panama (1, 3.8)
			E	*S.* Anatum (1, 3.8)
				S. Lexington (3, 11.5)
				S. Weltevreden (1, 3.8)
			G	*S.* Worthington (1, 3.8)
Chicken meat	40	30 (75)	B	*S.* Agona (3, 10)
				S. Derby (3, 10)
			C	*S.* Rissen (5, 16.7)
				S. Virchow (4, 13.3)
			D	*S.* Enteritidis (4, 13.3)
				S. Panama (1, 3.3)
			E	*S.* Anatum (10, 33.3)

Table 2
Serovars of *Salmonella* isolated from human patients.

Sample	n	Group	Serovars (number, %)
Salmonella isolation	54	B	*S.* Agona (2, 3.7)
			S. Derby (1, 1.9)
			S. Stanley (10, 18.5)
			S. Typhimurium (1, 1.9)
		C	*S.* Albany (1, 1.9)
			S. Bovismorbificans (3, 5.6)
			S. Braenderup (3, 5.6)
			S. Hadar (1, 1.9)
			S. Rissen (11, 20.4)
			S. Virchow (5, 9.3)
		D	*S.* Eastbourne (1, 1.9)
			S. Enteritidis (4, 7.4)
		E	*S.* Give (2, 3.7)
			S. Krefeld (2, 3.7)
			S. Lexington (1, 1.9)
			S. Weltevreden (2, 3.7)
		G	*S.* Worthington (2, 3.7)
		I	*S.* Hvittingfoss (2, 3.7)

human patients (Table 2).

All isolates from the three sources were resistant to Str and Sulfa. All the isolates from chicken meat were also resistant to Tet. The percentages of Tet resistance of the isolates from pork meat and from human patients were 88.5% and 92.6%, respectively. None of the isolates from any of the sources was resistant to Nor or Cip. Resistance to Amx, Chl, Gm, and Sxt was 15.4, 15.4, 3.9, and 15.4%, respectively for the isolates from pork (Table 3). The resistance to those antimicrobial agents was 30.0, 26.7, 6.7, and 20.0%, respectively, for the isolates from chicken meat (Table 4) and 27.8, 20.4, 5.6, and 31.5%, respectively, for the isolates from human patients (Table 5).

The statistical analyses of comparative drug resistance rates between pork, chicken meat, and human isolates found no difference in rates for any of the four agents tested (Amx, Chl, Gm, and Stx) with p>0.05 for each antimicrobial agent.

DISCUSSION

Salmonella contamination was found more frequent in chicken meat than in pork (75% and 65%) in this study. The prevalence in chicken meat was close to the rate reported in a study in 1997 in which 72% of chicken meat in the capital city of Thailand contained *Salmonella* (Boonmar *et al*, 1998b). In this study, the most prevalent serovar in chicken meat was *S.* Anatum (33%). This

Table 3
Antimicrobial susceptibility test of *Salmonella* isolated from pork.

Serovars (number of samples)	% Resistant								
	Amx	Chl	Nor	Cip	Gm	Sxt	Tet	Str	Sulfa
S. Anatum (1)	-	100	-	-	-	-	100	100	100
S. Lexington (3)	-	-	-	-	-	-	-	100	100
S. Panama (1)	100	100	-	-	-	100	100	100	100
S. Rissen (16)	12.5	12.5	-	-	6.2	12.5	100	100	100
S. Stanley (3)	-	-	-	-	-	-	100	100	100
S. Weltevreden (1)	-	-	-	-	-	-	100	100	100
S. Worthington (1)	100	-	-	-	-	100	100	100	100
Total (26)	15.4	15.4	0	0	3.8	15.4	88.5	100	100

Table 4
Antimicrobial susceptibility test of *Salmonella* isolated from chicken meat.

Serovars (number of samples)	% Resistant								
	Amx	Chl	Nor	Cip	Gm	Sxt	Tet	Str	Sulfa
S. Agona (3)	100	-	-	-	-	-	100	100	100
S. Anatum (10)	-	66.7	-	-	-	-	100	100	100
S. Derby (3)	33.3	-	-	-	66.7	66.7	100	100	100
S. Enteritidis (4)	-	-	-	-	-	100	100	100	100
S. Panama (1)	100	100	-	-	-	100	100	100	100
S. Rissen (5)	-	-	-	-	-	-	100	100	100
S. Virchow (4)	100	-	-	-	-	-	100	100	100
Total (30)	30	26.7	0	0	6.7	20	100	100	100

agrees with a previous study which had used chicken meat samples from the same markets and which also reported *S*. Anatum as the most frequently identified serovar (Angkititrakul *et al*, 2003).

The results showed a very low prevalence of *S*. Weltevreden. It was identified in only 2 out of 54 human isolates and 1 out of 26 pork isolates. This finding is not consistent with a report by Bangtrakulnonth *et al* (2004) in which *S*. Weltevreden was the most frequently isolated serovar among salmonellosis patients in Thailand. It is possible that this serovar may have a different prevalence depending on the specific location and distribution of livestock.

The most prevalent serovar found in human patients in our study was *S*. Rissen (20.4%) which was also the most prevalent serovar found in pork (61.5%) and the second most prevalent serovar in chicken meat (16.7%).

The rates of antimicrobial resistance found in pork, chicken meat, and human patients were not statistically different. All the isolates were resistant to Str and Sulfa. Resistance to other antimicrobial agents was closely related among the three sources. Overall resistance rates to individual drugs, however, appeared to be different to those reported in earlier studies and in studies conducted in different parts of the country. During the period 1996-1999, resistance to Tet and Sulfa among Thai patients with *Salmonella* was found to be 59% and 37%, respectively (Isenbarger *et al*, 2002). In our study, comparable resistance rates were much higher

Table 5
Antimicrobial susceptibility test of *Salmonella* isolated from human patients.

Serovars (number of samples)	% Resistant								
	Amx	Chl	Nor	Cip	Gm	Sxt	Tet	Str	Sulfa
S. Agona (2)	100	50	-	-	-	100	100	100	100
S. Albany (1)	100	100	-	-	-	100	100	100	100
S. Bovis-morbificans (3)	-	-	-	-	-	-	100	100	100
S. Braenderup (3)	-	-	-	-	-	-	100	100	100
S. Derby (1)	100	-	-	-	-	100	100	100	100
S. Eastbourne (1)	-	-	-	-	-	-	-	100	100
S. Enteritidis (4)	100	50	-	-	-	-	100	100	100
S. Give (2)	-	-	-	-	-	-	100	100	100
S. Hadar (1)	-	-	-	-	-	100	100	100	100
S. Hvittingfoss (2)	-	-	-	-	-	-	100	100	100
S. Krefeld (2)	100	100	-	-	-	100	100	100	100
S. Lexington (1)	-	-	-	-	-	-	-	100	100
S. Rissen (11)	9.1	36.4	-	-	18.2	72.7	100	100	100
S. Stanley (10)	10	10	-	-	10	10	100	100	100
S. Typhimurium (1)	100	-	-	-	-	-	100	100	100
S. Virchow (5)	-	-	-	-	-	-	100	100	100
S. Weltevreden (2)	-	-	-	-	-	-	-	100	100
S. Worthington (2)	100	-	-	-	-	50	100	100	100
Total (54)	27.8	20.4	0	0	5.6	31.5	92.6	100	100

(92.6% and 100%, respectively). A survey in live pigs, chickens, and livestock farmers in northern Thailand reported that the resistance rate to Cip was 12.5% (Hanson *et al*, 2002). However, our study found no resistance to Cip or Nor.

The fact that this study showed that serovar types and antimicrobial resistance rates were similar between the different sources of *Salmonella* isolates suggests that salmonella contamination in pork and chicken meat may be the major cause of salmonellosis in humans. To confirm this hypothesis, further examination of the genetic traits of the isolates is needed.

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