RECOVERY OF *SALMONELLA* USING A COMBINATION OF SELECTIVE ENRICHMENT MEDIA AND ANTIMICROBIAL RESISTANCE OF ISOLATES IN MEAT IN THAILAND

Aroon Bangtrakulnonth[1], Srirat Pornrungwong[1], Chaiwat Pulsrikarn[1], Sumalee Boonmar[2] and Keiji Yamaguchi[3]

[1]WHO National Salmonella and Shigella Center, National Institute of Health, Department of Medical Science, Ministry of Public Health, Nonthaburi; [2]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand; [3]Department of Microbiology, Hokkaido Institute of Public Health, Hokkaido, Japan

Abstract. From November 2004 to March 2005, 50 samples (chicken, pork and beef) of registered meat and non-registered meat were purchased from supermarkets and retail markets located in Bangkok, Thailand. Each sample was evaluated for *Salmonella* spp by a conventional method using combination of selective enrichment media (RV+MSRV) and compared with selective enrichment medium alone (DIASALM). Our study revealed the performance of RV+MSRV for the detection of *Salmonella* spp was significantly better than those of DIASALM alone since the recovery of *Salmonella* spp in both groups of meat was high using RV+MSRV, particularly in the registered meat. In addition, the recovery of serovars in registered meat was significantly higher than those in non-registered meat. Antimicrobial resistance of 62 *Salmonella* isolates in both groups of meat was determined for 10 antimicrobial drugs using the disk diffusion method. The results show that 100% of isolates from both groups were susceptible to amoxicillin/clavulanic acid, ciprofloxacin, cefotaxime and norfloxacin and 50-60% of isolates from both groups were resistant to tetracycline, streptomycin and ampicillin. Sixty percent of *Salmonella* isolates from meat showed multiresistance antimicrobial patterns.

INTRODUCTION

A rapid, accurate technique for isolation of *Salmonella* spp from humans, animals, food and environment specimens is important for the detection of salmonellosis. Over the past decade, several immunological, molecular and bacteriological techniques were developed. Semisolid media are suitable for isolation of *Salmonella* spp in food (De Midici *et al*, 1998), stool (Aspinall *et al*, 1992), poultry (Braun *et al*, 1998) and environmetal samples (Read *et al*, 1994). Voogt *et al* (2001) reported that the combination of selective enrichment media was significantly better compaired to the media alone for the detection of *Salmonella* spp in poultry feces.

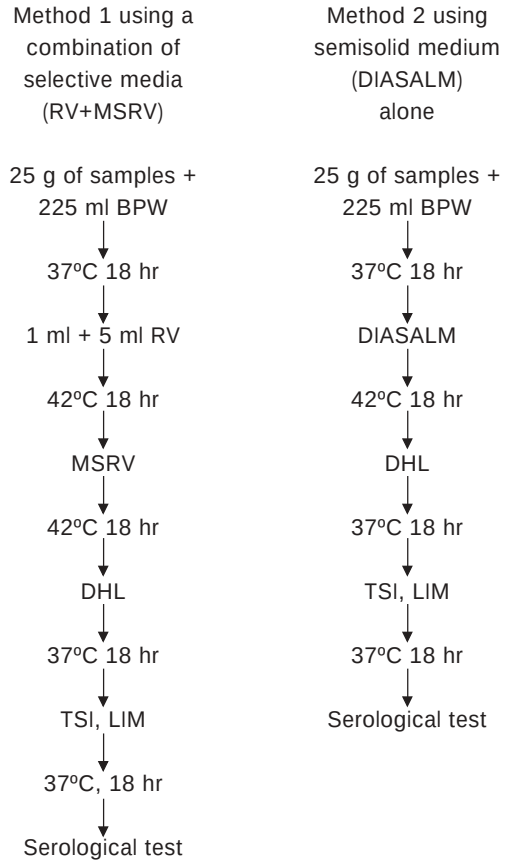
The objectives of this study were to compare the results of combination media (RV+MSRV) and semisolid medium alone (DIASALM) for the detection of *Salmonella* spp in meat, determine the prevalence of *Salmonella* spp in registered meat and in non- registered meat, and study the patterns of antimicrobial resistance of *Salmonella* isolates in meat.

MATERIALS AND METHODS

From November 2004 to March 2005, 25

Correspondence: Sumalee Boonmar, Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
Tel: +66 (0) 2942-8200 ext 4404; Fax: +66 (0) 2942-8200 ext 4414
E-mail: fvetslb@ku.ac.th

samples of registered meat and 25 samples of non-registered meat purchased from supermarkets and retail markets located in Bangkok were studied for *Salmonella* spp using standard culture methods. Each sample was isolated following two methods, Method 1 and Method 2.



Method 1 pre-enrichment was carried out by adding 25 g of each meat sample to 225 ml Buffer Peptone Water (BPW) and incubated at 37ºC for 18 hours. Then 1 ml of pre-enrichment culture was incubated in 5 ml Rappaport Vassiliadis (RV) broth at 42ºC for 18 hours. After incubation, the RV culture was transferred onto Modified Semisolid Rappaport Vassiliadis (MSRV) and incubated at 42ºC for 18 hours. Bacteria identified as *Salmonella* on MSRV were streaked onto Desoxycholate Hydrogen Sulfide Lactose agar (DHL) followed by incubation at 37ºC for 18 hours. Colonies identified on DHL as being *Salmonella* were confirmed biochemically using Triple Sugar Iron (TSI) and Lysine Indole Motility (LIM), then serovars were evaluated by serological test at the WHO National Salmonella and Shigella Center, NIH, Thailand. In Method 2, each sample was evaluated as in Method 1, except after the BPW pre-enrichment culture, the culture was transferred onto Diagnostic Semisolid Salmonella medium (DIASALM) instead of MSRV, and the RV broth culture step was not performed.

All isolates were tested for antimicrobial drug resistance by the disk diffusion method as described by Bauer *et al* (1966) with Mueller-Hinton agar (Oxoid) plates. Ten types of antimicrobial disks (Oxoid) containing 10 µg of ampicillin, 20/10 µg of amoxicillin/clavulanic acid, 30 µg of chloramphenicol, 5 µg of ciprofloxacin, 30 µg of cefotaxime, 30 µg of nalidixic acid, 10 µg of norfloxacin, 10 µg of streptomycin, 30 µg of tetracycline and 25 µg of trimethoprim+ sulfamethoxazole were used. The breakpoint for the antimicrobial drugs was based on the guidelines established by the National Committee on Clinical Laboratory Standards (2002).

RESULTS

The combination of selective enrichment media (RV+MSRV) was more effective than the semisolid medium alone (DIASALM), since the percentage of *Salmonella* spp in registered and non-registered meat using Method 1 was higher than Method 2. Particularly in registered meat, it was found that the number of serovars was higher than those in non-registered meat (Table 1). *S.* Anatum, *S.* Rissen and *S.* Vichow were the most common serovars found in registered meat, however *S.* Anatum, *S.* Stanley and *S.* Rissen were found in non-registered

meat (data not shown).

The susceptibility and resistant rates to 10 antimicrobial drugs are shown in Tables 2 and 3. One hundred percent of *Salmonella* isolates from both groups of meat were susceptible to AUG, CIP, CTX and NOR; 95% were susceptible to NA, 90% to C and 85% to TMSX in registered meat but 88% to C, 76% to TMSX and 64% to NA in non-registered meat.

Sixty percent of *Salmonella* isolates from registered meat were resistant to T, 50% to S and AMP. Forty-seven percent of isolates in non-registered meat were resistant to T, 43% to S, and 38% to AMP.

Table 1
Recovery of *Salmonella* spp in contaminated meat.

Meat group	Percent of *Salmonella* spp in contaminated meat (number of serovars)	
	Method 1 RV+MSRV → DHL	Method 2 DIASALM → DHL
Registered meat	64 (8 serovars)	12 (3 serovars)
Non-registered meat	92 (17 serovars)	88 (19 serovars)

Table 2
Percentages of antimicrobial drug susceptibility and resistance in 20 isolates of registered meat.

Isolates	Antimicrobial drugs									
	AMP	AUG	C	CIP	CTX	NA	NOR	S	T	TMSX
No. susceptible	10	20	18	20	20	19	20	10	8	17
Percentage	50	100	90	100	100	95	100	50	40	85
No. resistant	10	0	2	0	0	1	0	10	12	3
Percentage	50	0	10	0	0	5	0	50	60	15

AMP- Ampicillin, AUG-Amoxicillin/clavulanic acid, C-Chloramphenicol, CIP-Ciprofloxacin,CTX-Cefotaxime, NA-Nalidixic acid, NOR-Norfloxacin, S-Streptomycin, T-Tetracycline, TMSX-Trimethoprim/sulfamethoxazole

Table 3
Percentages of antimicrobial drug susceptibility and resistance in 42 isolates of non-registered meat.

Isolates	Antimicrobial drugs									
	AMP	AUG	C	CIP	CTX	NA	NOR	S	T	TMSX
No. susceptible	26	42	37	42	42	27	42	11	19	32
Percentage	62	100	88	100	100	64	100	26	45	76
No. resistant	16	0	5	0	0	12	0	18	20	10
Percentage	38	0	12	0	0	28	0	43	47	24

AMP- Ampicillin, AUG-Amoxicillin/clavulanic acid, C-Chloramphenicol, CIP-Ciprofloxacin,CTX-Cefotaxime, NA-Nalidixic acid, NOR-Norfloxacin, S-Streptomycin, T-Tetracycline, TMSX-Trimethoprim/sulfamethoxazole

Table 4
Patterns of antimicrobial multiresistance in isolates of meat.

Pattern	No of isolates in registered meat	No of isolates in non-registered meat
AMP+T	1	3
AMP+NA	0	3
AMP+S	1	1
T+C	1	0
T+TMSX	0	1
T+S	2	4
NA+S	0	1
AMP+T+S	4	0
AMP+S+NA	0	2
T+TMSX+S	0	1
AMP+T+C+S	0	1
AMP+T+TMSX+S	1	2
T+TMSX+C+S	0	2
AMP+T+TMSX+NA	1	0
AMP+T+TMSX+NA+S	0	2
AMP+T+TMSX+C+S	1	2

Table 5
Number and percentage of antimicrobial multiresistance in isolates of meat.

Number of drugs	Number of isolates (percentage)	
	20 isolates of registered meat	42 isolates of non-registered meat
2	5 (25)	13 (31)
3	4 (20)	3 (7)
4	2 (10)	5 (12)
5	1 (5)	4 (10)
Total	12 (60)	25 (60)

Table 4 shows 16 different antimicrobial multiresistance (more than two drugs) drug patterns in both groups of meat. The pattern AMP+T+S was the most frequent among 20 isolates from registered meat and the pattern T+S was the most frequent among 42 isolates from non-registered meat. Sixty percent of isolates in each group showed multiresistant patterns (Table 5).

DISCUSSION

A comparison MSRV and SCM for the isolation of *Salmonella* in meat and meat products has been reported in Thailand. They found MSRV were more effcetive than SCM (Boonmar *et al*, 1995, 1997). Voogt *et al* (2001) reported the combination of semisolid medium (MSRV or DIASALM) and selective enrichment broth (RV) was more sensitive in the detection of *Salmonella* in poultry feces compared with RV alone. They also found no significant difference between the results using MSRV and DIASALM. Our results are similar to the results of Voogt *et al* (2001) in that the recovery of *Salmonella* spp in both groups of meat was high using a combination of selective media (RV+ MSRV). In addition, the recovery of serovars in registered meat was significantly higher than those in non- registered meat (8 serovars *vs* 3 serovars) ($p<0.001$). The registered meat was purchased from guaranteed companies where GMP, HACCP processes and ISO 9001/2000 were used in the processing. Although the limit of antibiotics, residues and inhibitor chemicals against bacteria growth were controlled in the processing, it is possible bacteria in contaminated meat from the farms can be grown under the pre-enrichment process.

The present study also showed the results of antimicrobial resistance and found that 50-60% of *Salmonella* isolates from both groups of meat were resistance to tetracycline and streptomycin. This is similar to a previous study (Boonmar *et al*, 2000) which reported 50-100% of *Salmonella* isolates from beef, pork and chicken meat were resistant to streptomycin and doxycycline. Willinga *et al* (2002) reported only 5.7% of 35 *Salmonella* isolates of chicken meat in USA were resistant to strep-

tomycin, tetracycline and sulfamethoxazole but 100% of isolates were susceptible to ciprofloxacin, cefotaxime and nalidixic acid. In addition, 47% of 45 *Salmonella* isolates of turkey meat were resistant to streptomycin and tetracycline. White *et al* (2001) also reported that resistant strains of *Salmonella* were common in retail ground meat in Washington D.C. They found that 53% of *Salmonella* isolates were resistant to at least 3 antibiotics and 16% of isolates were resistance to ceftriaxone. Most antimicrobial resistant *Salmonella* isolates in humans come from eating contaminated food. Boonmar *et al* (1998) described a significant increase in antibiotic resistant of *Salmonella* isolates from human beings and chicken meat in Thailand, therefore poultry proceducers should reduce antimicrobial use to a minimum and stop feeding antimicrobials to healthy birds.

REFERENCES

Aspinall ST, Hindle MA, Hutchinson DN. Improved isolation of *Salmonella* from faeces using a semisolid Rappaport-Vassiliadis medium. *Eur J Clin Microbiol Infect Dis* 1992; 11: 936-9.

Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; 45: 493-6.

Boonmar S, Bangtrakulnonth A, Marnrim N, Kusum M, Suksitviwattana A. The isolation of *Salmonella* in fresh meat by standard conventional method and MSRVmethod. Proceedings of the 22 nd TVMA, 20-22 November 1995: 218-30.

Boonmar S, Bangtrakulnonth A, Marnrim N, Amatayakul C. Isolation of *Salmonella* in meat product by Standard Conventional Method (SCM) and Modified Semisolid Rappaport Vassiliadis method (MSRV). *Food (Thai)* 1997; 2: 88-97.

Boonmar S, Bangtrakulnonth A, Pornruangwong S, Samosornsuk S, Kaneko K, Ogawa M. Significant increase in antibiotic resistance of *Salmonella* isolates from human beings and chicken meat in Thailand. *Vet Microbiol* 1998; 62: 73-80.

Boonmar S, Bangtrakulnonth A, Khositanon W, Pornruangwong S, Watanatraibhob P, Suphasindhu V. Antimicrobial susceptibilities of *Salmonella* isolates from beef, pork, chicken and rats in Thailand. *Kasetsart Vet* 2000; 10: 6-13.

Braun C, Kostka V, Balks E, Redmann T, Helmuth R. Comparative studies of diagnostic bacteriological methods for the recovery of *Salmonella* from faecal samples from flocks of layers. *J Vet Med Ses b* 1998; 45: 245-50.

De Medici D, Pezzotti G, Marfoglia C, Caciolo D, Foschi G, Orefice L. Comparison between ICS-Vidas. MSRV and standard cultural method for *Salmonella* recovery in poultry meat. *J Food Prot* 1998; 45: 205-10.

National Committee on Clinical Laboratory Standard (NCCLS). Performance standards for antimicrobial susceptibility testing. Twelfth informational supplement. 2002.

Read SC, Irwin RJ, Poppe C, Harris J. A comparison of two methods for isolation of *Salmonella* from poultry litter samples. *Poult Sci* 1994; 73: 1617-21.

Voogt N, Raes M, Wannet WJB, Henken AM, Van De Giessen AW. Comparison of selective enrichment media for the detection of *Salmonella* in poultry faeces. *Lett Appl Microbiol* 2001; 32: 89-92.

White D, Shaohua Z, Sudler R, *et al*. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *N Engl J Med* 2001; 345: 1147-54.

Willinga D, Bermudez N, Hopkins E. Poultry on antibiotics: hazards to human health. [Cited 2005 Nov 7]. Available from: URL: www.siearraclub.org/antibiotic.2002