

THE OCCURRENCE OF SALMONELLAE IN BEAN SPROUTS IN - THAILAND

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Abstract. Detection of *Salmonella* spp. in 344 samples of bean sprouts was attempted by pre-enriched in buffered peptone water (BPW) or lactose broth (LB) at 37°C, followed by selective enrichments, selenite cystein broth (SCB) and Rappaport medium (RV) at 43°C. Four selective media, bismuth sulphite agar (BSA), brilliant green agar (BGA), *Salmonella-Shigella* agar (SSA) and MacConkey agar (MA) were used for isolation. Salmonellae were isolated from 30 (8.7%) samples. They were serotypes *lexington* (56.7%), *orion* (16.7%), *senftenberg* (16.7%), *tennessee* (3.3%), *poona* (3.3%) and *weltevreden* (3.3%). Pre-enrichment in LB, followed by enrichment in SCB and streaking on to BSA gave the highest recoveries. Fifteen of 30 strains isolated were sensitive to all 14 antibiotics tested. Seven strains were resistant to one, and a strains showed multiple resistance to 2-4 antimicrobial agents.

INTRODUCTION

Food-borne disease due to *Salmonella* is a major public health hazard throughout the globe. The number of cases has increased markedly, particularly in Americas and continental Europe, and *Salmonella enteritidis* and *S. typhimurium* are the most common serotypes (Anon, 1984, 1985, 1988; Cowden *et al*, 1989; Guinee and Valkenberg, 1975; Humphrey, 1990; Perales and Audicana, 1989; Rodrigue *et al*, 1990). Meat, poultry and eggs were found to be the main sources of infection.

In Thailand, enteric fever increased significantly from 15 cases per thousand in the last decade to 34 cases per thousand in 1987, while the isolation rate of *S. typhi* and *S. paratyphi* decreased markedly (Jayanetra *et al*, 1988). Other *Salmonella*-serotypes were considered to play a major role in the illness (Echeverria *et al*, 1989; Varavithya *et al*, 1990). Although the important sources of infection were not elucidated, many serotypes associated with enteritis were isolated from pork, beef, chicken and vegetables (Rasrinaul *et al*, 1988). Foods of animal origin have most frequently been implicated as sources of infection, but vegetables eg bean sprouts, peppers and coconuts also cause

salmonellosis (Andersson and Jong 1989; Andersson *et al* 1989; Wilson and MacKenzie 1955).

Bean sprouts are usually served raw in Thailand. We are aware that this vegetable may be contaminated with *Salmonella* spp. and could be a hazard to the public. Therefore, the purpose, of the present study was to determine the incidence of *Salmonella* spp. in bean sprouts. Also included is an assessment of the productivity of *Salmonella* isolation methods and susceptibility to 14 antimicrobial agents of the isolated organisms.

MATERIALS AND METHODS

Samples:

Three hundred and forty-four samples of bean sprouts were collected monthly, between March and September 1990, from 4 open markets, Sam Yan, Wang Hin, Bangkok Noi and Bang Khen, in Bangkok. Samples were collected in a sterile plastic bag and transported to the laboratory within 1 hour and examined immediately.

Detection of *Salmonella*:

Two methods were used to demonstrate the presence of *Salmonella* spp.

Procedure 1 : Two hundred and twenty-two out of 344 samples of bean sprouts were pre-enriched

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in buffer peptone water (BPW) (Edel and Kampelmacher, 1973). Aliquots of 25 g were homogenized in a stomacher with 225 ml of BPW and incubated for 20-24 hours at 37°C. For comparison purpose, 1 ml volumes of the pre-enrichment broths were transferred to 10 ml of selenite cystein broth (SCB; Difco) and Rappaport medium (RV) (Vassiliadis *et al*, 1981) and incubated in a waterbath at 43°C for 24 hours. The broth was then subcultured on bismuth sulphite agar (BSA; Difco), brilliant green agar (BGA; Difco), *Salmonella-Shigella* agar (SSA; Difco) and MacConkey agar (MAC; Difco) at 37°C for 24 hours. Three typical colonies on each selective medium were inoculated into triple sugar iron agar (Difco) and lysine iron agar (Difco). The isolates were subsequently confirmed using biochemical tests and serology (Cowan 1974; ICMSF, 1978).

Procedure 2 : One hundred and twenty-two samples were examined as described above but lactose broth (LB) was used as the pre-enrichment medium.

Drug susceptibility:

In vitro sensitivity to 14 agents, ampicillin

(AM), tetracyclin (TC), erythromycin (ER), neomycin (NE), carbenicillin (CB), streptomycin (SM), colistin (CL), furazolidone (FR), gentamycin (GM), kanamycin (KM), chloramphenicol (CP), penicillin (PN), polymixin B (PB) and nalidixic acid (NA) was determined by the method of Bauer *et al* (1966). Antibiotic discs and Mueller-Hinton agar used were commercial products (Difco).

RESULTS

The number of positive samples detected by each procedure is summarized in Table I. The most successful isolation technique was enrichment in SCB and subculture on BSA. Of the 222 samples examined by pre-enrichment in BPW, 17 (7.7%) were positive, while 13 (10.7%) out of 122 samples pre-enriched in LB, were positive. In total, 30 (8.7%) samples were found to contain *Salmonella* spp. Table 2 demonstrates the serotypes of *Salmonella* found and media used for their detection. Six *Salmonella* serotypes were recovered in all and *S. lexington* was the most common organism, which accounted for 56.7% of

Table 1

Detection of *Salmonella* spp. with BPW and LB pre-enrichment media followed by enrichment in SCB and RV and subcultured on BSA, BGA, SSA and MAC.

Positive selective media	BPW ^a		LB ^b	
	SCB	RV	SCB	RV
BSA BGA SSA MAC	-	-	1 ^c	-
BSA BGA SSA	2	-	1	-
BSA BGA MAC	-	-	1	-
BSA SSA MAC	1	-	-	-
BSA SSA	-	-	2	-
BSA MAC	-	-	1	-
BSA	6	5	3	-
BGA	1	-	1	-
SSA	1	-	-	-
MAC	1	-	3	-
Total ^d	12	5	13	0

a Two hundred and twenty-two samples of bean sprouts were analyzed.

b One hundred and twenty-two samples of beab sprouts were analyzed.

c Number of positive sample.

d Thirty samples (8.7%) out of 344 samples were positive.

Table 2

Salmonella serotypes and media used for their isolation.

No. of tested bean sprouts	Isolation media	Serotypes	No. of positive samples	
222	BPW--SCB--BSA	<i>S. lexington</i>	4	
		<i>S. orion</i>	1	
		<i>S. senftenberg</i>	1	
		<i>S. tennessee</i>	1	
		<i>S. weltevreden</i>	1	
	BPW--SCB--BGA	<i>S. orion</i>	2	
		--SSA	<i>S. lexington</i>	2
		--MAC	<i>S. lexington</i>	1
	BPW--RV--BSA	<i>S. lexington</i>	2	
		<i>S. senftenberg</i>	1	
<i>S. orion</i>		1		
122	LB--SCB--BSA	<i>S. lexington</i>	6	
		<i>S. poona</i>	1	
		<i>S. orion</i>	1	
	LB--SCB--BGA	<i>S. lexington</i>	1	
		<i>S. senftenberg</i>	1	
		--MA	<i>S. lexington</i>	1
		<i>S. senftenberg</i>	2	

the positive samples, followed by *S. orion* (16.7%), *S. senftenberg* (16.7%), *S. tennessee* (3.3%), *S. poona* (3.3%) and *S. weltevreden* (3.3%). Table 3 shows the resistance patterns of the isolated salmonellae. Fifteen strains of 30 isolated were resistant to one or more antibiotics.

DISCUSSIONS

It is well known that foods of animal origin are the main sources of salmonellosis throughout the world (Cowden *et al.*, 1989; Flower, 1988; St Louis *et al.*, 1988). Besides these sources, food of non-animal origin such as vegetables were occasionally implicated as the sources of infection (Andersson and Jong, 1989; Andersson *et al.*, 1989). Coconut was reported as a potential carrier of salmonellae (Daniels-Bosman and Huisman 1961; Schaffner *et al.*, 1967; Velaudapillai *et al.*, 1964). Tamminga *et al.* (1978) detected salmonellae in 23 out of 103 samples of vegetables, while Rasrinaul *et al.* (1988) demonstrated that less than

1% of vegetables collected in Bangkok were contaminated with *Salmonella* spp. No sample of bean sprouts was included in their studies. We found that 8.7% of bean sprouts contained salmonellae, *S. lexington* was the most common serotype. *S. lexington* was also among the ten most frequently isolated salmonellae from food and humans in Thailand (Jayanetra *et al.*, 1988).

Many reports demonstrated that RV medium was superior to the other enrichment media for *Salmonellae*-detection (Harvey and Price 1981; Vassiliadis 1983; Vassiliadis *et al.*, 1981, 1987). However in our laboratory following pre-enrichment in BPW, SCB ensured a higher isolation rate than RV medium. When lactose broth was used for pre-enrichment, using RV media no salmonellae could be detected, whereas nonetheless 13 (10.7%) of a total of 122 samples were found positive by SCB. One of the reasons for this discrepancy could be that we examined a vegetable product-while most reports refer to foods of animal origin (Perales and Audicana 1989; Vassiliadis *et al.*, 1981). Moreover it has been demonstrated repeatedly

Table 3
Resistance patterns of *Salmonella* isolated from bean sprouts.

Resistance patterns	Number of strains (%)
<i>S. lexington</i> (17*)	
Sensitive	10 (58.8)
AM, TE, ER, CL	1 (5.9)
AM, TE, ER	1 (5.9)
AM, ER, CL	1 (5.9)
AM, ER	3 (17.6)
ER	1 (5.9)
<i>S. orion</i> (5)	
Sensitive	2 (40)
ER	3 (60)
<i>S. senftenberg</i> (5)	
Sensitive	3 (60)
AM, ER, NE, CL	1 (20)
ER	1 (20)
<i>S. poona</i>	
ER	1 (100)
<i>S. tennessee</i>	
ER	1 (100)
<i>S. weltevreden</i>	
TE, ER, SM	1 (100)

* Number of isolates tested

that results obtained in different laboratories using the same kind of samples and methods could vary widely (Edel and Kampelmacher, 1973; Skovgaards *et al*, 1985; Vassiliadis *et al*, 1987).

The incidence of antibiotic resistance among the our isolates was not high. Multiple resistance was found in 6 strains of *S. lexington* and one strain of each of *S. senftenberg* and *S. weltevreden*. Our results were similar to those obtained in salmonellae isolated from patients in Ramathibodi hospital (Jayanetra *et al*, 1988). However, the WHO National *Salmonella* and *Shigella* Center (Banghagulnonth, personnal communication) found that most of the afore-mentioned *Salmonella* serotypes isolated from humans were resistant to TC, SM and NA.

In conclusion, bean sprouts sold in Bangkok markets were found to be a source of salmonellosis. Isolation of salmonellae from this vegetable was successful by pre-enrichment in BPW or LB at 37°C and enrichment in SCB at 43°C followed by

plating on to BSA and MAC. Fifty percent of the organisms were resistant to one or more drugs.

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