FIRST ISOLATION OF *SALMONELLA BLOCKLEY* IN THAILAND

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Abstract. The first isolation of *Salmonella blockley* in Thailand was found in 2 strains of animal feed samples and 3 strains of chicken feather samples from a private poultry company in 1989. From 1987 to 1992, the number of *S. blockley* isolates increased and found in various sources. The major sources were the stools of diarrheal patients, mainly children. Another source of *S. blockley* was frozen chicken meat which increased every year studied. *S. blockley* isolated from human and other sources showed a high percentage resistance to streptomycin, tetracycline, kanamycin and chloramphenicol and a low percentage resistance to ampicillin and cotrimoxazole. Thus, *S. blockley* must now be listed as a possible cause of *Salmonella* food poisoning in Thailand.

INTRODUCTION

Salmonellosis is a disease of a public health importance and economic significance. *Salmonella* infections have substantially increased in Thailand and worldwide over the past decade (Bamrasnaradura Infectious Diseases Hospital Report, 1988; Rodrigue *et al*, 1990). This may be due to improved detection and reporting systems. In Thailand, the prevalence of salmonellae causing diarrhea was highest (17%) in children aged under 5 months (Varavithya *et al*, 1990). In most cases of food poisoning the etiologic agents are rarely confirmed due to the fact that foods associated with diarrheal illness are usually not available for culture or laboratory examination (Annual Epidemiology Surveillance Report, 1989; Rasrinual *et al*, 1988). The isolation of salmonellae is mostly reported from animals, animal feeds, water and environmental sources (Boriraj *et al*, 1988; Phan-Urai *et al*, 1987).

Surveillance and epidemiological investigations have traditionally relied on biochemical and serological methods for the primary identification of bacterial strains. Approximately 4,000 - 7,000 strains of *Salmonella* are sent for serotyping from government and and private laboratories to the WHO Salmonella and Shigella Center, National Institute of Health (NIH), Nonthaburi, Thailand each year (Boriraj *et al*, 1988). Many different common serovars have been repeatedly reported from NIH year by year (Boriraj *et al*, 1988; Phan-Urai *et al*, 1987). A new serovar, *S. blockley* has not been identified among the common serovars usually found in the past. In the present study, we noticed the emergence of this new serovar *S. blockley* in 1986. Therefore, the prevalence of the *S. blockley* isolation from various resources as well as its antibiotic resistance was analyzed during 1986 - 1992.

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MATERIALS AND METHODS

Bacterial strain

A total of 1,738 strains of *S. blockley* obtained from various institutes, *ie* hospitals; the Veterinary Department, the Fisheries Department, Ministry of Agriculture; the Food Analysis Department, Ministry of Public Health; and private company laboratories in Bangkok and provinces, were confirmed for *Salmonella* identification and serotyping. The strains were isolated from diarrheal patients, frozen chickens, frozen seafood, meat, offal, animal feed, feathers, and the environment during 1986 - 1992.

Identification : All strains were transferred on to Endo agar (Difco, Michigan, USA) plates and incubated overnight at 37°C. The suspected colony was identified for *Salmonella* by triple sugar iron agar (TSI; Difco).

The suspected positive reacting on TSI agar for salmonellae were further identified by biochemical tests, *ie* dextrose, mannitol, lactose, Simmon's citrate, lysine decarboxylase, arginine dihydrolase, indole, H_2S, and motility as described by WHO (1987).

Serotyping : The identified salmonellae were serotyped by the Gard Technique (Gard, 1938). For 0 serotyping, *Salmonella* 0 antigen was prepared from culture on Endo agar plates. Serotyping was performed by slide agglutination test with *Salmonella* A-65, and *Salmonella* A-I polyvalent antisera. The positive serotyped strains were subsequently slide-agglutinated with group antisera (A, B, C, D, E....65). *S. blockley* was agglutinated with group C and the positives were further agglutinated with 0-factor, 0 : 6 and 0 : 8 (0 : 6, 0 : 7, 0 : 8, 0 : 14, 0 : 20).

For H serotyping, culture from Endo agar plate was spotted at the center of swarm agar plate according to Gard's method [10 ml of melted agar (0.7%) in a Petri dish 10 × 50 mm]. After incubation at 37°C for 18 hours, the motile strains swarmed over the plates were tested for H antigens with H polyvalent antisera (H : a, H : b, H : c, H : d,....H : k, H : r,.....H : z66) by slide agglutination test. *S. blockley* which showed positive agglutination with H : k monovalent (phase 1 antigen) were then subcultured on the second swarm agar plate containing 0.09 ml of dilution 1 : 1600 H : k antiserum. After incubation at 37°C for 18 hours, the phase 1 antigen was agglutinated with H : k antiserum in the plate. The other H antigen showing the swarm appearance on that agar plate was used as the phase 2 antigen. That phase 2 antigen was slide-agglutinated with phase 2 antisera (*ie*, H : 1,2, H : 2, H : 5, H : 6, H : 7 and H : z6). *S. blockley* showed H : 1,5 positive. The culture from the second swarm agar plate was transferred to the third swarm agar plate containing 0.09 ml of dilution 1 : 1600 H : k and H : 1,5 antisera. After incubation, the homologous culture showed no swarming on the agar plate. Serotypes of the tested strains were recorded with the Table of *Salmonella* serotyping scheme (Ewing, 1986).

Antibiotic sensitivity : Antibiotic sensitivity testing of *S. blockley* was performed by the disc method as described by Bauer *et al* (1966). Seven kinds of antibiotic discs were used in this study: chloramphenicol (CP), tetracycline (TC), streptomycin (SM), kanamycin (KM), ampicillin (AMP), cotrimoxazole (trimethoprim - sulfamethoxazole, SXT), and nalidixic acid (NA).

RESULTS

A number of *S. blockley* from various sources isolated in Thailand during 1986 - 1992 are shown in Fig 1. Five strains of *S. blockley* were first reported in Thailand in 1986. In 1987, 190 strains were isolated with 55.8% (106/190) originated from humans. Since then, the isolation of *S. blockley* increased twice in number. However, during 1989 - 1992, the number of reported *S. blockley* declined slightly.

The isolation rate of *S. blockley* from total *Salmonella* strains was low (0.1%) in 1986 (Table 1). Then the isolation rate of *S. blockley* increased and the highest rate was up to 6.2% in 1988.

Sources of isolation

In 1986, 2 strains of *S. blockley* isolated from animal feeds and 3 strains isolated from chicken feather were sent from the private poultry company (Table 2). In

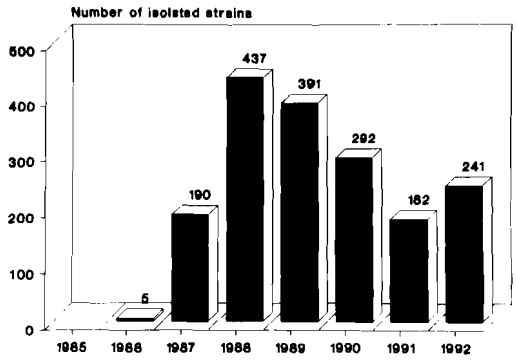

Fig 1–Number of isolated *Salmonella blockley* from all sources in Thailand, 1986 - 1992.

Table 1

Isolation rates of *Salmonella blockley* during 1986 - 1992.

Year	No. of isolated *Salmonella*	No. (%) of *S. blockley*
1986	4878	5 (0.1%)
1987	6734	190 (2.8%)
1988	7054	437 (6.2%)
1989	6424	391 (6.1%)
1990	6101	292 (4.8%)
1991	5110	182 (3.6%)
1992	5569	241 (4.3%)

Table 2

Number of strains of *Salmonella blockley* isolated from various sources in Thailand, during 1986 - 1992.

Sources	1986	1987	1988	1989	1990	1991	1992
Animal feed	2	1	34	14	11	-	-
Animal[a]	3	9	22	10	9	12	47
Frozen chicken meat	-	68	201	205	123	72	88
Human	-	106	156	160	140	85	88
Environment[b]	-	6	24	2	7	-	6
Food[c]	-	-	-	-	2	13	12
Total	5	190	437	391	292	182	241

[a] Chicken, pig, and partridge
[b] River, pond, sludge, sewage, and litter
[c] Thai sausage, chicken curry, chicken fried with ginger, soup

1987, *S. blockley* was isolated from 5 sources. Two major sources were diarrheic patient stools, especially in children (55.7%) and exported frozen chicken meat (35.8%). The rest was found in animals (5%), environment (3%) and animal feeds (0.5%). During 1987-1992, *S. blockley* isolated from human and from frozen chicken meat was found in high number.

Among animals from which *S. blockley* was isolated were chickens, pigs, and partridges. *S. blockley* was isolated from the environment *eg* river, pond, sludge, sewage and litter. *S. blockley* was also isolated from cooked food (0.7% - 7%) *eg* Thai sausage, chicken curry, chicken fried with ginger, soup, etc.

Antibiotic sensitivity test

All *S. blockley* were assessed for their susceptibility to 7 antimicrobial agents. During 1986-1992, the antibiotic resistance of *S. blockley* from human sources is shown in Table 3. The resistance of *S. blockley* to streptomycin was high and ranged from 96%-100%, tetracycline ranged from 95%-100% and kanamycin ranged from 84%-98%, and chloramphenicol ranged from 63%-86%. *S. blokcley* was resistant to ampicillin, cotrimoxazole, and nalidixic acid in lower percent (Table 3).

Antibiotic resistance of *S. blockley* isolated from other sources during 1986-1992 is shown in Table 4.

Table 3

Antibiotic resistance of *Salmonella blockley* isolated from human, during 1987 - 1992.

Year	No. of strains tested	% of resistant strains to						
		CP	TC	SM	KM	AMP	SXT	NA
1987	22	86	95	100	95	5	5	ND[a]
1988	74	72	96	97	84	18	15	36
1989	85	79	99	99	95	1	5	20
1990	82	77	100	100	98	5	6	18
1991	27	63	96	100	93	7	4	7
1992	51	65	96	96	92	8	9	20

CP - chloramphenicol; TC - Tetracycline; SM - Streptomycin;
KM - Kanamycin; AMP - Ampicillin; SXT - Cotrimoxazole;
NA - Nalidixic acid
[a] Not done

Table 4

Antibiotic resistance of *Salmonella blockley* isolated from other sources[a] during 1986 - 1992.

Year	No. of strains tested	% of resistant strains to						
		CP	TC	SM	KM	AMP	SXT	NA
1986	2	100	100	100	100	0	0	ND[b]
1987	19	58	100	84	10	10	5	ND
1988	37	46	97	97	54	43	43	10
1989	28	79	93	96	96	0	0	7
1990	49	80	100	100	96	4	6	29
1991	20	75	95	95	100	5	5	5
1992	52	71	100	92	98	6	4	4

CP - chloramphenicol; TC - Tetracycline; SM - Streptomycin;
KM - Kanamycin; AMP - Ampicillin; SXT - Cotrimoxazole;
NA - Nalidixic acid
[a] Animal feed, animal, frozen chicken meat, environment and food
[b] Not done

The resistance rates of *S. blockley* from other sources to 7 antimicrobial agents were quite similar to those from human sources. The resistance of *S. blockley* from other sources to streptomycin was high and ranged from 92%-100%, tetracycline ranged from 93%-100%, kanamycin ranged from 54%-100%, and chloramphenicol ranged from 46%-100%. *S. blockley* was resistant to ampicillin, cotrimoxazole, and nalidixic acid to a lesser degree (Table 4).

DISCUSSION

S. blockley is one of over 2,000 serovars belonging to species *S. enteritidis* (Le Minor and Popoff, 1988). Recently, *S. enteritidis* serovar has been a major etiologic agent in outbreaks of gastroenteritis in the United States, the United Kingdom and parts of Europe. The common serovars that caused bacterial food poisoning reported in Thailand were *S. derby, S. typhimurium, S. weltevreden, S. agona, S. krefeld, S. virchow* (Boriraj *et al*, 1988).

S. blockley had not been isolated in Thailand before 1986. In the last 5 years, *S. blockley* has become one of the commonly recognized salmonellae causing bacterial food poisoning. This is the first report on the existence of *S. blockley* in Thailand. It was first isolated in 1986 from animal feeds and chicken feathers from a private chicken farm and then the identification was confirmed in this study. It was believed that this *Salmonella* serovar was imported from abroad since all animal feeds were imported. The chicken feathers might be cross-contaminated with this serovar from feed sources. Since then, many strains of *S. blockley* have been isolated from various sources, mostly from chickens, chicken products and humans.

Thus, the spread of *S. blockley* probably started from imported animal feed contaminated with *S. blockley*. The chickens were infected by eating this animal feed. However, these farm chickens did not have any illness. Fresh and frozen chicken meat could be also contaminated during the process of cutting, part sorting and packaging. Then man could become infected by eating the contaminated meat and hen products such as eggs, intestinal organs, as with other serovars of *Salmonella* (Pavia and Tauxe, 1991). Most of the cooked food from which *S. blockley* was isolated included chicken or pork as an ingredient, namely chicken curry, Thai sausage, chicken soup.

Antibiotics are being given to livestock in Thailand in an uncontrolled manner (Rasrinaul *et al*, 1988). Cotrimoxazole and chloramphenicol should be used to treat humans rather than raise livestock (US Food and Drug Administration, 1982). The tracing of a recent outbreak of chloramphenicol-resistant *Salmonella* in California to dairy farms where chloramphenicol had been used demonstrated that the use of this antibiotic raise livestock can result in an increase in chloram-

phenicol- resistant *Salmonella* infections in persons in the community (Spika *et al*, 1987). In this study, *S. blockley* isolated from either humans or other sources showed similar antibiotic resistance patterns. They were highly resistant to streptomycin, tetracycline, kanamycin (80%-100%) and moderately resistant to chloramphenicol (40%-80%). The finding of similar drug resistance of *S. blockley* isolated from humans and other sources suggests that they might come from the same original source. However, the plasmid profile of these strains should be determined in order to prove this claim (Rasrinaul *et al*, 1988; Rodrigue *et al*, 1992). On comparison with those *S. blockley* reported in Japan, the resistance of *S. blockley* to streptomycin, kanamycin, tetracycline and chloramphenicol was not so high (66.2%) (Yoshida *et al*, 1992).

S. blockley has now become one additional possible cause of *Salmonella* food poisoning. Food inspectors and poultry farmers should be aware of its existence, and as well the clinician should recognize this new serovar and its drug resistance. As international movement of materials, persons and livestock is now so common, the spread of new microorganism strains and diseases is readily possible. Therefore, law enforcement and regulation concerning biological standards of imported products should be emphasized.

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