

Comparison of Plasmid Profiles and Antimicrobial Resistance Patterns of *S. enteritidis* Isolates in Thailand

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ABSTRACT

Two hundred isolates of *S. enteritidis* from human and chicken meat in Thailand between 1990 and 1997 were studied for antimicrobial susceptibility using disk diffusion method. The results showed that 40% of the isolates from both human and chicken meat were susceptible to all 10 antimicrobial agents, on the other hand 44-50% of the isolates from human and chicken meat were resistant to cefotaxime (CTX). Only 40% of the isolates from both susceptible group and CTX resistant group were sampled to analyse for plasmid using modified method of Kado and Liu. Most isolates contained a single 55 kilobases (kb) plasmid. Our study revealed that even the isolates from human and from chicken meat had different antimicrobial susceptibility patterns, they contained the same size of plasmid which indicated a close correlation between isolates and were derived from the same source.

Key words: *S. enteritidis*, epidemiology study, plasmid profile, antimicrobial resistance pattern

INTRODUCTION

Recently, *Salmonella* serotype *enteritidis* has become the predominant serotype in many countries and has the important role for salmonellosis in both human and animals (Rodrigue *et al.*, 1990). Human infections with *S. enteritidis* have been increasing worldwide since 1980 and have been shown to be related mainly to consumption of eggs and egg products including contaminated chicken meat (Humphrey *et al.*, 1989).

There are many useful methods for epidemiological study of bacteria such as phage typing, plasmid profiles, biotype, pulsed field gel electrophoresis and antimicrobial resistance patterns (Powell *et al.*, 1994). Plasmid analysis and

antimicrobial resistance patterns were reported in epidemiological study of salmonellosis (Riley and Cohen, 1982; Stubbs *et al.*, 1994; Nakamura *et al.*, 1986).

The objective of the present study was to compare plasmid profiles and antimicrobial resistance patterns of *S. enteritidis* isolates from human and chicken meat in Thailand.

MATERIALS AND METHODS

Salmonella isolates were collected at the faculty of Veterinary Medicine, Kasetsart University between 1990 and 1997. Only 200 selected *S. enteritidis* isolates from human and chicken meat were confirmed by serology and biochemical

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method. All isolates were tested for antimicrobial resistance pattern using 10 antimicrobial agents : amikacin, cefotaxime, ciprofloxacin, norfloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, gentamicin, ofloxacin, ampicillin and amoxicillin/clavo and also using Disk diffusion method (Barry and Thornsberry, 1991).

Only 40 isolates of both susceptible and CTX resistant group were sampled to analyse plasmid by the modified method of Kado and Liu (1981). In brief, bacterial cells were grown overnight in 3 ml of broth at 37° C, harvested by centrifugation, and suspended in 100 ul of E buffer. The cells were then lysed by the addition of 200 ul of freshly prepared lysing solution, incubated for 1 h at 55°C and extracted with 600 ul of phenol/chloroform (1:1 v/v). After centrifugation, the supernatant was subjected to 1% agarose gel electrophoresis for detection and sizing of plasmid DNA.

RESULTS

Antimicrobial resistance patterns

All 200 *S. enteritidis* isolates from human and chicken meat were tested for antimicrobial resistance patterns. It was found that 40% of the isolates from human were susceptible to all 10 antimicrobial agents, on the other hand 44% were resistant to CTX, 4% were resistant to CTX-AN, 4% were resistant to only AN and the other 8% were resistant to more than three drugs. For the isolates from chicken meat, 40% of isolates were susceptible to all 10 antimicrobial agents, but 50% were resistant to CTX, 2% were resistant to CTX-SXT and C-SXT, the other 6% were resistant to CTX-CIP (Table 1).

Plasmid profiles

Most of the plasmid from *S. enteritidis* isolates of human and chicken meat derived from antimicrobial susceptible group and resistance group were 55 kb in size (Table 2).

Table 1 Antimicrobial resistance of 200 *S. enteritidis* isolates from human and chicken meat between 1990 and 1997.

Source of isolates	Total no. of isolates	Total no. of sensitive isolates	No. of isolates resistant to the following antimicrobial ^a					
			CTX	CTX-AN	AN	CTX-SXT	CTX-CIP	C-SXT
Human	100	40	44	4	4	-	-	-
Chicken meat	100	40	50	-	-	2	6	2
								More than three drugs
								8
								-

^a The following antimicrobial agent were tested : amikacin(AN), cefotaxime(CTX), ciprofloxacin(CIP), norfloxacin(NOR), trimethoprim/sulfamethoxazole(SXT), chloramphenicol(C), gentamicin(G), ofloxacin(OFX), ampicillin(AM) and amoxicillin/clavo(AMC)

Table 2 Plasmid size of *S. enteritidis* isolates from human and chicken meat between 1990 and 1997.

Source of isolates	No. of isolates	Antimicrobial resistance isolates	Plasmid size
Human	10	-	55
Human	10	CTX	55
Chicken meat	10	-	50,55
Chicken meat	10	CTX	48,55

DISCUSSION

Boonmar *et al.* (1998) reported that 76% of *S. enteritidis* isolates from human and 73% of the isolates from broiler chickens in Thailand between 1990 and 1997 were phage type 4 and the study of pulsed field gel electrophoresis showed that they contained an indistinguishable pattern by Bln I-digested. Their studies indicated the spread of a genetically identical clone of *S. enteritidis* in human and poultry in Thailand.

However, the present study showed that 40% of isolates from both human and chicken meat were susceptible to all 10 antimicrobial agents but 44 to 50% were resistant to CTX. Plasmid profile was analysed from both groups of isolates and they contained 55 kb plasmid.

It is contrast to the result of Stubbs *et al.* (1994) which reported that most of the *S. enteritidis* PT 8 derived from chicken meat contained 55 kb plasmid and also almost were susceptible to 13 antimicrobial agents, except some isolates that resistant to ampicillin- carbenicillin lacking the 55 kb plasmid. The present study showed that even the isolates from susceptible or resistant to antimicrobial agents contained the same plasmid profile. However, there are some publications reported that plasmid was not analysed from the *Salmonella* isolates (Holmberg *et al.*, 1984).

The study of Nakamura *et al.* (1986) confirmed that plasmid analysis was accepted as a means of identifying relatedness or unrelatedness of isolates to *Salmonella*, *Shigella*, *Enterobacter*,

and *Campylobacter* (Brunner *et al.*, 1983; Holmberg *et al.*, 1984) for example *S. typhimurium* isolated from animals reared in limited areas and exhibiting identical or similar plasmid patterns originated from one source and that isolates from such limited areas exhibiting quite different plasmid patterns were derived from different sources (Nakamura *et al.*, 1985).

ACKNOWLEDGMENTS

We are grateful to the Kasetsart University Research and Development Institute (KURDI) for the financial support.

LISTERATURE CITED

- Barry, A.L. and C. Thornsberry. 1991. Susceptibility test : diffusion test procedures, pp. 1117-1125. In A. Balows, W.J. Hausler, Jr., K.L. Herrmann, H.D. Isenberg, and H.J. Shadomy (eds.). Manual of Clinical Microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
- Boonmar, S., A. Bangtrakulnonth, S. Pornrunangwong, J. Terajima, H. Watanabe, K. Kaneko, and M. Ogawa. 1998. Epidemiological analysis of *Salmonella enteritidis* isolates from humans and broiler chickens in Thailand by phage typing and pulsed-field gel electrophoresis. J. Clin. Microbiol. 36 : 971-974.
- Brunner, F., A. Margadant, R. Peduzzi, and J.C.

- Piffaretti. 1983. The plasmid pattern as an epidemiologic tool for *Salmonella typhimurium* epidemic : comparison with the biotype. J. Infect. Dis. 148 : 7-11.
- Holmberg, S.D., I.K. Wachsmuth, F.W. Hickman-Brenner, and M.L. Cohen. 1984. Comparison of plasmid profile analysis, phage typing, and antimicrobial susceptibility testing in characterizing *Salmonella typhimurium* isolates from outbreaks. J. Clin. Microbiol. 19 :100-104.
- Humphrey, T.J., J.G. Cruickshank, and B. Rowe. 1989. *Salmonella enteritidis* phage type 4 and hens' eggs. Lancet i : 281.
- Kado, C.I., and S.T. Liu. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145 : 1365-1373.
- Nakamura, M., K. Ohmae, S. Sato, S. Suzuki, and S. Ikeda. 1985. Isolation of *Salmonella* from apparently healthy fattening male dairy calves and fattening pigs and stability of plasmids in the isolates. Jpn. J. Vet. Sci. 45 : 379-384.
- Nakamura, M., S. Sato, T. Ohya, S. Suzuki, and S. Ikeda. 1986. Plasmid profile analysis in epidemiological studies of animal *Salmonella typhimurium* infection in Japan. Jpn. J. Clin. Microbiol. 23 : 360-365.
- Powell, N.G., E.J. Threlfall, H. Chart, and B. Rowe. 1994. Subdivision of *Salmonella enteritidis* PT 4 by pulsed-field gel electrophoresis : potential for epidemiological surveillance. FEMS Microbiol. 119 : 193-198.
- Riley, L.W., and M.L. Cohen. 1982. Plasmid profiles and *Salmonella* epidemiology. Lancet.i : 537.
- Rodrigue, D.C., R.V. Tauxe, and B. Rowe. 1990. International increase in *Salmonella enteritidis* : a new pandemic ? Epidemiol. Infect. 105 : 21-27.
- Stubbs, A.D., F.W. Hickman-Brenner, D.N. Cameron, and J.J. Farmer. 1994. Differentiation of *Salmonella enteritidis* phage type 8 strains : evaluation of three additional phage typing systems, plasmid profiles, antibiotic susceptibility patterns, and biotyping. J. Clin. Microbiol. 32 :199-201.

Received date : 24/05/02

Accepted date : 28/06/02