ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND PHAGE TYPES OF *SALMONELLA TYPHI* FROM VIETNAM

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Abstract. A retrospective study of the patterns of antimicrobial susceptibility and phage types of 111 *Salmonella typhi* strains isolated in 1996 from Vietnam was carried out. The strains were tested for susceptibility to chloramphenicol, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, ceftazidime, ceftriaxone and ciprofloxacin. Simultaneous resistance to chloramphenicol, ampicillin, tetracycline and trimethoprim-sulfamethoxazole were present in 84 strains (75.7%). Nalidixic acid resistance was only observed in 2 multidrug-resistant strains (1.8%). Twenty-one strains (18.9%) were completely susceptible to all drugs tested. All 111 strains were susceptible to ceftazidime, ceftriaxone and cipropfloxacin. The MIC values for chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole corresponded with the results by disk diffusion method. On Vi phage-typing, 5 different phage types (28, A, D1, E1 and M1) were found in 12 strains (10.8%). However, most *S. typhi* strains were indistinguishable by this typing technique because they were degraded Vi-positive or untypeable Vi-positive strains (35.1% and 54.1%, respectively). There were no correlations between antimicrobial resistance patterns and phage types in the tested *S. typhi* strains in this study.

INTRODUCTION

Typhoid fever is a worldwide health problem, especially prevalent in developing countries. Globally, there are approximately 16 million cases of typhoid fever with 600,000 deaths annually (Ivanoff, 1995). The regions with a high incidence of this disease (>100/100,000 persons/year) include Southcentral and Southeast Asia (Crump *et al,* 2004). In some developing countries of Asia and Africa, the annual incidence of infection may reach 1% with case fatality rates as high as 10%. About 70% of all fatalities from typhoid fever occur in Asia (WHO, 2005).

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A combination of effective antimicrobial therapy, improved sanitation and hygiene, and vaccines reduce significantly the morbidity and mortality from typhoid fever. Under selective antibiotic pressure the organism has developed different mechanisms of antibiotic resistance. Owing to the development of bacterial resistance to chloramphenicol during the 1970s and 1980s, treatment with this drug was widely replaced by ampicillin and trimethoprim-sulfamethoxazole. However, by the 1980s and 1990s, *S. typhi* developed resistance simultaneously to all drugs used for first-line treatment, namely, chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (Bhutta *et al,* 1992). The widespread emergence of resistance to drugs used to treat typhoid fever led to large epidemics, particularly in Asia, and complicated the treatment of this serious infection (Parry, 2004).

In Vietnam, resistance to chloramphenicol was first observed in the south in 1971, and this resistance spreaded rapidly to two-thirds of *S. typhi* isolated (Brown *et al,* 1975; Meyruey *et al,* 1975). Ampicillin and trimethoprim-sulfamethoxazole were clinically effective alternative drugs (Butler *et al,* 1977). Multidrug-resistant (MDR) typhoid fever [simultaneous resistance to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole (CmApTmSu) and other antibiotics] was first detected in the late 1980s and emerged as a problem in 1992 causing several large outbreaks over the succeeding years (Hoa *et al,* 1998). The initial outbreak of MDR typhoid fever was described in Kien Giang Province (southern Vietnam) in 1993 (Nguyen *et al*, 1993). By 1994, over 80% of all *S. typhi* strains isolated in southern Vietnam were multidrug-resistant (Vinh *et al,* 1996) while MDR strains were reported in less than 5% and 10% of cases in central and northern Vietnam, respectively (Nguyen and Tran, 1994; Pham and Dang, 1994). Three other MDR typhoid outbreaks occurred in southern Vietnam from 1993 to 1997. Some of the MDR strains from the outbreaks were associated with nalidixic acid resistance (Nguyen *et al*, 1993; Tran *el al,* 1995; Vinh *el al,* 1996).

Strains within *S. typhi* are separated into a number of phage types by their patterns of susceptibility to lysis by a series of variants of a single phage, Vi-phage II, which has been adapted to the different types of typhoid bacillus. There is a high degree of correlation between the phage-type and the epidemic source of typhoid fever. Consequently, phage typing has been one of the most effective tools for the classification of the pathogen at the intraspecific level. Determination of the phage type obtained from patients is valuable for epidemiological study of infections (Anderson and Williams, 1956). So far about 106 different phage types of *S. typhi* have been defined and are designated by letter or number (Hampton *et al,* 1998).

In the present study, we aimed to identify the patterns and levels of antibiotic resistance in clinical strains of *S. typhi* isolated in Vietnam in 1996 and to investigate Vi-phage types and their correlation to resistance patterns in selected strains.

MATERIALS AND METHODS

Bacterial strains

One hundred eleven strains of *S. typhi* isolated in 1996 from blood, bone marrow and stool of typhoid patients admitted to national hospitals in Hanoi (North), Hue (Center) and Ho Chi Minh City (South), Vietnam were selected for study. Bacterial identification was carried out at the time of isolation by biochemical reactions and agglutination with specific antisera for *S. typhi*. The strains were stored on brain-heart infusion broth with 20% glycerol at -70ºC before further work was performed. For this study, all these strains were confirmed biochemically as being *S. typhi* on the following media: triple sugar ion agar (Merck KGaA, Darmstadt, Germany), lysine ion agar (Becton, Dickinson, France), motility indole ornithine medium (Difco, Detroit, USA), urea agar (Becton, Dickinson, France) and Simmons' citrate agar (Difco, Detroit, USA) and by slide agglutination with antisera specific for group D and Vi antigens (S & A Reagents Lab, Thailand).

Antimicrobial susceptibility testing

A total of 111 *S. typhi* strains were tested for their susceptibility to antimicrobial agents (chloramphenicol, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, ceftazidime, ceftriaxone and ciprofloxacin; all from Oxoid) by a modified Kirby- Bauer disk diffusion method (Vandepitte *et al*, 1991). The minimum inhibitory concentrations (MICs) of chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole were determined by using E-test strips following the manufacturer's instructions (AB BIODISK, Solna, Sweden). *Es-*

Table 1
Antimicrobial resistance patterns for *Salmonella typhi* strains.

Resistance pattern[a]	Strain		Strain by region		
	Number	%	North	Center	South
CmApTeTmSu	82	73.9	38	37	7
CmApTeTmSuNa	2	1.8	0	0	2
CmTe	5	4.5	1	3	1
Te	1	0.9	0	1	0
Complete susceptibility	21	18.9	13	4	4
Total	111	100	52	45	14

[a] Cm, chloramphenicol; Ap, ampicillin; Te, tetracycline; Tm, trimethoprim; Su, sulfamethoxazole; Na, nalidixic acid

cherichia coli ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (the MICs for which are known) were used as controls for the potency of antimicrobial agents.

Phage typing

Phage typing of all the clinical strains was performed by routine test dilutions of the typing phages of *S. typhi* (Anderson and Williams, 1956) in the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand.

RESULTS

Antimicrobial susceptibility

Of the 111 *S. typhi* strains tested for their susceptibility to 9 antimicrobial agents (chloramphenicol, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, ceftazidime, ceftriaxone and ciprofloxacin), only 21 strains (18.9%) were completely susceptible to all drugs tested, whereas 90 strains (81.1%) exhibited resistance to one or more antibiotics. There were 84 MDR strains (75.7%) with a resistance pattern of CmApTeTmSu, but 2 strains (1.8%) obtained from the South were also resistant to nalidixic acid. Resistance to chloramphenicol and tetracycline only was observed in 5 strains (4.5%). One strain (0.9%) from central Vietnam possessed single resistance to tetracycline. However, none of the strains were resistant to ceftazidime, ceftriaxone or cipropfloxacin. Detailed antimicrobial resistance profiles are summarized in Table 1.

The MICs for chloramphenicol ranged from 1.5 to >256 μg/ml, the MICs for ampicillin were from 0.19 to >256 μg/ml and the MICs for trimethoprim-sulfamethoxazole were from 0.006 to >32 μg/ml. A good correlation between disk diffusion technique and the E-test was observed in the tested strains. The resistant strains were those that showed an MIC ≥32 μg/ml for chloramphenicol and ampicillin and an MIC ≥4 μg/ml for trimethoprim-sulfamethoxazole.

The MIC values of the two control strains, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were within the expected ranges.

Phage typing

Of the 111 strains analyzed by Vi phage-typing, 12 (10.81%) were typeable with 5 different phage types (28, A, D1, E1 and M1), in which phage type A was only recognized in 3 strains from northern Vietnam, phage types 28 and D1 were detected in 2 strains from central Vietnam and phage type D1 was exhibited in 4 strains from the north and 1 strain from the south. Thirty-nine (35.14%) were degraded Vi-positive and 60 were untypeable Vi-positive strains (54.1%) (Table 2).

Table 2
Summary of phage types for *Salmonella typhi* strains.

Phage type[a]	Strain		Strain by region		
	Number	%	North	Center	South
28	1	0.9	0	1	0
A	3	2.7	3	0	0
D1	1	0.9	0	1	0
E1	5	4.5	4	0	1
M1	2	1.8	2	0	0
DVS	39	35.1	21	16	2
UVS	60	54.1	22	27	11
Total	111	100	52	45	14

[a]DVS, Degraded Vi-positive strain; UVS, Untypeable Vi-positive strain

Table 3
Distribution of resistance patterns and phage types in *Salmonella typhi* strains.

Resistance pattern	Phage type	Number of strain
CmApTeTmSu	28	1
	A	1
	D1	1
	E1	2
	DVS	31
	UVS	46
CmApTeTmSuNa	UVS	2
CmTe	DVS	2
	UVS	3
Te	UVS	1
Complete susceptibility	A	2
	E1	3
	M1	2
	DVS	6
	UVS	8

DISCUSSION

Typhoid fever had been endemic in Vietnam for many years and is especially common in the densely populated rice-growing areas of the Mekong Delta and urban areas of Ho Chi Minh City (formerly Saigon). During the 1990s, MDR typhoid fever emerged widely in the south, most *S. typhi* strains isolated from sporadic and epidemic cases exhibit resistance to first-line drugs used for treatment of typhoid fever, and strains with reduced susceptibility to fluoroquinolones (indicated by resistance to nalidixic acid) have been reported (Vinh *et al,* 1996; Wain *et al,* 1997). Out data agree with previous studies (Vinh *et al,* 1996; Wain *et al,* 2003). The multidrug-resistant pattern was common (75.7%). However, increased MDR patterns were observed in strains obtained from both northern and central areas (73.1% and 82.2%, respectively). Data obtained in these regions previously had MDR in less than 10% of cases (Nguyen and Tran, 1994; Pham and Dang, 1994). The availability and affordability or effective drugs and the unrestricted consumption of these drugs without prescription in Vietnam likely led to the selection of resistant strains of *S. typhi*. The resistance to nalidixic acid was present in only 2 strains from the south, and not detected in strains from the two other regions. All strains were susceptible to ceftazidime, ceftriaxone and cipropfloxacin. Third-generation cephalosporins and fluoroquinolones are clinically effective alternatives in treating MDR typhoid fever at this time (Chinh *et al,* 2000; Parry *et al,* 2002).

Epidemiological analysis of typhoid fever is important in monitoring its transmission. Typing is a useful tool in epidemiological investigations of the infections. Vi phage-typing is a

conventional phenotypic typing system used for this purpose. Different Vi-phage types, such as A, D4, E1, E2, E3, M1, M2, M4, 46, and 56 have been previously reported in the *S. typhi* population in Vietnam. Vi-phage types E1 and E3 are predominant among MDR strains. Nevertheless, a number of strains are phage untypeable (Connerton *et al,* 2000; Le *et al,* 2004). Various analyses of phage types indicates some types (E1, A, C, D_1, F_1) have global distribution (Anderson and Williams, 1956). E1 is the most common type of MDR strain of *S. typhi* in India, Pakistan, Bangladesh and the Arabian Gulf (Pillai and Prakash, 1993; Hermans *et al*, 1996; Hampton *et al,* 1998).

In the present study, the analysis of *S. typhi* by Vi phage-typing divided 10.8% of the strains into 5 different phage types, in which phage types D1 and 28 have not been previously reported in Vietnam. No type was predominant. The data suggest the strains typeable by Vi phages were epidemiologically unrelated. There were multiple sources of typhoid fever due to several different clones of *S. typhi* in Vietnam. Both phage types D1 and 28 may have been recently introduced into Vietnam. There were significant proportions of degraded Vi-positive strains (35.1%) and untypeable Vi-positive strains (54.1%). The findings indicate that degraded Vi-positive strains were sensitive to many Vi-typing phages and could not be typed. The presence of untypeable Vi-positive strains may be due to the fact that they were resistant to Vi-phage II used and sensitive to Vi-phages other than Vi-phage II (Anderson and Williams, 1956). As a result, most *S. typhi* strains were indistinguishable by Vi phage-typing. This conventional phenotypic typing method presented nonsignificant discriminatory power in subdividing the strains of *S. typhi* in the present study. To provide useful information for epidemiological typing of the *S. typhi* strains, the Vi phage-typing method must be complemented by more sensitive and discriminative molecular techniques, such as plasmid profile typing, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) or pulsed-field gel electrophoresis (PFGE).

No particular phage types correlated with antimicrobial resistance patterns (Table 3).

In conclusion, multidrug-resistance was encountered predominantly in *S. typhi* strains obtained in Vietnam in 1996. All these strain remained susceptible to the third generation cephalosporins and fluoroquinolones. Most strains were not differentiated by Vi phage-typing and there was no relationship between resistance patterns and phage types.

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