ANTIMICROBIAL RESISTANCE OF *SHIGELLA* SPP ISOLATED FROM DIARRHEAL PATIENTS BETWEEN 1989 AND 1998 IN VIETNAM

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Abstract. Shigellosis is an important cause of infectious diarrhea in Vietnam, caused mainly by *Shigella flexneri*. This study provides for the first time in the international literature, data on the development of antimicrobial resistance in *Shigella* between 1988 to 1998, including data reported to the National Program for Surveillance of Antimicrobial Resistance (NPSAR). Our studies show that about 80% of the *Shigella* strains tested were resistant to ampicillin, chloramphenicol, oxytetracycline, trimethoprim and sulfonamides. This combination of drugs was also the most common antibiogram among multiple-resistant *S. flexneri* (57%). Resistance to tetracyclines, sulfonamides and, in particular trimethoprim ($p<0.001$), increased during the study period. Our findings indicate that tetracyclines and co-trimoxazole (a combination of a sulfonamide and trimethoprim), which are recommended and commonly used drugs for the treatment of shigellosis in Vietnam, may have limited therapeutic effect. In contrast to neighboring countries, low percentages of resistance were found to nalidixic acid and norfloxacin (3-5%) and no resistance was found to ciprofloxacin, indicating that nalidixic acid with its low cost and safety in children could be recommended for the treatment of shigellosis. The NPSAR provides a useful picture of the levels and development of antimicrobial resistance in Vietnam and should receive continued support for further improvement by increasing the number of provinces covered, the numbers of isolates tested from rural areas, and the communication of results to medical practitioners and others prescribing and/or selling antimicrobials.

INTRODUCTION

Shigella is increasingly recognized as an important enteric pathogen with global impact, causing about 164 million episodes of shigellosis each year throughout the world (Kotloff *et al*, 1999). The incidence of shigellosis is highest in developing countries where general standards of living and sanitary conditions are usually poor (Yohannes and Limenih, 1980). Antimicrobial treatment of shigellosis can reduce morbidity, mortality and transmission of the disease. However, the progressive increase of antimicrobial resistance in this enteric pathogen is great of concern.

Over the years, *Shigella* spp in many parts of the world have developed resistance to most antimicrobial agents used for treatment. Sulfonamides, tetracyclines, ampicillin and trimethoprim-sulfamethoxazole (co-trimoxazole) were initially highly efficacious, cheap and available antimicrobials. However, high levels of resistance to these drugs have emerged among *Shigella* spp in many parts of the world (Agarwal *et al*, 1984; Hoge *et al*, 1998; Somsak *et al*, 1991; Hossain *et al*, 1998; Flores *et al*, 1998; Parry *et al*, 1998).

As in other developing countries, shigellosis is an important cause of infectious diarrhea in Vietnam. *Shigella* has, since 1989, been listed for surveillance of antimicrobial resistance in the National Program for Surveillance of Antimicrobial Resistance (NPSAR). Studies have shown that *S. flexneri* is the most common isolated species, accounting for about 60-80 % of all *Shigella* spp isolated from human fecal specimens (Qui, 1998; Isenbarger *et al*, 1999).

The *Program of Antibiotic Sensitivity Testing Study* was established in 1988. The Program was funded by the Swedish International Cooperation Agency (SIDA) under the supervision of the Ministry of Health. It was initially conducted mainly in northern Vietnam. In 1992, the program was renamed as the *National Program for Surveillance on Antimicrobial Resistance* (NPSAR) with the aim of covering the entire country. The objective of the NPSAR is to investigate and document the antimicrobial susceptibility of the most common pathogenic bacteria, including *Shigella*.

The objectives of our study were twofold: a) to study the trends of antimicrobial resistance in *Shigella* spp isolated from 1989 to 1998 based on data from the NPSAR; b) to determine the susceptibility of *S. flexneri* strains isolated from diarrheal patients in different geographic areas of Vietnam between 1995 and 1999.

MATERIALS AND METHODS

The National Program for Surveillance of Antimicrobial Resistance (NPSAR) of *Shigella* spp

Data on the antimicrobial susceptibility of *Shigella* were obtained from the NPSAR data collected between 1989 and 1998. Every two years, NPSAR publish antimicrobial susceptibility data collected at 20 district and provincial hospitals participating in NPSAR. All hospitals have trained personnel and are provided with consumables for antimicrobial susceptibility testing according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS), including antimicrobial disks and Mueller Hinton II agar. *Shigella* strains are isolated from human fecal samples by internationally accepted standardized methods and generally present as single cases at hospital outpatient departments. However, strains may only be characterized to the genus level. About 10 % of the strains tested are sent for quality control by bacterial identification and antimicrobial susceptibility testing at the regional centers located in northern, central and southern Vietnam. Dispite being a national program, NPSAR received data from only some 15 of 61 provinces in the country; furthermore, the microbiology laboratories may not always have had available all of the different antimicrobial disks listed in Table 1.

Table 1 shows the numbers of strains tested and the percentages of *Shigella* strains each year from 1989 to 1998; resistance to a total of 14 antimicrobials is evident. The majority of the strains in Table 1 are *S. flexneri*. However, as the species of some strains were not determined, all strains were designated as *Shigella* spp. According to the NCCLS guidelines, strains in NPSAR are categorized as being resistant of intermediate susceptibility, or sensitive. Only strains defined as resistant were included in Table 1.

Collection of *Shigella flexneri* strains

As *S. flexneri* is the most commonly isolated species in Vietnam, a total of 150 *S. flexneri* strains were selected for susceptibility testing. Strains were isolated between 1995 and 1999 from sporadic cases of diarrhea in different geographic areas of Vietnam, including 100 strains from northern, 20 strains from southern and 30 strains from central Vietnam. Of the 100 strains isolated in the north, 95 were from a cohort study conducted in one district from 1997 to 1999 by the National Institute of Hygiene and Epidemiology (NIHE), Hanoi. Isolates were identified by biochemical tests at the NIHE and serotyped with full sets of antisera (Denka Seiken Co Ltd, Tokyo, Japan).

Antimicrobial susceptibility testing

Each of the 150 *S. flexneri* strains was tested for antimicrobial susceptibility by the disk diffusion method according to the instructions of the disk manufacturer (Oxoid Ltd, United Kingdom) and the NCCLS guidelines, (NCCLS, 2000). The following antimicrobial agents were used (disk concentrations and ab-

breviations shown in brackets): ampicillin (10 μg; AMP); chloramphenicol (30 μg; CHL); sulfonamides (300 mg; SUL); oxytetracycline (30 μg; OTE), trimethoprim (5 μg; TMP), ciprofloxacin (5 μg; CIP); nalidixic acid (30 μg; NAL); gentamicin (10 μg; GEN); furazolidone (50 μg; FUR) and erythromycin (15 μg; ERY).

Statistical analyses

The program of Statistical Products for the Social Services (SPSS 10.0) was used for the data analyses. The probability that the percentages of antimicrobial resistance in different study periods were significant different was calculated by the chi-square test.

RESULTS

Development of antimicrobial resistance in *Shigella* spp from 1989 to 1998

A total of 14 antimicrobial agents, including 6 different classes of antimicrobials were used for testing in the NPSAR. However, susceptibility testing for some antimicrobials, *eg* sulfisomidin, cephalothin, amikacin, was conducted for only parts of the period (Table 1). Data on resistance to ampicillin, chloramphenicol, sulfisomidin, co-trimoxazole, tetracycline and gentamicin were available for all the 10 years. *Shigella* spp showed high mean percentages of resistance to ampicillin (87%), chloramphenicol (82%), tetracycline/doxycycline (87%/90%), co-trimoxaxole (78%), and sulfisomidin (94%) through out the study period. Resistance to co-trimoxazole increased significantly from 25% in 1989, to 58% in 1990, and to 94% in 1994 (χ^2, $p<0.001$) and remained high from 1994 to 1998 (84-91%) (Fig 1).

Nalidixic acid- and norfloxacin-resistant *Shigella* spp were first recognized in 1993 (Table 1). However, resistance to nalidixic acid has since remained low - 3 to 5% annually - and only 3% of strains were found to be resistant to norfloxacin in 1994 and 1998. No resistance was found to ciprofloxacin in the period studied from 1996 to 1998.

The percentages of resistances to the antimicrobials of the beta-lactam group varied between 17 and 49% for cephalothin between 1990 to 1995 (Table 1). Ceftriaxone resistance was not found among the 55 strains tested in 1995; however 7% and 9% of strains were resistant in 1997 and 1998 respectively.

Among the three aminoglycosides tested, resistance to gentamicin varied from 2 to 17%, with the highest percentages reported in the most recent study period (Table 1). The mean percentages of resistance to tobramycin and amikacin were 22% and 10% respectively during the period studied from 1994 to 1998.

Unfortunately, antimicrobial resistance patterns, including common multiple resistance patterns, could not be obtained from the NPSAR as data for single strains were not reported.

Antimicrobial susceptibility of *S. flexneri*

The *S. flexneri* isolates showed high mean percentages of resistance to ampicillin (79%), chloramphenicol (79%), oxytetracycline (83%), trimethoprim (78%) and sulfonamides (80%) (Fig 2). Only 2 strains isolated in 1996 and 1997 in southern and northern Vietnam were found to be resistant to nalidixic acid. No isolates were resistant to ciprofloxacin. Only one strain isolated in 1998 showed resistance to gentamicin. The susceptibility of *S. flexneri* confirms the antimicrobial susceptibility of *Shigella* spp as reported to the NPSAR (Table 1).

The most common antimicrobial susceptibility pattern of *S. flexneri* included resistance to ampicillin, chloramphenicol, oxytetracycline, trimethoprim and sulfonamides (57%). Resistance to 6 antimicrobials was shown by only one strain isolated in 1998 in northern Vietnam. Eleven strains (8%) were sensitive to all antimicrobials tested. Strains from northern Vietnam collected in the cohort study showed patterns and levels of resistance similar to those of the *S. flexneri* strains from other

Table 1
The numbers of strains tested and percentages of *Shigella* spp. found resistant to 14 antimicrobials as reported from 1989 to 1998 to the National Program on Surveillance of Antimicrobial Resistance (NPSAR).

Antimicrobial agents	Year of isolation									
	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Ampicillin										
n[a]	79	239	161	154	456	519	420	363	127	149
R[a] (%)	77	75	76	89	95	89	89	84	93	89
Chloramphenicol										
n	81	251	170	194	495	579	468	370	143	215
R (%)	79	74	77	84	89	77	76	85	86	85
Sulfisomidin										
n			129		124		12			
R (%)			97		89		100			
Co-trimoxazole										
n	79	243	165	184	484	575	466	361	143	213
R (%)	25	58	73	79	92	94	91	90	85	84
Tetracycline										
n	82	154	72		113		138	35	33	18
R (%)	83	88	93		92		83	89	85	83
Doxycline										
n						62		267	78	61
R (%)						98		88	94	85
Cephalothin										
n		16			32	161	212			
R (%)		19			41	17	18			
Tobramycin										
n						58	38	32	28	32
R (%)						24	34	16	22	13
Gentamycin										
n	84	232	148	126	158	260	255	110	32	41
R (%)	4	4	5	6	14	2	2	13	9	17
Amikacin										
n						26	56	79	30	38
R (%)						0	9	17	10	11
Ceftriaxone										
n							55		31	39
R (%)							0		7	9
Nalidixic acid										
n			5	21	234	329	347	215	60	157
R (%)			0	0	3	3	3	4	3	6
Norfloxacine										
n			15	35	95	127	55	337	141	141
R (%)			0	0	2	3	0	0	0	3
Ciprofloxacine										
n								22	33	58
R (%)								0	0	0

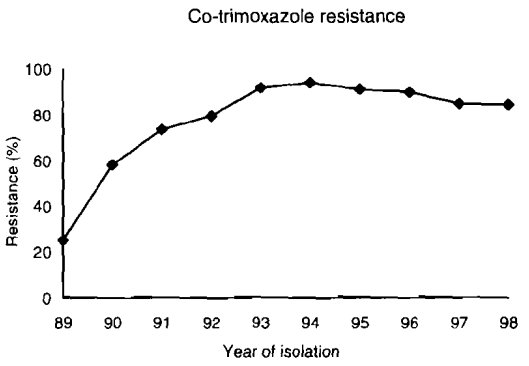

Fig 1–Trend of co-trimoxazole resistance in *shigella* isolated in Vietnam from 1989 and 1998.

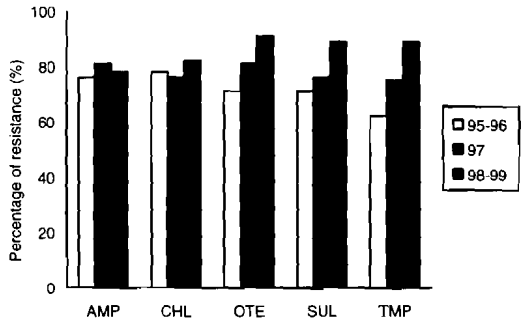

Fig 2–The development of antimicrobial resistance in *Shigella flexneri* isolated in 1995-1996; 1997 and 1998-1999.

parts of the country and to those *Shigella* spp included in the NPSAR.

The development of *S. flexneri* resistant to five selected antimicrobials is shown in Fig 2 for three groups of strains including 23 strains from 1995-1996; 68 strains from 1997; and 54 strains from 1998-1999. The percentage of *S. flexneri* resistant to ampicillin and chloramphenicol remained high (≈80%), whereas resistance to oxytetracycline, sulfonamides, and co-trimoxazole increased throughout the study period (Fig 2). However, only the 14% increased resistance to co-trimoxazole between each study period was significant (χ^2, $p<0.05$). The percentage of *S. flexneri* showing multiple resistance to 4 and 5 antimicrobials increased during the study periods with resistance to 5 antimicrobials increasing from 48% in 1995-1996 to 57% in 1997 and to 60% in 1998-1999 (χ^2, $p<0.05$).

Strains from the cohort study included 51 strains isolated in 1997 and 44 strains isolated from 1998-1999. Resistance to sulfonamides and co-trimoxazole increased significantly between 1997 and 1998-1999 by 20% and 22% respectively (χ^2, $p<0.05$), whereas a 12% increase in oxytetracycline resistance and a 5% increase in chloramphenicol resistance were not significant.

DISCUSSION

Our studies of the antimicrobial susceptibility of *Shigella* spp based on the data as reported to the NPSAR and the collection of *S. flexneri* showed that about 80% of the strains tested were resistant to ampicillin, chloramphenicol, oxytetracycline, sulfonamides, and trimethoprim. This combination of drugs was also the most common antibiogram among multiple-resistant *S. flexneri* (57%), which is the major species causing shigellosis in Vietnam; furthermore, the results showed that resistance to tetracyclines, sulfonamides and, in particular, trimethoprim increased during the study period from 1989 to 1998. Our findings indicate that tetracyclines and co-trimoxazole (trimethoprim-sulfamethoxazole), which are recommended and commonly-used drugs for the treatment of shigellosis in Vietnam, may have limited therapeutic effect. However, it should be noted that despite *in vitro* resistance, a curative effect of co-trimoxazole has been reported in some studies (Flores *et al*, 1998; Song *et al*, 1992). Co-trimoxazole has been extensively used in Vietnam for the treatment of shigellosis since early 1980 when high levels of ampicillin resistance emerged (Song *et al*, 1992; Vinh, 1992).

Based on the common resistance patterns found, we are currently carrying out studies of the genetic mechanisms of resistance in *Shigella* spp, in particular the role of plasmids and class 1 integrons. The 3′ conserved seg-

ment (3'-CS) of class 1 integrons normally contains the *qacEΔ1* and *sul1* genes, which encode resistance to quaternary ammonium compounds and to sulfonamides respectively (Dalsgaard *et al*, 2000). As the majority of *Shigella* spp were resistant to sulfonamides, resistance gene cassettes inserted in class 1 integrons may be important in encoding resistance in *Shigella*.

A low percentage of resistance was found for both nalidixic acid and norfloxacin (3-5%); all strains tested were sensitive to ciprofloxacin. These results suggest that nalidixic acid, a first-generation quinolone, may be used effectively to treat shigellosis in Vietnam. In view of its low cost and its safety in pediatric use, nalidixic acid is preferable to second- and third-generation quinolones (*eg* norfloxacin and ciprofloxacin) which are prohibitively expensive and not safe for use in children (Thisyakorn and Rienprayoon, 1992); furthermore, bacterial strains resistant to the newer quinolones are also resistant to nalidixic acid, whereas strains resistant to nalidixic acid are often not resistant to the newer quinolones. Currently, nalidixic acid is seldomly used to treat shigellosis and other bacterial gastro-intestinal diseases because of its limited availability throughout the country. However, the new β-lactamases (*eg* the cephalosporins) seem effective although the low level of resistant strains should be carefully studied.

The antimicrobial susceptibility patterns found in Vietnam seem similar to those reported from Thailand and elsewhere in southeast Asia: common findings of multiple-resistance to ampicillin, chloramphenicol, oxytetracycline, co-trimoxazole, and sulfonamides. In contrast to other countries in the region, which report relatively high and increasing resistance to nalidixic acid, low level or no resistance was found in Vietnam to nalidixic acid and the flouroquinolones (Lin and Chang, 1992; Parry, 1998; Dutta *et al*, 1998; Chu *et al*, 1998).

Since the establishment of NPSAR in 1992, the major costs of carrying out the surveillance were met by the Swedish International Cooperation Agency (SIDA). In the future, it will be important that the Vietnamese authorities and others continue in their support of NPSAR, including training personnel, mantaining quality control at provincial and national levels, and providing the necessary consumables. To be a truly national program, the number of provinces reporting data to NPSAR should be significantly increased from the current 15 of 61 provinces. It should be noted that data in NPSAR mostly represent the antimicrobial susceptibility of *Shigella* spp isolated from patients in urban areas with access to district or provincial hospitals; little is known about the antimicrobial resistance of the bacterial pathogens that affect the remaining 80% of the populationess – people who live in rural areas. However, as farmers living in rural areas are likely to have access to antimicrobials than people living in urban areas, levels of antimicrobial resistance in rural areas might be expected to be different. In spite of these limitations, the NPSAR seems to provide a reliable picture of antimicrobial resistance among major bacterial pathogens in Vietnam. In the future, increased efforts should be made to disseminate the results of the NPSAR and to make available practical recommendations for antimicrobial treatment for use by practitioners at community, district and provincial level.

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