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Prevalence and Antimicrobial Susceptibility of Serogroup D Nontyphoidal *Salmonella* in a University Hospital in Taiwan

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The incidence of serogroup D *Salmonella* has been increasing in Taiwan. Most of these isolates belonged to *Salmonella enterica* serovar Enteritidis and showed a relatively higher rate of resistance to sulfamethoxazole-trimethoprim than to other antimicrobial agents. The results of molecular experiments indicated that genes responsible for the resistance were located on plasmids. The resistance may occur via horizontal gene transfer. Furthermore, the first identification of ciprofloxacin and ceftriaxone resistance in serogroup D *Salmonella* in our hospital is also than they did to other antimicrobial agents cause for concern.

Nontyphoidal *Salmonella* is recognized as one of the principal causes of foodborne infections worldwide. Among the more than 2,000 *Salmonella* serovars, serovar Enteritidis was one of the top two serovars reported in the United States (6). Most cases of gastroenteritis caused by serovar Enteritidis occur sporadically or as limited outbreaks, but recent reports of large, hospital- and nursing home-associated outbreaks emphasize the importance of serovar Enteritidis infections as a major public health problem (4, 7).

Antimicrobial resistance among nontyphoidal *Salmonella* has been a serious problem worldwide. In the United States, the number of *Salmonella* organisms that were resistant to one or more antimicrobials rose significantly from 16% in the 1980s to 31% in the 1990s (3). A similar situation has been found in the United Kingdom (10). Serovar Enteritidis is more susceptible to available antimicrobial agents than other common *Salmonella* serotypes (3, 10).

In a previous study, Su et al. found that for nontyphoidal serogroup D *Salmonella* isolates (serovar Enteritidis in particular), the level of resistance to ampicillin and chloramphenicol was approximately 10% and to sulfamethoxazole-trimethoprim was approximately 20% (8). To further study the associated mechanism, records of clinical *Salmonella* isolates in the Department of Clinical Pathology of Chang Gung Memorial Hospital (CGMH) (a 3,500-bed university-affiliated teaching hospital) in Taoyuan, Taiwan, obtained between 1997 and 2002 were retrospectively reviewed. Throughout the study period, standard methods were used for the isolation and identification of bacteria and no major changes were made. Antimicrobial susceptibility levels were investigated, and results were defined according to those suggested by the National Committee for Clinical Laboratory Standards (5). The chi-square test was used to determine the significance of differences.

Furthermore, a total of 20 isolates of serogroup D *Salmonella*, including 18 serovar Enteritidis and 2 serovar Dublin isolates, were randomly collected between 2001 and 2002 for molecular analysis. Of the isolates, 15 were from blood, 3 were from feces, 1 was from pus, and the remaining 1 was from sputum. Plasmid profiles were determined by a method described earlier (2). The oligonucleotide primers synthesized according to the published DNA sequences of *spvC*, a conserved gene located on the virulence plasmid of *Salmonella*, were used in PCR to detect the presence of the plasmid (1). The following resistance genes were detected by PCR using methods described earlier (1, 2): *sulI* and *sulII* for sulfamethoxazole resistance and *dfr* for trimethoprim resistance. Moreover, class I integron gene cassettes were detected using PCR with primers derived in previous work (11). PCR products were sequenced using an ABI 377 automatic sequencer (Perkin-Elmer, Applied Biosystems). The search for homologous sequences was done using FASTA software available at the GenBank database website on the Internet. DNA-DNA

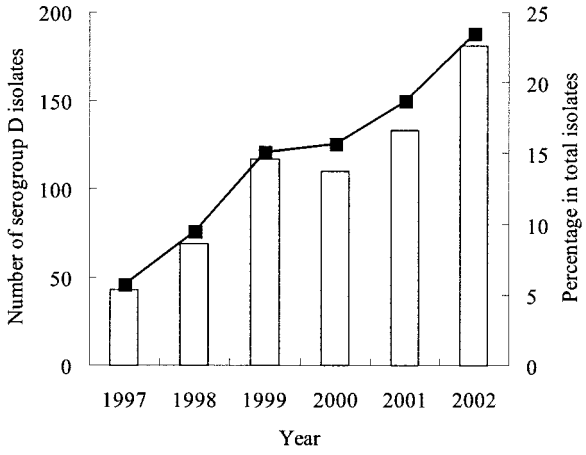

FIG. 1. Prevalence and antimicrobial susceptibility of serogroup D *Salmonella* isolates in CGMH between 1997 and 2002. Annual numbers of isolates obtained (bars) and the annual incidence (per total *Salmonella* isolates) (line) of serogroup D *Salmonella* infections are indicated.

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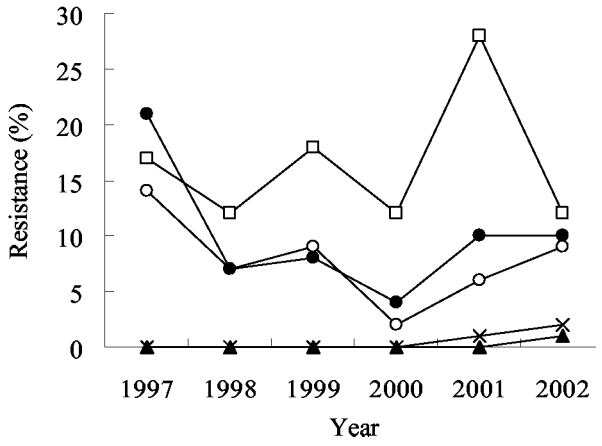

FIG. 2. Secular trends in antimicrobial resistance to ampicillin (○), chloramphenicol (●), sulfamethoxazole-trimethoprim (□), ciprofloxacin (▲), and ceftriaxone (×) in serogroup D *Salmonella* isolates over time.

hybridization was performed by a method described earlier (2) to examine whether the resistance was plasmid mediated.

According to the laboratory records available in CGMH, serogroup D formed the third largest group (after serogroups B and C) among all *Salmonella* isolates. In contrast to serogroups B and C, however, infections caused by serogroup D *Salmonella* have been increasing in recent years; the number of isolates obtained was 43 in 1997, while after 1999 a significant ($P < 0.01$) increase to more than 100 in each year was noted (Fig. 1). Compared to total *Salmonella* isolates, the percentage of serogroup D isolates increased approximately fourfold (from 6 to 24%). Su et al. have confirmed that most serogroup D isolates belonged to serovar Enteritidis (9).

Figure 2 shows the trend of antimicrobial resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole among nontyphoidal serogroup D *Salmonella* isolates over the study period. The average rate of resistance to sulfamethoxazole-trimethoprim was 16.5%, which was higher than that to ampicillin (7.8%) or chloramphenicol (10%) ($P < 0.05$). None of the serogroup D isolates was resistant to ciprofloxacin or ceftriaxone until 2001 to 2002, when one ciprofloxacin-resistant isolate and three ceftriaxone-resistant isolates were first identified.

Analysis of plasmid profiles showed that all 18 of the serovar Enteritidis isolates examined harbored a 60-kb serotype-specific virulence plasmid. Plasmids of other sizes were found in some isolates (Table 1). Of the 18 serovar Enteritidis isolates, 4 were resistant to sulfamethoxazole-trimethoprim (Table 1) and 3 (isolates 336, 338, and 340) carried *dfr* and *sulI* genes, which were responsible for trimethoprim and sulfamethoxazole resistance, respectively. DNA-DNA hybridization showed that both *dfr* and *sulI* were located on a 125-kb plasmid. PCR targeted at the class I integron produced a 1.5-kb amplicon in the three strains that carried the *dfr* and *sulI* genes, and the amplicon was found (by sequencing) to contain the *dfr* and *aadA2* genes that confer resistance to trimethoprim and streptomycin, respectively. DNA-DNA hybridization showed that the integron was located on the 125-kb plasmid as well. One serovar Dublin isolate (isolate 189) and three serovar Enteritidis isolates (isolates 329, 334, and 343) also contained the *dfr* gene. Among these four isolates, three (isolates 189, 334, and 343) remained susceptible to sulfamethoxazole-trimethoprim because a *sul* gene was lacking. Serovar Enteritidis strain 329 was resistant to sulfamethoxazole-trimethoprim, as this strain contained a *sulII* gene along with the *dfr* gene. Another serovar Enteritidis strain (isolate 332) that was susceptible to sulfamethoxazole-trimethoprim also harbored a *sulII* gene but not a *dfr* gene. DNA-DNA hybridization showed that the *sulII* gene was located on a smaller, 40-kb plasmid in the two isolates.

TABLE 1. Characteristics of the 20 isolates of serogroup D *Salmonella* studied

Strain	Serotype	No. of plasmids	Size(s) (kb)	Resistance gene			Class I integron	Resistance to SXT[a]
				sulI	*sulII*	*dfr*		
189	Dublin	1	80	−	−	+	−	−
295	Dublin	1	80	−	−	−	−	−
294	Enteritidis	1	60	−	−	−	−	−
328	Enteritidis	1	60	−	−	−	−	−
329	Enteritidis	4	60, 40, 25, 20	−	+	+	−	+
330	Enteritidis	1	60	−	−	−	−	−
332	Enteritidis	4	60, 40, 25, 20	−	+	−	−	−
333	Enteritidis	2	60, 45	−	−	−	−	−
334	Enteritidis	1	60	−	−	+	−	−
335	Enteritidis	1	60	−	−	−	−	−
336	Enteritidis	2	125, 60	+	−	+	+	+
338	Enteritidis	3	125, 60, 45	+	−	+	+	+
339	Enteritidis	1	60	−	−	−	−	−
340	Enteritidis	2	125, 60	+	−	+	+	+
342	Enteritidis	1	60	−	−	−	−	−
343	Enteritidis	3	125, 60, 45	−	−	+	−	−
346	Enteritidis	2	60, 10	−	−	−	−	−
347	Enteritidis	2	60, 10	−	−	−	−	−
348	Enteritidis	1	60	−	−	−	−	−
350	Enteritidis	1	60	−	−	−	−	−

[a] SXT, sulfamethoxazole-trimethoprim.

The study confirmed the emergence and rapid increase in numbers of nontyphoidal serogroup D *Salmonella* (particularly serovar Enteritidis) infection in Taiwan in the past 6 years. Concurrently, in our hospital the number of serogroup B isolates began to decline gradually after 1995 (8). If this trend continues, the incidence of nontyphoidal serogroup D-induced salmonellosis in Taiwan will soon surpass that of serogroup B infection, as has been the situation in the United States and Europe (6, 7). Serovar Enteritidis is known to be closely associated with layer and broiler flocks, and the infection is generally believed to be derived from chicken and chicken products, including eggs (6, 7). In Taiwan, there was an epidemic of swine mouth-foot disease in 1996 to 1997. This resulted in a decreased consumption of pork and, in turn, an increased consumption of poultry in the community. The results of a recent study have shown that approximately 88% of broiler flocks and 49% of broilers in Taiwan were contaminated with *Salmonella* (H. J. Tsai and C. H. Chou, Abstr. Proc. 4th Int. Symp. Typhoid Fever and Other Salmonellosis, Taipei, Taiwan, abstr. P12, 1999). Popularized Western-style food such as mayonnaise may also be a factor contributing to the increase. From the public health standpoint, such a rapid increase in serovar Enteritidis infection necessitates a more detailed surveillance of the situation and better methods for precise identification of the organism.

Our findings confirm those of earlier studies indicating that most serogroup D strains (serovar Enteritidis in particular) were susceptible to a wide range of antimicrobial agents (3, 4, 10). Nevertheless, a higher rate of resistance to sulfamethoxazole-trimethoprim was observed in Taiwan (8). This study showed that all resistance genes found were located on plasmids. Furthermore, a class I integron carrying a *dfr-aadA2* gene cassette was detected in the resistant isolates. The location of the integron on the plasmid might contribute to horizontal dissemination of the antibiotic resistance gene cassette. In an earlier study (9), a predominant genotype among clinical isolates of serovar Enteritidis was identified by pulsed-field gel electrophoresis, indicating that the emergence of serovar Enteritidis in Taiwan was mainly due to dissemination of clones that were endemic in Taiwan. Taken together, these results provide evidence suggesting that the resistance to sulfamethoxazole-trimethoprim developed due to the acquisition of resistance genes by the preexisting susceptible serovar Enteritidis strains via horizontal gene transfer.

Although most isolates remained susceptible, the first identification of ciprofloxacin and ceftriaxone resistance in serogroup D *Salmonella* isolates in 2001 and 2002 is cause for concern. Active monitoring of serogroup D *Salmonella* for resistance to antimicrobial agents is crucial because of the public health implications derived from the increasing prevalence of such organisms.

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