大韓微生物學會誌: 第21卷 第4號 1986
J. Korea Soc. Microbiol., Vol. 21, No. 4, 1986

Prevalence and Drug Resistance of *Shigella* in Taegu Area of Korea

Do-ki Chun, Jong-Wook Park, Seong-Il Suh, Dong Taek Cho, Sung-Yong Seol[1] and Yoo-Chul Lee[1]

Departments of Microbiology, Keimyung University School of Medicine, and Kyungpook National University School of Medicine[1], Taegu, Korea

= 국문초록 =

대구지방에서 분리된 *Shigella*의 양상과 항균제 내성

계명대학교 의과대학 미생물학교실
경북대학교 의과대학 미생물학교실[1]

전도기 · 박종욱 · 서성일 · 조동택[1] · 설성용[1] · 이유철[1]

1973년부터 1985년 사이에 대구지방에서 분리된 *Shigella*는 약 1,200주였는데, 대부분이 *Shigella flexneri*였고, *S. sonnei*는 약 20%였으며, *S. dysenteriae*와 *S. boydii*는 극히 적었다. 분리균의 95%이상이 chloramphenicol(Cm), tetracycline(Tc), streptomycin(Sm), sulfisomidine(Su), ampicillin(Ap), trimethoprim(Tp) 등의 전부 또는 일부 약제에 내성이었으며, kanamycin, nalidixic acid(Na)와 rifampin(Rf)에 내성인 균주는 소수 있었으나, cephaloridine, gentamicin, amikacin등에 내성인 균주는 없었다. 1973년에 분리된 균은 약 절반이 약제내성이었으나, 1977년 이후 분리된 균은 95% 이상이 약제내성이었다. 1977년 까지는 Cm, Tc, Sm 및 Su의 4제 내성균이 가장 많았으나 1978년 부터는 Cm, Tc, Sm, Su, Ap 및 Tp의 6제 내성균이 가장 많았다. 약 75%의 균이 그 약제내성을 접합에 의하여 *Escherichia coli*에 전달하였으므로 이 약제내성은 R plasmid에 의한 것으로 생각되었다. 대다수의 균에 있어서 약제내성 전부를 *E. coli*에 전달하였으나, Na와 Rf의 내성은 전달되지 않았다. 일부 균주의 약제내성은 *E. coli*에 전달될 때 분리되는 일이 있었으나, 그 수는 극히 적었다. *Shigella*의 R plasmid는 대부분이 비적합성군(incompatibility group) FII에 분류되었으나, 극소수는 B군에 속하였고 군별이 안되는 것도 있었다.

Key Words: Shigella, Drug resistance, Korea.

INTRODUCTION

Asian countries in subtropical and temperate zones have been known for a long time as areas where enteric infections constitute a major medical problem, and shigellosis has been an important epidemic water-borne disease sacrificing many lives of infected patients. Dysentery-like disease was first described in Korea in 13th century, but it was not well differentiated from acute enteritis or simple diarrhoea, and the nature of the infection was not determined for a long time.

Dysentery has been studied with its infective nature from the beginning of the 20th century in Korea, and known as one of the most prevalent epidemic water-borne infections. The major cause of the wide spread incidence of dysentery and other water-borne infections has been considered to be originated from poor sanitary conditions; such as poor running water and sewage disposal systems, use of well water for drinking, and the lack of knowledge in preventive medicine for the control of enteric infections.

* This study was partly supported by the Han-Kok Research grant, and aided by the technical assistance of Sung Duck Paik.

The establishment of the international method of classification of *Shigella* (14, 17, 18) brought about the marked development in bacteriology and epidemiology of shigellosis, and much detailed information has appeared on the distribution of species and serotypes in various parts of the world. Even though the gradual improvement of sanitary conditions and the introduction of effective antimicrobial drugs resulted in the marked decrease of large epidemic in Korea in recent decades, sporadic incidences of shigellosis have frequently been encountered, endemic in nature especially among children.

Shigellosis has not been considered a serious infection in Korea and many patients were frequently treated at home with commercially available antimicrobial drugs without physician's prescriptions (5, 8). Thus, only small numbers of severe patients were subjected for the bacteriological study in hospitals. Therefore, isolated *Shigella* strains were very small in numbers as compared with the real number of patients, and it is not easy to know the actual detailed figures for the incidence of shigellosis, the distribution of species, and the drug resistance *Shigella* in Korea.

The emergence of microorganisms resistant to antimicrobial drugs has been an important problem in clinical medicine. When sulfonamide was introduced, the drug was found to be fairly effective against infections with many pathogenic organisms including *Shigella*, and a large amount of sulfonamide was extensively used for the treatment of bacillary dysentery. However, the effectiveness of this drug lasted for only several years as the result of the rapid emergence of *Shigella* strains resistant to this drug (21, 23). Thereafter, chloramphenicol (Cm), tetracycline (Tc), streptomycin (Sm), and other drugs effective for *Shigella* were successively introduced. Then, organisms singly or multiply resistant to these drugs also emerged rapidly as they were widely used, and the isolation of strains multiply resistant to these drugs increased by years (22, 28). The multiplicity of drug resistance frequently noted in *Shigella* in the 1960's was quadruple resistance to Cm, Tc, Sm, and sulfonamide (22, 23). Multiple drug resistance was in most cases found to be transferred from *Shigella* to *Escherichia coli* and other organisms by mixed cultivation, and it was proved to be mediated by extrachromosomal elements; plasmids (1, 11, 22, 31).

This report summarizes the results previously observed in our laboratories on the isolation, species distribution, and drug resistance of *Shigella*, and adds the recent findings of our study on strains isolated in the Taegu area.

MATERIALS AND METHODS

Isolation of *Shigella*

The organisms were isolated in the laboratories of Kyungpook National University School of Medicine and Keimyung University School of Medicine. Stool specimens were collected from patients suspected of enteric infections and cultured on MacConkey or eosin methylene blue agar plates. Colonies suspected to be *Shigella* on these media were picked, purified, and confirmed biochemically and immunologically by the method described by Edwards and Ewing (13). Isolated strains were maintained in rubber-stoppered soft nutrient agar stabs, and typical colonies were selected on MacConkey agar plates before use. One strain represents one sporadic incidence or one epidemic. Antibiotic-resistant strains were maintained on nutrient agar containing Tc.

Antimicrobial susceptibility tests

Freshly prepared solutions of Cm, Tc, Sm, sulfisomidine (Su), ampicillin (Ap), kanamycin (Km), gentamicin (Gm), amikacin (Ak), cephaloridine (Cr), trimethoprim (Tp), nalidixic acid (Na), and rifampin (Rf) were used, and the agar dilution method was applied throughout the study using Steers multiple inoculator (29). Overnight cultures of test strains in brain heart infusion (BHI) broth were diluted 100 fold with buffered saline and inoculated on Mueller-Hinton (MH) agar plates containing serially diluted drugs. After overnight incubation at 37°C, the breakpoint of drug resistance was determined as described by the National Committee for Clinical Laboratory Stan-

dards (24).

Detection of conjugative R plasmids

R plasmids were detected by the procedure described by Chun et al. (9) using Na-resistant *E. coli* ML1410 (19) and Rf-resistant RG488 (16) as recipients. Single colonies of donor and recipient strains were cultured overnight in BHI broth. One drop of each culture was inoculated in 5 ml of BHI broth and incubated at 37°C for 3.5 h with gentle shaking. One milliliter of donor and four ml of recipient cultures were mixed and conjugated for 18 h at 36°C, and then spread on selective MH or BHI agar plates containing 50ug/ml of Na or Rf and one of the drugs to which the donor strain was resistant. After incubation at 37°C for 24h, plates were inspected for colonies of resistant *E. coli*. Five to ten colonies were picked, purified on MacConkey agar plates, confirmed to be *E. coli*, and tested for the resistance patterns.

Incompatibility test

The incompatibility group was determined by colony test (12, 28, 30) with standard R plasmids previously used (7). *E. coli* carrying R plasmids transferred from *Shigella* was mated to *E. coli* carrying standard R plasmids of each incompatibility group whose resistance markers were distinguishable from those of the donor. Ten transconjugant clones obtained on selective media were picked and purified by successive single colony isolation and tested, by double ditch plates, for the presence of incoming and resident plasmids. When resident plasmid was eliminated from all

Table 1. Species distribution and drug resistance of *Shigella* isolated in years from 1973 to 1979

Species	No. of strains isolated in:							
	1973	1974	1975	1976	1977	1978	1979	Total (%)
S. dysenteriae	1	3	2	3	0	0	1	10 (3)
S. flexneri	2	10	17	11	7	79	153	279 (80)
S. boydii	0	0	0	1	0	0	0	1
S. sonnei	5	8	10	5	14	16	1	59 (17)
Total	8	21	29	20	21	95	155	349 (100)
Resistant to one or more drugs (%)	4	15	25	15	20	93	151	323
	(50)	(71)	(86)	(75)	(95)	(98)	(97)	(93)
Susceptible to all drugs tested	4	6	4	5	1	2	4	26

Table 2. Patterns and conjugal transfer of drug resistance of *Shigella* (1973-1977 isolates)

Resistance pattern	No. of strains	No. of strains transferred resistance (%)	Pattern of resistance transferred
CmTcSmSuAp[a]	5	5 (100)	CmTcSmSuAp
CmTcSmSu	46	4 (96)	CmTcSmSu
CmSmSuAp	3	3 (100)	CmSmSuAp
CmSmSu	8	3 (38)	CmSmSu
TcSmSu	5	2 (40)	TcSmSu
SmSu	6	1 (17)	SmSu
Tc	2	0	
Su	4	1 (25)	Su
Total (%)	79	59 (75)	

Abbreviation of drugs, see text.

transconjugant clones, the cross was mated in the opposite direction. Elimination of the resident plasmid in both directions was taken as the evidence that two plasmids belonged to the same incompatibility group. When the resident plasmid was eliminated from most but not all of the tested transconjugants, the apparent doubles were tested for stability. After overnight growth in drug-free media, they were inoculated on agar plates and 20 colonies were tested for the continued presence of markers of each plasmid. If the doubles were stable, they were used as donors to other strains of *E. coli*, each R plasmid being selected separately in transconjugants. If one or both R plasmids were separately transferred from the doubles, the two were recorded as being compatible. When both plasmids were always transferred simultaneously, they were considered as the recombinant of both plasmids.

RESULTS

Shigella strains isolated during the years of 1973 to 1979 are shown in Table 1. Only small numbers of strains were isolated in 1973 through 1977, with increased numbers of isolation in 1978 and 1979. Eighty percent of them was classified into *S. flexneri* and only 17% was *S. sonnei*. *S. dysenteriae* and *S. boydii* were isolated only occasionally.

Drug resistance patterns and the conjugal transfer of resistance were studied on strains isolated in 1973 through 1977 (Table 2). Among 99 strains, 79 (80%) were resistant to one or more drugs, and the majority of them resistant to two or more drugs. The most frequently found pattern was 46 (58%) strains resistant to four drugs (Cm, Tc, Sm, and Su), and the others were 8 or less of the other combinations of resistant drugs. Seventy-five percent of strains transferred their resistance to *E. coli* by conjugation, and the transferred patterns of resistance were the same with the original patterns.

Strains isolated in 1978 and 1979 were tested for

Table 3. Patterns and conjugal transfer of drug resistance of *Shigella* (1978-1979 isolates)

Resistance pattern	No.of strains	No. of strains transferred resistance (%)	Pattern of resistance transferred
CmTcSmSuApTpKm	1	1 (100)	CmTcSmSuApTpKm
CmTcSmSuApTpNa	39	36 (92)	CmTcSmSuApTp
CmTcSmSuApTp	162	119 (74)	CmTcSmSuApTp
CmTcSmSuTp	12	11 (92)	CmTcSmSuTp
CmTcSmSuAp	1	1 (100)	CmTcSmSuAp
CmTcSmSu	25	20 (80)	CmTcSmSu
CmTcSmAp	3	0	
Su	1	0	
Total (%)	244	188 (77)	

Table 4. Species distribution and drug resistance of *Shigella* isolated in years from 1980 to 1985

Species	No. of strains isolated in:						
	1980	1981	1982	1983	1984	1985	Total
S. dysenteriae	2	0	2	0	1	0	5 (0.6)
S. flexneri	255	135	71	89	65	41	656 (79.3)
S. boydii	1	0	0	0	0	1	2 (0.2)
S. sonnei	36	30	26	39	25	8	164 (19.8)
Total	294	165	99	128	91	50	827
Resistant to one or more drugs (%)	284 (97)	161 (98)	96 (97)	122 (95)	89 (98)	50 (100)	802 (97)
Susceptible to all drugs tested	10	4	3	6	2	0	25

Table 5. Patterns and conjugal transfer of drug resistance of *Shigella* (1980-1981 isolates)

Resistance pattern	No. of strains	No. of strains transferred resistance (%)	Pattern of transferred resistance	Incompatibility group[a]
CmTcSmSuApTpNaRf	5	NT[b]		
CmTcSmSuApTpNa	40	15 (38)	CmTcSmSuApTp	FII
CmTcSmSuApTpRf	5	5 (100)	CmTcSmSuApTp	FII
CmTcSmSuApTp	271	236 (87)	CmTcSmSuApTp	FII
CmTcSmSuTpKm	13	13 (100)	CmTcSmSuTpKm	FII
CmTcSmSuTpRf	6	6 (100)	CmTcSmSuTp	FII
CmTcSmSuTpNa	1	0		
CmSmSuApTpRf	1	1 (100)	CmSmSuApTp	FII
CmTcSmSuAp	7	5 (71)	CmTcSmSuAp	FII
CmTcSmSuTp	72	33 (46)	CmTcSmSuTp	FII
CmSmSuApTp	1	0		
CmTcSuApTp	2	2 (100)	CmTcSuApTp	FII
CmTcSmSu	5	2 (40)	CmTcSmSu	B
		1 (20)	CmTcSmSu	FII
CmTcSmAp	8	2 (25)	CmTcSmAp	UC[c]
CmTcSuTp	2	1 (50)	CmTcSuTp	FII
TcSmSu	5	0		
CmTcAp	1	0		
Total	445	322 (73)[d]		

[a] Some plasmids selected at random were subjected for incompatibility.
[b] Not tested without having appropriate recipient strain.
[c] Unclassified.
[d] Percentage of 440 strains tested.

resistance patterns and the transferability of drug resistance (Table 3). Among 244 drug-resistant strains, approximately two-thirds were resistant to six drugs (Cm, Tc, Sm, Su, Ap, and Tp), followed by 39 strains resistant to 7 drugs (Cm, Tc, Sm, Su, Ap. Tp, and Na). Strains resistant to four drugs (Cm, Tc, Sm, and Su) which were the most frequent pattern among strains of 1973 to 1977, have since decreased to only 25 occupying the third place. The complete patterns of resistance to drugs except Na were almost always co-transferred to *E. coli* in 77% of drug-resistant strains, when recipient strains were selected with any of the drugs except Na. The resistance to Na was never transferred to *E. coli* by conjugation.

Strains isolated during the years of 1980 to 1985 are listed in Table 4. With the highest number of isolation in 1980, the number of strains thereafter showed a tendency of gradual decrease by years, with only 50 strains in 1985. The most prevalent species in this period was also *S. flexneri* occupying 79% of total strains, and the next frequent species was *S. sonnei*. The other two species were only rarely isolated. Eighty hundred and two strains among 827 were resistant to one or more drugs tested, and only 25 were susceptible to all drugs tested.

All drug-resistant strains among 1980 and 1981 isolates were multiply resistant to three or more drugs with 17 resistance patterns (Table 5). The most frequent pattern was R type (Cm Tc Sm Su Ap Tp) with 271 (61%) strains among 445 drug-resistant strains. The next most frequent was R type (Cm Tc Sm Su Tp) with 72 (16%) strains, followed by R type (Cm Tc Sm Su Ap Tp Na) (9%). The other patterns of resistance were 13 strains or less. Four hundred and forty drug-resistant strains except five strains which were resistant to both Na and Rf and other drugs, were tested for the transmissibility of the resistance to *E. coli* by conjugation, and the resistance of 322 (73%) strains was found to be conjugative. However, autotransferability was very poor in R types (Cm Tc Sm Su Ap Tp Na) and (Cm Tc Sm Su Tp). The complete patterns of original resistance to drugs except Na and Rf were almost

Table 6. Patterns and conjugal transfer of drug resistance of *Shigella* (1982-1983 isolates)

Resistance pattern	No. of strains	No. of strains transferred resistance (%)	Pattern of transferred resistance	Incompatibility group
CmTcSmSuApTpKmNa	2	1 (50)	CmTcSmSuApTp	FII
			SmSuTpKm	UC
			Km	UC
CmTcSmSuApTpKm	7	7 (100)	CmTcSmSuApTpKm	FII or NC
			CmTcSmSuApTp	FII
			ApKm	UC
			Km	UC
CmTcSmSuApTpNa	3	2 (67)	CmTcSmSuApTp	FII
			CmTcSmSuAp	FII
			TcSmSu	UC
CmTcSmSuApTpRf	6	6 (100)	CmTcSmSuApTp	FII
CmTcSmSuApTp	106	90 (85)	CmTcSmSuApTp	FII
			CmTcSmSuTp	FII
			SmSuApTp	UC
CmTcSmSuTpNa	1	1 (100)	CmTcSmSuTp	FII
CmTcSmSuTpRf	2	1 (50)	CmTcSmSuTp	FII
CmTcSmSuAp	5	2 (40)	CmTcSmSuAp	FII
CmTcSmSuTp	50	22 (44)	CmTcSmSuTp	FII
CmTcSmApTp	1	0		
CmSmSuApTp	1	0		
CmTcSmSu	5	4 (80)	CmTcSmSu	B
CmTcSmAp	24	2 (8)	CmTcSmAp	UC
CmTcSuTp	2	1 (50)	CmTcSuTp	FII
CmTcSu	2	2 (100)	CmTcSu	B
TcSmSu	1	0		
Total (%)	218	141 (65)		

always co-transferred, when recipients were selected with any of the drugs except Na and Rf. The resistance to Na and Rf was never transferred to *E. coli* by conjugation.

The incompatibility of R plasmids was tested on randomly selected limited numbers of R plasmids having different patterns of resistance.

The majority of plasmids tested were classified into *Inc.* FII, but there were two plasmids showing different results. Among three plasmids having markers of Cm, Tc, Sm, and Su, two were classified into *Inc* B and one into FII. Two plasmids having markers of Cm, Tc, Sm, and Ap were not classified with our standard plasmids used, since they were compatible with FII, B, and other plasmids.

Table 6 shows the results obtained on strains isolated in 1982 and 1983. Sixteen patterns of drug resistance were noted. The most frequent pattern was that conferring resistance to Cm, Tc, Sm, Su, Ap, and Tp with 106 (49%) among 218 drug-resistant strains. The next most frequent was the pattern with markers of Cm, Tc, Sm, Su, and Tp with 50 strains, and then pattern of Cm, Tc, Sm, and Ap followed. One hundred and forty-one (65%) strains transferred their resistance to *E. coli* by conjugation.

However, as observed in Table 6, the transferability of resistance patterns varied by the patterns with different markers. In contrast to the previous results, the transferred resistance of some R plasmids with various makers showed some different patterns. In most cases, the transferred patterns were the same as in the original patterns, and they were classified into *Inc* FII. The different patterns of transferred resistance were very rare in numbers and they were mostly unclassified. The different patterns of transferred resistance were most frequently encountered in R plasimids having the Km marker.

Table 7. Patterns and conjugal transfer of drug resistance of *Shigella* (1984-1985 isolates)

Resistance pattern	No. of strains	No. of strains transferred resistance (%)	Pattern of transferred resistance	Incompatibility group
CmTcSmSuApTpNaRf	1	NT		
CmTcSmSuApTpKmNa	1	1 (100)	CmTcSmSuApTp	FII
			CmTcSmSuKm	UC
CmTcSmSuApTpKm	5	5 (100)	TcSmSuApTpKm	UC
			SmSuApTpKm	UC
CmTcSmSuApTpNa	20	20 (100)	CmTcSmSuApTp	FII
			CmTcSmSuAp	FII
			TcSmSu	UC
CmTcSmSuApTp	25	20 (80)	CmTcSmSuApTp	FII
			CmSmSuApTp	FII
			CmTcSmSuTp	FII
			TcSmSuApTp	FII
			SmSuApTp	UC
CmTcSmSuApNa	1	1 (100)	CmTcSmSuAp	UC
CmTcSmSuTpNa	2	2 (100)	CmTcSmSuTp	FII
CmTcSmSuTpRf	1	1 (100)	CmTcSmSuTp	FII
CmTcSmSuAp	21	4 (19)	CmTcSmSuAp	FII
CmTcSmSuTp	25	19 (76)	CmTcSmSuTp	FII
CmTcSmSuNa	1	1 (100)	CmTcSmSu	FII
CmTcSmApRf	1	0		
CmTcSmAp	27	0		
CmSmSuTp	1	1 (100)	CmSmSuTp	FII
CmTcSu	5	0		
TcSmAp	1	0		
Su	1	0		
Total (%)	139	75 (54)		

Table 8. Frequency of resistance to drugs of *Shigella* in years from 1977 to 1981

Drug	No. (%) of resistant strains				
	1977 (21)[a]	1978 (95)	1979 (155)	1980 (296)	1981 (166)
Chloramphenicol	20 (95)	94 (99)	151 (97)	280 (95)	162 (98)
Tetracycline	20 (95)	94 (99)	151 (97)	285 (96)	159 (96)
Streptomycin	20 (95)	94 (99)	151 (97)	285 (96)	156 (94)
Sulfisomidine	20 (95)	94 (99)	152 (98)	286 (97)	161 (97)
Ampicillin	0	70 (74)	138 (89)	228 (77)	120 (72)
Trimethoprim	0	80 (84)	136 (88)	264 (89)	157 (95)
Kanamycin	0	1 (1)	0	10 (3)	4 (2)
Nalidixic acid	0	22 (23)	19 (12)	34 (12)	12 (7)
Rifampin	0	0	0	0	17 (10)
Susceptible to all drugs tested	1	1	3	10	4

[a] Number of strains isolated in the year.

Results obtained on strains isolated in 1984 and 1985 are shown in Table 7. Among 17 patterns of resistance, five patterns included 20 or more strains, and strains having other resistance patterns were five or less. Seventy-five (54%) of the strains among 139 drug-resistant ones transferred their resistance to *E. coli* by conjugation. Distribution of transferred patterns of resistance was almost the same as observed in Table 6. Most of them have the same patterns of resistance except Na and Rf to the original patterns and were classified into *Inc* FII. However, in some cases of

Table 9. Number of *Shigella* strains resistant to drugs (1982-1985)

Drug	No. of drug-resistant strains									
	S. flexneri					*S. sonnei*				
	1982 (71)[a]	1983 (89)	1984 (65)	1985 (41)	Total (%) (266)	1982 (26)	1983 (39)	1984 (25)	1985 (8)	Total (%) (98)
Chloramphenicol	69	83	64	41	257 (97)	26	38	24	7	95 (97)
Tetracycline	69	83	64	41	257 (97)	26	38	23	6	93 (95)
Streptomycin	69	84	63	25	241 (91)	24	38	24	6	92 (94)
Sulfisomidine	65	65	47	30	207 (78)	26	37	25	7	95 (97)
Ampicillin	68	82	64	40	254 (96)	0	5	1	0	6 (6)
Trimethoprim	65	56	30	21	172 (65)	26	32	23	7	88 (90)
Kanamycin	4	1	2	4	11 (4)	0	4	0	0	4 (4)
Nalidixic acid	2	3	2	11	18 (7)	1	0	1	2	4 (4)
Rifampin	4	2	1	1	8 (3)	2	0	1	0	3 (3)

[a] Number of strains isolated in the year in parentheses.

strains conferring resistance to Cm, Tc, Sm, Su, Ap, and Tp with or without resistance to Km, Na, or Rf, the transferred resistance showed some segregated patterns and most of these patterns were not classified into known incompatibility groups with our standard plasmids.

Table 8 shows the frequency of resistance to each drug of strains isolated in the years from 1977 to 1981. Strains resistant to Cm, Tc, Sm, and Su were more than 95% from 1977, and these results were supposed to be true even before 1977, as suggested in Table 2. No strain resistant to Ap and Tp was noted among strains isolated in 1977, but the number of strains resistant to these two drugs appeared in high proportion of 74 and 84% and thereafter continued the similar levels. Na-resistant strains appeared from 1978, but the number of strains was around 10% except the isolates of 1978. Strains resistant to Km and Rf appeared only rarely, and no strain resistant to Gm, Ak, and Cr was isolated during the period of study.

Frequency of resistance to each drug in *S. flexneri* and *S. sonnei* among strains from 1982 to 1985 was compared (Table 9). As noted in the preceeding table, strains resistant to Km, Na, and Rf were only occasionally isolated. Strains resistant to Cm, Tc, Sm, Su, and/or Tp were isolated in high proportions in both *S. flexneri* and *S. sonnei*. One exceptional finding is strains resistant to Ap in *S. sonnei* which were only rarely isolated, while Ap-resistant *S. flexneri* was found in high rates.

DISCUSSION

Large epidemics and sporadic incidences of shigellosis were reported frequently in Korea, but only few reports were available on detailed bacteriological and epidemiological studies until 1950's. Durig the Korean war, large numbers of *Shigella* were isolated in the U.N. Forces laboratories from U.N. Forces personnel, refugees, prisoners of war, and others (5). The most prevalent species was *S. flexneri* occupying 91% of total strains, with the most prevalent serotype 4, and other species were rarely isolated. Thereafter, some reports on the isolation of *Shigella* appeared from various parts of Korea (5, 6). However, the number of isolated strains was very small and it was difficult to know the detailed distribution of species in Korea. At that time, the resistance to antimicrobial drugs was not well studied.

We collected *Shigella* strains in the Taegu city area from the 1970's and studied systematically. *S. flexneri* occupied the majority of strains, and *S. sonnei* was less than 20%, while the latter species was overwhelming in western countries at that time (2). We noted the increased isolation of *Shigella* strains from 1978 to 1984 than before 1978. Since laboratories engaged in the isolation were the same throughout the study, the increased isolation of strains may reflect the increased incidence of shigellosis in this period. A tendency of decreased isolation was noted from 1985, sug-

gesting the decreased incidence of shigellosis.

Strains resistant to drugs showed a tendancy to increase by years from 50% in 1973 to more than 95% after 1977. A majority of strains were resistant to four drugs (Cm, Tc, Sm, and Su) until 1977, with very few strains of the other combination of multiple resistance, but strains multiply resistant to six drugs (Cm, Tc, Sm, Su, Ap, and Tp)with or without combinations of Na and Rf occupied more than 80% of strains from 1978, with the marked decrease of strains resistant to four drugs (Cm, Tc, Sm, and Su). We observed the high incidence of Ap- and Tp-resistant strains from 1978 (10, 28), and this caused the marked increase of strains resistant to six drugs. These results also suggest that shigellae can easily become resistant to some newly introduced drugs, such as Ap and Tp. In western countries, Tp was reported effective for the treatment of shigellosis (3, 27), but we noted the high incidence of Tp-resistant strains from 1978 (10). One interesting finding is the very small number of Ap-resistant strains in *S. sonnei,* as compared to the high incidence in *S. flexneri.* This is a contrast to the reports of Neu et al. (25) and Lerman et al. (20) who noted the high incidence of Ap-resistant *S. sonnei. Shigella* was susceptible to aminoglycosides with the rare occurrence of Km-resistant strains. They were also susceptible to Cr, and shigellosis can be treated with these drugs. Na and Rf can also be used.

Resistance to drugs in most *Shigella* strains can be transferred to *E. coli* by conjugation and the resistance was considered to be mediated by R plasmids (10, 28). Olarte et al. (26) noted two R plasmids in *S. dysenteriae* 1 isolated in Mexico; one was responsible for resistance to Cm, Tc, Sm, and Su, and the other caused resistance to Ap, but the resistance to Ap and Tp and other drugs was almost always co-transferred in our strains. This result suggests that the linkage of resistance genes in *S. dysenteriae* in Mexico differs from our strains of *S. flexneri* and *S. sonnei*, and shows the close linkage of genes governing resistance to Ap and Tp, and other drugs in our strains. The difficulty of obtaining segregants lacking one or more R plasmid markers by the method of Bochner and Ames (4) also suggests the close linkage of genes governing resistance in our strains (7). However, in some strains among recent isolates, transferred resistance was segregated into several patterns, especially in R plasmids conferring resistance to Km and other drugs. In these cases, the segregated patterns occur rarely (less than (5%), and most segregants differ from the original plasmids in the incompatibility group. For these segregants, further study is needed.

The incompatibility group B plasmids predominate in *Shigella* in Central America, including Mexico, and in the United Kingdom (12, 15), but we identified only small numbers of *Inc B* plasmids having markers of Cm, Tc, Sm, and Su, suggesting that this is rare in Korea. *Inc* FII and related groups are common in Asia, Africa, and other parts of the world (15, 32, 33). The majority of our plasmids in shigellae were classified into *Inc* FII. Our report is the only one in Korea at present, and further study is necessary to know the detailed distribution of incompatibility groups in Korea. We were unable to determine the incompatibility groups of some segregated patterns of resistance in transconjugants with our standard plasmids used.

During the incompatibility studies, we found in most cases numbers of recombinants of both incoming and resident plasmids in recipient cultures. This result may be a cause of the broadening of resistance patterns and the yearly increase of multiply drug-resistant shigellae. The predominant multiplicity of resistance increased from four drugs in 1977 to six drugs after 1977, as observed in this report and the other results (7, 10).

SUMMARY

Shigella strains isloated in the Teagu area during the period from 1973 to 1985 were studied for species distribution, drug resistance, and R plasmids. Approximately 1,200 strains were isolated during this period, and most of them were classified into *Shigella flexneri. S. sonnei* occupied

less than 20%, and *S. dysenteriae* and *S. boydii* were very rarely isolated. More than 95% of them were resistant to one or more of these drugs; chloramphenicol (Cm), tetracycline (Tc), streptomycin (Sm), sulfisomidine (Su), ampicillin (Ap), and trimethoprim (Tp). Strains resistant to kanamycin, nalidixic acid (Na), and rifampin (Rf) were rare, and no strain was resistant to cephaloridine, gentamicin, and amikacin. Approximately half of the isolates were resistant to drugs in 1973, but the rate of resistant strains increased to more than 95% from 1977. Strains resistant to the four drugs (Cm, Tc, Sm, and Su) occupied the majority of resistant strains until 1977, but the most prevalent multiplicity of drug resistance increased to six drugs (Cm, Tc, Sm, Su, Ap, and Tp) from 1978 with the marked increase of Ap- and Tp-resistant strains. Approximately 75% of them transferred resistance to *Escherichia coli* by conjugation, and the resistance was considered to be mediated by R plasmids. Almost all of them transferred the complete patterns of resistance to drugs except Na and Rf. However, among some strains of recent isolates, small numbers of segregants of transferred resistance were observed. The R plasmids in *Shigella* were mostly classified into *Inc* FII, and only small numbers into *Inc* B. Segregants were in most cases unclassified.

REFERENCES

1) Anderson ES: Origin of transferable drug resistance factor in *Enterobacteriaceae*. *Brit. Med. J.* **ii:** 1289-1291, 1965.
2) Aoki Y: *Shigella* spectrum in Japan and surrounding countries: a enlarged review. *Trop. Med.* **11:** 45-56, 1969.
3) Barada FA, Jr., and Guerrant RL: Sulfamethoxazole versus ampicillin treatment of acute invasive diarrhea in adults. *Antimicrob. Agents Chemother.* **17:** 961-964, 1980.
4) Bochner BR, and Ames BN: Positive selection for loss of tetracycline resistance. *J. Bacteriol.* **143:** 926-933, 1980.
5) Chun D: A review of *Salmonella* and *Shigella* in Korea. *Endemic Dis. Bull. Nagasaki Univ.* **6:** 125-138, 1964.
6) Chun D: Classificantion of dysentery bacilli and their prevalence in Korea (in Korean). *J. Korean Med. Assoc.* **13:** 704-710, 1970.
7) Chun D, Cho DT, Seol SY, Suh MH, and Lee YC: R plasmids conferring multiple drug resistance from *Shigella* isolated in Korea. *J. Hyg.* (Camb.) **92:** 153-160, 1984.
8) Chun D, and Seol SY: Drug resistance and R plasmids of *Salmonella* and *Shigella* in Korea. *Trop. Med.* **20:** 123-129, 1979.
9) Chun D, Seol SY, Cho DT, and Tak R: Drug resistance and R plasmids in *Salmonella typhi* in Korea. *Antimicrob. Agents Chemother.* **11:** 209-213, 1977.
10) Chun D, Seol SY, and Suh MH: Transferable resistance to trimethoprim in *Shigella*. *J. Infect. Dis.* **143:** 742, 1981.
11) Datta N, and Kontomichalou P: Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. *Nature* **208:** 239-241, 1965.
12) Datta N, and Olarte J: R factors in strains of *Salmonella typhi* and *Shigella dysenteriae* 1 isolated during epidemics in Mexico: classification by compatibility. *Antimicrob. Agents Chemother.* **5:** 310-317, 1974.
13) Edwards PR, and Ewing WH: Identification of *Enterobacteriaceae*, 3rd ed., Burgess Publ. Co., Minneapolis, Minn., U.S.A., 1972.
14) Ewing WH: *Shigella* nomenclature. *J. Bacteriol.* **57:** 633-638, 1949.
15) Frost JA, and Rowe B: Plasmid-determined antibiotic resistance in *Shigella flexneri* isolated in England and Wales between 1974 and 1978. *J. Hyg.* (Camb.) **90:** 27-32, 1983.
16) Grant RB, Bannatyne RM, and Shapley AJ: Resistance to chloramphenicol and ampicillin of *Salmonella typhimurium* in Ontario, Canada. *J. Infect. Dis.* **134:** 254-261, 1976.
17) International *Enterobacteriaceae* Subcommittee Report, 1950.
18) International *Enterobacteriaceae* Subcommittee Report, 1954.
19) Ishiguro N, Oka C, and Sato G: Isolation of citrate-positive variants of *Escherichia coli* from domestic pigeons, pigs, cattle, and horses. *Appl. Environ. Microbiol.* **36:** 217-222, 1978.

20) Lerman SJ, Waller JM, and Simms DH: Resistance of shigellae to ampicillin and other antibiotics: South Bronx, New York (1971-1972). *J. Pediatrics* **83:** 500-501, 1973.

21) Mitsuhashi S: Transmissible drug resistance factor R. *Gumma J. Med. Sci.* **14:** 169-209, 1965.

22) Mitsuhashi S: Review: the R factor. *J. Infect. Dis.* **119:** 89-100, 1969.

23) Mitsuhashi S, Harada K, Hashimoto H, and Egawa R: Drug resistance of *Shigella* prevalent in Japan. *Jpn. J. Exp. Med.* **31:** 47-52, 1961.

24) National committee for Clinical Laboratory Standards: Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-T. NCCLS, Villanova, Pa., U.S.A., 1983.

25) Neu HC, Cerubin CE, Longo ED, and Winter J: Antimicrobial resistance of *Shigella* isolated in New York City in 1973. *Antimicrob. Agents Chemother.* **7:** 833-835, 1975.

26) Olarte J, Filloy L, and Galindo E: Resistance of *Shigella dysenteriae* type 1 to ampicillin and other antimicrobial agents: strains isolated during a dysentery outbreak in a hospital in Mexico city. *J. Infect. dis.* **133:** 572-575, 1976.

27) Rudoy RC, Nelson JD, and Haltalin KC: In vitro susceptibility of *Shigella* strains to trimethoprim and sulfamethoxazole. *Antimicrob. Agents Chemother.* **5:** 439-443, 1974.

28) Suh MH, Seol SY, Cho DT, and Chun D: Characterizatin of R plasmids and antimicrobial drug resistance of *Shigella* (in Korean). *J. Korean Soc. Chemother.* **2:** 97-114, 1984.

29) Steers E, Flotz EL, and Graves BS: Inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Cemother.* **9:** 307-311, 1959.

30) Uhlin BE, and Noerdstroem K: Plasmid incompatibility and control of replication: copy mutant of the R factor Rl in *Escherichia coli* K-12. *J. Bacteriol.* **124:** 641-649, 1975.

31) Watanabe T, and Fukasawa T: Episome-mediated transfer of drug resistance in *Enterobacteriaceae.* IV. Interaction between resistance factor and F-factor in *Escherichia coli* K-12. *J. Bacteriol.* **83:** 727-735, 1962.

32) Watson CE: Infections drug resistance in *Shigella* in Cape Town. *South Afr. Med. J.* **41:** 728-731, 1967.

33) Yoshikawa M, Nagashima S, and Mitsuhashi S: Genetic distinction of R factors derived from shigellae and salmonellae. *Jpn. J. Microbiol.* **15:** 425-436, 1971.