

DRUG RESISTANCE PATTERN OF SHIGELLA ISOLATED IN TAIWAN DURING 1970-1971

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In 1954 the first strain of shigella resistant to 4-drugs i.e. tetracycline, chloramphenicol, streptomycin and sulfonamide, was reported by Kitamoto⁽¹⁾. Since then the 4-drug resistant strains were more and more frequently isolated. Moreover, triple, double, single, or other type of resistant strains were more frequently encountered every year. This phenomenon of multiple drug resistance has become an increasingly serious problem in the treatment and epidemiology.

In view of lack of recent reports on Taiwan except several earlier one; Yang, et al.⁽¹³⁾ in 1954, Hwang and Hsu⁽¹⁴⁾ in 1961, Hsu⁽¹⁵⁾ in 1961, and Yu, et al.⁽²⁾ in 1962, we undertook the present experiment, in which we studied the serotyping, drug resistance and the relation between the serotype and resistant pattern on about 200 strains isolated from clinical materials and fish market sewage.

MATERIALS AND METHODS

I. Collection of specimens:

A. Clinical material: Rectal swabs or stools taken into buffered glycerol saline solution were collected from diarrheal patients in Provincial Keelung Hospital, Jen-Ai Municipal Hospital, Chung-Hsin Municipal Hospital and Mackay Memorial Hospital from February 1970 to July 1971.

B. Sewage: Taipei Central Market sewage water were collected for bacterial culture from April to October 1970.

II. Bacterial isolation:

A. Clinical Materials were streaked on EMB agar, MacConkey agar and SS agar

(Difco) and also placed in selenite-F broth (Eiken) for enrichment.

B. The fish market sewage water (10 ml.) were concentrated by centrifugation at 3000 rpm. for 15 minutes, the sediment was streaked on EMB agar, MacConkey agar and SS agar and also placed in selenite-F broth for enrichment.

After 24 hr. incubation at 37°C, suspicious colonies were transferred to Kligler Iron agar (Eiken), and the selenite-F broth enrichment was streaked on EMB agar and SS agar for locating the suspicious colonies. The isolated bacteria were identified by biochemical test^(3,4,5,16) and serological test.

III. Serological typing:

The suspicious strains were identified by slide agglutination test with Bacto-Shigella Group antisera and Type antisera (Difco).

IV. Sensitivity test:

Sensitivity test were were performed on Mueller-Hinton agar (Eiken) by Bauer-Kirby's method⁽⁶⁾. The drugs tested were chlormphenicol, tetracycline, streptomycin, kanamycin, colistin, sulfamethoxazol, ampicillin, hetacillin, cephalothin and bactrin. The zones of inhibition were read by caliper micrometer, and the results were interpreted in Table 1.

RESULTS

A total of 187 strains of Shigella was isolated: 166 strains were isolated from patients and 21 strains were isolated from sewage.

I. Distribution of serotype: Table 2 Shows the numbers of isolates from both patients

Table 1. Interpretation of Single Disk Sensitivity Test

Drug	Abbrev.	Disk potency	Prepared by	Diameter of zone of inhibition interpreted as sensitive
Chloramphenicol	CP	10 mcg	Eiken	more than 8.5 mm.
Tetracycline	TC	10 mcg	Eiken	more than 8.5 mm.
Streptomycin	SM	10 mcg	Eiken	more than 8.5 mm.
Kanamycin	KM	10 mcg	Eiken	more than 8.5 mm.
Colistin	CL	100 u	Eiken	more than 8.5 mm.
Sulfamethoxazol	SX	100 mcg	Eiken	more than 8.5 mm.
Ampicillin	AM	25 mcg	Bristol	more than 14.0 mm.
Hetacillin	HE	25 mcg	Bristol	more than 14.0 mm.
Cephalothin	CR	30 mcg	BBL	more than 14.0 mm.
Bactrin	S×T	25 mcg	Oxoid	more than 14.0 mm.

and sewage. Most of 187 strains isolated from patients and sewage belonged to type B and type D, especially type B(2), (101 strains), type B(1) (24 strains), type B(4) (11 strains) and type D (45 strains) were prevalent. Serotypes A and type C, which have not been reported in Taiwan before, were not found in our experiment either.

Table 2. Distribution of serotypes among 187 strains of *Shigella* spp. isolated in Taiwan in 1970-1971

Mannitol fermentation	Serotype	Source		
		Patient	Sewage	Total
Negative	A	0	0	0
Positive	B(1)	24	0	24
	B(2)	96	5	101
	B(3)	4	0	4
	B(4)	11	0	11
	B(5)	0	0	0
	B(6)	2	0	2
	C	0	0	0
	D	29	16	45
Total		166	21	187

II. Drug sensitivity: The drug sensitivity of the isolated strains are shown in Table 3. 177 of the 187 strains (94.65%) were resistant

to SX, 161 strains (86.09%) to TC and SM. 160 strains (85.56%) to CP. 10 strains (5.35%) to CR. 8 strains (4.09%) to CL, HE and AM. 3 strains (1.06) to KM, and one strain (0.53%) to S×T.

In this experiment, nearly 90% of the isolated strains were observed to be resistant to SX, TC, SM and CP, which have been used for the treatment of dysentery frequently. It should be noted that not only the strains isolated from patients, but also those from sewage were resistant to these drugs. Especially 8 of CL resistant strains were isolated only from sewage and not from patients.

Further classification concerning resistance pattern to CP, TC, SM and SX is shown in Table 4.

We found the following 8 patterns:

1. Pattern I. Resistant to all 4 drugs of CP, TC, SM, and SX: 156 strains (83.42%). Among them 120 strains belonged to serotype B (84.51%) and 36 strains to serotype D (80%).

2. Strains resistant to three of the above mentioned drugs:

(A) Pattern II. Resistant to TC, SM, and SX but sensitive to CP: 3 strains (1.60%).

(B) Pattern III. Resistant to CP, SM, and SX but sensitive to TC: 3 strains (1.60%).

Drug Resistance Patterns of *Shigella* Isolated in TaiwanTable 3. Drug resistance patterns of 187 strains of *Shigella* spp. to tested drugs

Antibiotic	Sources		
	Patient (%)	Sewage (%)	Total (%)
Chloramphenicol	146 (87.95)	14 (66.67)	160 (85.56)
Tetracycline	142 (85.54)	19 (90.48)	161 (86.09)
Streptomycin	146 (87.95)	15 (71.43)	161 (86.09)
Sulfamethoxazole	157 (94.58)	20 (95.24)	177 (94.65)
Bactrin	1 (0.60)	0	1 (0.53)
Kanamycin	1 (0.60)	2 (9.52)	3 (1.60)
Colistin	0	8 (38.09)	8 (4.09)
Ampicillin	3 (1.81)	5 (23.81)	8 (4.09)
Hetacillin	3 (1.81)	5 (23.81)	8 (4.09)
Cephalothin	2 (1.20)	8 (38.09)	10 (5.35)

Table 4. The drug resistance patterns to four drugs (CP, TC, SM, SX) of tested strains

Pattern	Antibiotic				Serotype				Total (%)
					Type B		Type D		
	CP	TC	SM	SX	Patient	Sewage	Patient	Sewage	
I	R	R	R	R	115	5	28	8	156 (83.42)
II	s	R	R	R	1	0	0	2	3 (1.60)
III	R	s	R	R	2	0	1	0	3 (1.60)
IV	R	R	s	R	0	0	0	1	1 (0.53)
V	R	s	s	R	1	0	0	0	1 (0.53)
VI	s	R	s	R	0	0	0	3	3 (1.60)
VII	s	s	s	R	8	0	0	1	9 (4.82)
VIII	s	s	s	s	9	0	1	1	11 (5.88)

(C) Pattern IV. Resistant to CP, TC, and SX but sensitive to SM: 1 strain (0.53%).

3. Strains resistant to two of the above mentioned drugs:

(A) Pattern V. Resistant to CP and SX but sensitive to TC and SM: 1 strains (0.53%).

(B) Pattern VI. Resistant to TC and SX but sensitive to CP and SM: 3 strains (1.60%).

4. Pattern VII. Resistant to one of the above mentioned drug, that is, resistant only to SX but sensitive to other three drugs: 9 strains (4.82%).

5. Pattern VIII. Sensitive to all four drugs: 11 strains (5.88%).

III. Unusual multiple drug resistance: Further classification of drug resistance patterns linked with usual antidiarrheal drugs (CP, TC, SM and SX) is described as follows:

1. The resistance to other drugs of the strains with quadruple resistance to CP, TC, SM and SX (pattern I of Table 4) is shown in Table 5.

Of the 156 strains investigated, 153 strains were sensitive to all other drugs (type I). Two strains were 5 drug-resistant, that is, one strain resistant to KM (type II), and the other to S×T (type III). Lastly, one strain was 6 drug-resistant, that is, resistant to AM and HE.

Table 5. Unusual multiple drug resistant strains linked with quadruple resistance (Pattern I; resistant to CP, TC, SM & SY)

Type	Antibiotic						Serotype				Total (%)
							Type B		Type D		
	S×T	KM	CL	AM	HE	CR	Patient	Sewage	Patient	Sewage	
I	s	s	s	s	s	s	112	5	28	8	153 (81.82)
II	s	R	s	s	s	s	1	0	0	0	1 (0.53)
III	R	s	s	s	s	s	1	0	0	0	1 (0.53)
IV	s	s	s	R	R	s	1	0	0	0	1 (0.53)

Table 6. Unusual multiple drug resistant strains linked with triple resistance

Pattern	Antibiotic						Serotype				Total (%)
							Type B		Type D		
	S×T	KM	CL	AM	HE	CR	Patient	Sewage	Patient	Sewage	
II	s	s	s	s	s	s	1	0	0	0	1 (0.53)
	s	R	R	s	s	R	0	0	0	1	1 (0.53)
	s	R	R	R	R	R	0	0	0	1	1 (0.53)
III	s	s	s	s	s	s	2	0	1	0	3 (1.60)
IV	s	s	R	R	R	R	0	0	0	1	1 (0.53)

Pattern II: resistant to TC, SM & SX. Pattern III: resistant to CP, SM & SX.

Pattern IV: resistant to CP, TC & SX.

2. The resistance to other drugs of the strains with triple resistance is shown in Table 6.

Out of three strains which were resistant to TC, SM and SX (Pattern II of Table 4), one strain resistant to KM, CL and CR (total 6 drug-resistant) and one strain resistant to 8 antibiotics, that is, in addition to the said 6 antibiotics also resistant to AM and HE were isolated from sewage, and one strain

isolated from patient was sensitive to other drug. The three strains isolated from patient, which were resistant to CP, SM and SX (Pattern III of Table 4) were sensitive to other drugs. And one strain isolated from sewage, which was resistant to CP, TC and SX (Pattern IV of Table 4) was also resistant to CL, HE, AM and CR (7 drug-resistant strain).

3. The resistance to other drugs of strains

Table 7. Unusual multiple drug resistant strains linked with double resistance

Pattern	Antibiotic						Serotype				Total (%)
							Type B		Type D		
	S×T	KM	CL	HE	AM	CR	Patient	Sewage	Patient	Sewage	
V	s	s	s	s	s	s	1	0	0	0	1 (0.53)
VI	s	s	R	R	R	R	0	0	0	1	1 (0.53)
	s	s	R	s	s	R	0	0	0	2	2 (1.07)

Pattern V: resistant to CP & SX. Pattern VI: resistant to TC & SX.

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linked with double resistance is shown in Table 7.

One strain isolated from patient which was resistant to CP and SX (Pattern V of Table 4) was sensitive to other drugs. Out of three strains isolated from sewage which were resistant to TC and SX (Pattern VI of Table 4), one was resistant to CL, HE, AM and CR (6 drug-resistant), while two were resistant to CL and CR (quadruple resistant).

4. The resistance to other drugs of strains linked with single resistance to SX (Pattern VII of Table 4) is presented in Table 8.

Out of 9 strains resistant to SX, only one strain isolated from sewage was also resistant to CL, HE, AM and CR (5 drug-resistant), while the other 8 strains isolated from patient were sensitive to other drugs.

5. The resistance to other drugs of strains sensitive to the usual antidiysenterial drugs (CP, TC, SM and SX) Pattern VIII of Table 4) is tabulated in Table 9.

Two strains, one isolated from patient and one from sewage, were resistant to CL, HE, AM and CR (quadruple drug resistant). One strain isolated from patient was resistant

to HE, AM and CR (triple drug resistant). Only 8 strains isolated from patient were sensitive to all drugs we tested.

It is interesting that almost all the strains were resistant to SX but sensitive to the mixture of SX and Trimethoprim (S×T). Further, we noted that most strains were sensitive to those newly used drugs.

DISCUSSION

If we compare our results with the reports by Nakatani⁽¹⁷⁾, Matuhara⁽¹⁸⁾, Research Committee on *Shigella* Infection of Japan⁽¹⁾, and with those reported by scholars of several countries at the 7th SEAME Tropical Medicine Seminar in Infectious Disease of the Gastrointestinal System in Southeast Asia and Far East held at Taipei in 1970^(7,8,9,10), we find that the percentage of drug resistance of shigella varies with places. In Malaysia it is the lowest: TC-resistance is 45.9%, SM-resistance 13.1%, and frequencies of resistance to CP and other antibiotics are all less than 10%. Next comes Vietnam: CP-resistance goes up to 56.2%, SM-resistance 48.7%, TC-resistance 37.4%, but CL-resistance is 22.1%. In Japan,

Table 8. Unusual multiple drug resistant strains linked with single resistance (Pattern VII: resistant to SX)

Type	Antibiotic						Serotype				Total (%)
							Type B		Type D		
	S×T	KM	CL	HE	AM	CR	Patient	Sewage	Patient	Sewage	
I	s	s	R	R	R	R	0	0	0	1	1 (0.53)
II	s	s	s	s	s	s	8	0	0	0	8 (4.28)

Table 9. Strains sensitive to the usual antidiysenterial drugs (CP, TC, SM & SX)

Type	Antibiotic						Serotype				Total (%)
							Type B		Type D		
	S×T	KM	CL	HE	AM	CR	Patient	Sewage	Patient	Sewage	
I	s	s	R	R	R	R	1	0	0	1	2 (1.07)
II	s	s	s	R	R	R	0	0	1	0	1 (0.53)
III	s	s	s	s	s	s	0	0	0	0	8 (4.28)

Hongkong and Taiwan the drug resistance of shigella is more serious. For instance, quadruple resistance to commonly used antidiysenterial drugs (such as CP, TC, SM and SX) was about 70% in Japan recently, and about 80% in Hongkong, and 83.42% in Taiwan. As for the frequency of resistance to other antibiotics, our results also showed the highest in the neighboring regions. And the multiple drug resistant pattern in our experiment was more complex than that of Japan and Hongkong.

On the other hand, since the studies of Yang et al.⁽¹³⁾ in 1954, Hwang and Hsu⁽¹⁴⁾ in 1961, Hsu⁽¹⁵⁾ in 1961, and Yu, et al.⁽²⁾ in 1962, there have been no reports on the resistant pattern to antidiysenterial drugs in Taiwan for nearly 10 years, except the studies of Liu, et al.⁽¹¹⁾ in 1969 and Pang⁽¹²⁾ in 1971, on certain antibiotics in which they made sensitivity test of some strains. So, we can not make a detailed comparison about the changes of drug-resistance patterns, during these years. However, we can see that in Taiwan, the drug resistance to commonly used antidiysenterial antibiotics (CP, TC, SM and SX) was always very high, and resistance to the recently introduced new drugs has been increasing as well, perhaps owing to the somewhat long history of their improper application and their availability at drug store without doctors prescription. It is important for the treatment and prevention of shigellosis to consider proper measure to cope with this serious situation in Taiwan.

As for the relation between the drug resistance pattern and the serotype of shigella, unlike the high percentage resistance of serotype D and relatively low percentage of serotype B in Japan⁽¹⁾, our experiment showed little difference between serotypes B and D.

It should also be noted that some strains isolated from sewage were found to be resistant to new drugs (such as CL, KM, HE, AM and CR) while strains isolated from patients

showed lower resistance to these new drugs, though at present we do not know the reason of it. If these strains have opportunity to infect human, they will cause great difficulty in treatment, a serious problem from the point of view of epidemiology of Shigellosis.

SUMMARY

From the specimens collected from hospital patients and sewage in northern Taiwan during the period of 1970-71, a total of 187 strains of *Shigella* was isolated and their serotyping and sensitivity to chemotherapeutic drugs were tested.

Of the 166 strains isolated from patients, 137 strains were Group B, and 29 strains were Group D. Of the 21 strains isolated from sewage, 5 strains were Group B, and 16 strains were Group D.

The result of the sensitivity test was as follows: 177 strains (94.65%) were resistant to Sulfamethoxazol, 161 strains (86.09%) to Tetracycline and Streptomycin, 160 strains (85.56%) to Chloramphenicol, 10 strains (5.35%) to Cephalothin, 8 strains (4.09%) to Colistin, Hetacillin and Ampicillin, 3 strains (1.60%) to Kanamycin, and only one strain (0.53%) was resistant to Bactrin.

As for drug resistance patterns to commonly used antidiysenterial drugs (such as chloramphenicol, tetracycline, streptomycin and sulfamethoxazol), 156 strains (83.42%) were resistant to all 4 drugs, where as only 11 strains (5.88%) were sensitive to those drugs. One strain isolated from sewage was resistant to tetracycline, streptomycin, sulfamethoxazol, kanamycin, colistin, ampicillin and cephalothin (8 drug-resistance) and one strain was a case of 7 drug-resistance, strains of other resistance patterns were also found.

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臺灣區分離之赤痢菌的抗藥性

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為瞭解臺灣的赤痢菌流行型別以及其對各種藥劑的耐性情形，於民國 59-60 年間從北部各醫院的腹瀉病人及臺北市中央市場的下水道採取檢體，做赤痢菌之分離培養及血清分型，並做其對各種藥劑之感受性試驗。

從病人共分離出赤痢菌166株，其中 Group B 有 137株；Group D 有29株，從下水道分離出21株，其中 Group B 有 5株，Group D 有 16株，總共 187株。但是沒有分離出屬於 Group A 及 Group C 的菌株。

此 187 株中對磺胺劑具有耐性者有 177 株 (94.65%)，對 Tetracycline 及 Streptomycin 具有耐性者各 161 株 (86.09%)，對 Chloramphenicol 具有耐性者有 160 株 (85.56%)，對 Cephalothin 具有耐性者有 10 株 (5.35%)，對 Ampecillin, Hetacillin 及

Colistin 具有耐性者各 8 株 (4.09%)，對 Kanamycin 具有耐性者有 3 株 (1.60%)；對 Bacterin 具有耐性者只有一株 (0.53%)。

關於多劑耐性情形來說，對常用的四種抗赤痢菌藥劑 (如 CP, TC, SM, SX) 同時具有耐性者有 156 株 (83.42%)，而對此四種藥劑均具有感受性者只有 11 株 (5.88%)，從病人分離出之菌株中有一菌株為對六種藥劑具有耐性，兩菌株對五種藥劑具有耐性，但是從下水道分離出的菌株中有一菌株為對八種藥劑具有耐性，另外有一株為對七種藥劑具有耐性，同時我們亦發現各種不同的耐性情形。關於血清型與藥劑耐性情形之關係，我們發現兩者之間無顯著的差異。

綜合以上，關於赤痢菌之耐性情形在臺灣較其他各國更為嚴重，所以對此問題解決的對策為目前克不容緩的研究課題。