Colicine types of *Shigella sonnei* isolated in Hong Kong

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SYNOPSIS Two-hundred and twenty-five strains of *Shigella sonnei* isolated from sporadic cases of bacillary dysentery were subjected to colicine typing. The result showed that types 15 and 12 were the prevailing types in Hong Kong and two new types which did not fit in with the colicine patterns of the current types were observed. A marked increase in the numbers of strains concurrently resistant to chloramphenicol, tetracycline, and streptomycin occurred in recent years. However, there was no correlation between drug resistance or biochemical pattern and colicine types. Consistency of colicinogenicity was confirmed by the fact that even a rough variant of a smooth strain had not changed its colicine pattern.

Abbott and Shannon (1958) reported that colicine production can be used as an epidemiological marker of *Shigella sonnei*. Since then this method has been widely used for typing of this organism in epidemiological studies. Gillies (1964) and Gillies and Brown (1966) reported respectively two new colicine types (types 14 and 15) and Aoki, Naito, Fujise, Muira, Iwanga, Ikeda, Jinnouchi, Marino, Miyahara, Motoki, and Tokiwa (1969) added another four new indicator strains which were derived from variants of *Escherichia coli* K-12 strain. Thus, the colicine typing of *Sh. sonnei* could be brought on to a more precise basis.

Though Huang and Chan-Teoh (1965) reported the incidence of shigella infection in Hong Kong, they made no reference to the colicine types of *Sh. sonnei* in their findings. Because of the increased incidence of its infection over other *Shigella* species in the Far East in recent years (Aoki *et al*, 1969) and because of the growing interest in its epidemiology, we studied the colicine types as well as the drug resistance and sugar-fermentation of 225 strains of *Sh. sonnei* collected in Hong Kong between the years 1965 and 1969. The strains included those isolated from patients admitted to the Queen Mary Hospital and those submitted to the Government Pathological Institute, the public health laboratory of the colony, for examination. The results of our findings are presented in this report.

Materials and Methods

SOURCES OF STRAINS

All strains of *Sh. sonnei* were isolated from stools or rectal swabs of patients of sporadic cases of bacillary dysentery. Only one representative strain of each outbreak of bacillary dysentery was included and those isolated from contacts were not accounted for. All, except six strains, were derived from clinical cases with direct complaints of gastroenteritis and diarrhoea. The six primarily non-dysenteric patients were admitted to hospital for appendicitis (2 cases), Hirschsprung's disease (2 cases), retroperitoneal tumour (1 case), and kidney transplant (1 case). They developed dysentery after admission.

All cultures conformed to the characterization of *Sh. sonnei* as described by Carpenter (1968), i.e. they were Gram-negative, non-motile rods, fermented glucose and mannitol but were late lactose and sucrose fermenters, and were indole, urease, H₂S, and phenylpyruvic reactions negative. They agglutinated with specific serum to high titres.

When not in use, the cultures were preserved in 0.3% semisolid nutrient agar stabs overlaid with thin layer of sterile liquid paraffin. Lyophilized cultures of the strains were also kept for reference.

INDICATOR STRAINS FOR COLICINE TYPING

The 19 indicator strains were 56, 17, 56/56, 2, R6, M19, 2/7, R5, Row, Row/E, Row/I, K-12-30/1, Row/K, 2/15, 2/64, 56/98, 38, 2M, and R1. Among

these, Row/E, Row/I, Row/K and K-12-30/I were mutants of *E. coli* K-12. More patterns could be obtained if K-12 mutants series was included (Aoki *et al*, 1969).

DRUG SENSITIVITY TEST

Oxoid Multodisks incorporated with different antibiotics and chemical agents were employed. The drugs used and their amount per disc are given in Table III. DST agar (Oxoid) was the medium used for sensitivity test.

SUGAR FERMENTATION REACTIONS

Sugar-fermentation activities were tested in peptone broth containing 1% of the sugars. Screw-capped bottles were used as containers so that the cultures could be kept for a long time with little evaporation. The final results of negative reaction were read after two months' incubation at 37°C.

Results

The results of colicine typing of 225 strains of *Sh. sonnei* are presented in Table I and the distribution of the types in Table II. As is seen, the prevalent colicine types of *Sh. sonnei* in Hong Kong were types 15 (43.1%) and 12 (24.9%), followed by types 16 (9.6%), 8 (7.7%), 9a (7.1%), 3a (3.1%), and 6 (1.5%). Types 3, 11, and 17 constituted 1% each

Colicine Type	No. of Strains	Percentage of All Strains	Percentage of Typable Strains
1a	—	—	—
1b	—	—	—
2	—	—	—
3	2	0.9	1.0
3a	6	2.7	3.1
4	—	—	—
4a	—	—	—
5	—	—	—
6	3	1.3	1.5
7	—	—	—
8	15	6.8	7.7
9	—	—	—
9a	14	6.2	7.1
10	—	—	—
11	2	0.9	1.0
12	49	21.7	24.9
13	—	—	—
13a	—	—	—
14	—	—	—
15	85	37.8	43.1
16	19	8.4	9.6
17	2	0.9	1.0
0	28	12.4	—

Table II Distribution of colicine types among 225 strains of *Sh. sonnei* isolated in Hong Kong

only. The number of non-typable strains (type 0) was high. They accounted for 12.4%.

Types 16 and 17 were new types which did not fit in with the patterns described by previous workers (Abbott and Shannon, 1958; Gillies and Brown, 1966; Aoki *et al*, 1969). In general, the colicine

Indicator Strains	Colicine Type										
	3	3a	6	8	9a	11	12	15	16	17	0
56	+	+	+	—	+	—	+	—	+	+	—
17	+	+	—	+	+	—	—	+	—	+	—
56/56	+	+	+	—	+	—	+	—	+	+	—
2	+	+	—	+	+	—	+	+	+	+	—
R6	+	+	+	—	+	—	+	—	+	+	—
M19	+	—	—	—	—	—	—	—	—	—	—
2/7	+	+	—	—	—	—	—	+	+	+	—
R5	+	+	—	+ ¹	+ ¹	—	+ ¹	+	+	+	—
Row	+	+	+	+	+	+	+	+	+	+	—
Row/E	— ¹	+	—	—	—	+	—	—	—	+	—
Row/I	+	+	+	+	+	+	+	+	+	+	—
K-12-30/1	+	+	—	+	+	+	+	+	+	+	—
Row/K	+	+	+ ¹	+	+	+	+	+	+	+	—
2/15	+	+	—	+	+	—	+	+	+	+	—
2/64	— ¹	— ¹	—	—	—	—	—	—	—	—	—
56/98	+	+	+	—	+	—	+	—	+	—	—
38	+	+	+ ¹	+	+	+	+	+	+	+	—
2M	+	—	+/-	—	—	—	—	+	+	+	—
R1	+	+	+	+	+	+ ¹	+	+	+	+	—
Total no. of strains	2	6	3	15	14	2	49	85	19	2	28

Table I Colicine types of 225 strains of *Sh. sonnei* isolated in Hong Kong

¹Do not conform with the findings of Gillies and Brown (1966) or of Aoki *et al* (1969).

patterns of our types agreed with those described in the literature with certain discrepancies. For example, the inhibition of the indicator strain R5 by types 8, 9a, and 12, and of indicator strain 38 by type 6, and R1 by type 11 has not been reported before. These variations occurred in all corresponding colicine types in our study. The occurrence of such variations might be due to transference of colicine factor from local strains of *E. coli* as discussed by Farrant and Tomlinson (1966).

The results of drug sensitivity tests are presented in Table III. Only 2.2% of the strains were sensitive to sulphamethoxazole but 94.7% were sensitive to

Antibiotic	Amount of Drug/Disk (μ g)	No. of Sensitive Strains	Percentage of Sensitive Strains
Chloramphenicol	10	36	16.0
Nitrofurantoin	50	211	93.8
Nalidixic acid	30	210	93.3
Ampicillin	25	200	88.9
Streptomycin	10	36	16.0
Tetracycline	10	42	18.7
Septin	25	213	94.7
Gentamicin	10	217	96.4
Sulphamethoxazole	23.75	5	2.2
Neomycin	10	225	100.0
Kanamycin	10	225	100.0
Polymyxin B	100	225	100.0

Table III Antibiotic sensitivity of 225 strains of *Sh. sonnei* isolated in Hong Kong

Septin (Burroughs and Wellcome) which is trimethoprim and sulphamethoxazole combined. More than 80% of the strains were resistant to chloramphenicol (C), tetracycline (T), or streptomycin (S). Of these, 158 out of 225 strains (70.2%) carried a CTS resistance pattern, ie, they were resistant to these three drugs at the same time. Most of the strains were sensitive to ampicillin, nalidixic acid and gentamicin, and all strains were sensitive to kanamycin, neomycin, and polymyxin B. Table IV compares the drug resistance of the strains tested in

	Period	
	1959-64	1968-69
	171 Strains	225 Strains
	Percentage of Resistant Strain	Percentage of Resistant
Chloramphenicol	30	84
Tetracycline	28	81
Streptomycin	39	84
Nitrofurantoin	4	6
Polymyxin B	7	0
Neomycin	0	0
Kanamycin	0	0

Table IV Comparison of drug resistance of *Sh. sonnei* isolated in two periods

the present series and those in a previous study. There was no correlation between colicine types and drug resistance patterns.

Of 150 strains tested, 119 (79.3%) fermented melibiose in two days, 124 (82.6%) fermented raffinose in 10 days, 144 (96.0%) fermented rhamnose in 24 hours, but only 18 (12.0%) fermented xylose within two months (Table V). There was no correlation between biochemical reactions and the colicine types either. Of the 18 xylose-fermenting strains, six belonged to type 12, 11 to type 16, and one to type 0. Our findings verified those of Aoki *et al* (1969) who found that colicine type 12 showed various fermentation and that xylose-positive types prevailed in Asia. Since all our strains were collected from sporadic cases of bacillary dysentery the result does not imply that drug resistance patterns and biochemical types are not useful as subsidiary markers for epidemiological study of strains isolated from outbreaks.

Colicine Type	No. of Strains Examined	No. of Strains Fermented			
		Melibiose	Raffinose	Rhamnose	Xylose
3	2	1	1	2	0
3a	4	2	3	2	0
6	2	2	2	2	0
8	10	10	9	10	0
9a	15	15	14	15	0
11	1	1	1	1	0
12	19	8	12	19	6
15	58	58	58	58	0
16	17	3	8	17	11
17	1	1	1	1	0
0	21	18	15	17	1
Total	150	119	124	144	18
Percentage of strains		79.3	82.6	96.0	12.0

Table V Fermentation reactions of 150 strains of *Sh. sonnei*

Our results also conformed with the observation of Gillies (1964) on the consistency of colicine patterns of *Sh. sonnei* strains. Rough progenies consistently produced the same colicine pattern of a smooth parent strain after long periods of stocking.

Discussion

The usefulness of colicine typing as a tool for epidemiological study of *Sh. sonnei* infection is generally recognized. Aoki *et al* (1969) added four indicators of *E. coli* K-12 mutant origin for typing thus enabling a clear distinction to be made between types 6 and 11 and types 4 and 14. They also established three new types, viz, types 4a, 9a, and 13a. Using their scheme we found not only that there were discrepancies existing in the colicine patterns of

Fig. 1 Percentage incidence of *Sh. flexneri* and *Sh. sonnei* isolated from stools.

our strains when compared with the known types, but also two new types (types 16 and 17) which did not fit in with the patterns of the types described by previous workers. Their existence was probably due to the natural occurrence of such variants of *Sh. sonnei* strains in Hong Kong or to mutation resulting from transference of colicine factor as indicated by Farrant and Tomlinson (1966).

In Japan the incidence of *Sh. sonnei* infection has increased and is responsible for more than half of the dysentery cases in large cities in that country. The increase in frequency of *Sh. sonnei* infection has also been felt in other Far Eastern countries (Aoki *et al.*, 1969). Although there was an appreciable increase in prevalence of *Sh. sonnei* infection in Hong Kong, it has not superseded *Sh. flexneri* in prevalence (Figure 1). It must be noted that our strains were those isolated from selected clinical and hospital groups and they do not actually reflect the true picture in the general population, for *Sh. sonnei* often produces a milder infection with transient diarrhoea, and asymptomatic cases may even be encountered. Infection of these types usually escapes attention whereas in Japan, when shigellae are found in the stools, despite the absence of

clinical symptoms, the law requires that the person must be isolated (Aoki, 1968). The rate of reported cases is, therefore, higher.

Aoki *et al.* (1969) reported that types 6 and 14 were the predominant colicine types of *Sh. sonnei* isolated in Japan, whereas types 8, 6, and 12 were predominant in Taiwan. In our findings, types 15 and 12 were the most prevalent in Hong Kong. The people in Taiwan and in Hong Kong are largely Chinese and the difference in prevalence of the colicine types appears to be related to the locality rather than to the ethnic group of the people. It must be noted that types 2, 4, and 7, which were frequently isolated in Britain, have not been found in the Far East. With traffic by air becoming heavier, the new types may be introduced from one geographical area to another in future.

Gillies (1964) reported that there was no evidence of change in colicine production either quantitatively or qualitatively when cultures were checked at intervals of six to 12 months over a period of four years. Naito, Kono, Fujise, Yakushiji, and Aoki (1966) found that among 76 strains of *Sh. sonnei* examined, only one strain changed its colicinogenic activity and five had changed in their sensitivity to colicine after storage. We confirmed the findings of Gillies and found that even rough variants of a strain maintained the colicinogenicity of a smooth parent strain. The stability of colicinogenicity is therefore regarded as a practical and well grounded method of identification of epidemic strains of *Sh. sonnei*.

The drug resistance of *Sh. sonnei* isolated in Hong Kong has been described by Huang and Chan-Teoh (1965). There was no change in the resistance patterns except that there was an increase in the percentage of strains resistant to chloramphenicol, tetracycline, and streptomycin combined. The incidence rose from 23.3% as reported in 1965 to 70.2% in the present series. This phenomenon was also observed in Japan and other Asian countries, and is probably due to transferable resistance *in vivo* from one species of enteric organism to another.

Though there was no correlation between colicine types and drug resistance patterns or between colicine types and biochemical types, colicine typing, when used together with drug resistance patterns and biochemical reactions, can be useful for subsidiary pinning down the source of an outbreak. In a recent outbreak of *Sh. sonnei* dysentery in an orphanage in Taipo, Hong Kong, all the 49 isolates from clinical and asymptomatic cases were identified as colicine type 15. They were all resistant to chloramphenicol, tetracycline, and streptomycin, but were sensitive to nitrofurantoin, nalidixic acid, and ampicillin. Biochemically, all strains fermented melibiose and rhamnose within 48 hours and raffinose within 10

days, but xylose was negative. The source of the outbreak was finally traced to the water supply of the orphanage because identical strains were isolated from a stream which supplied the water to the orphanage and a little girl who lived in the neighbouring area had polluted the water.

The problem remains of the presence of the 'unclassifiable' or type 0 strains. Since their colicine patterns are not known, the significance of colicine typing for epidemiological study of these strains is lost. However, when more indicator strains are found in the future, and further attempts are made by combining their colicinogenic activity against the indicators and their sensitivity to colicine produced by the indicators (Naito *et al*, 1966) the numbers of unclassified strains can then be narrowed down. When compared with those reported in Britain or in Japan, the percentage of type 0 strains in our series (12.4%) was unusually high. The high rate of isolation was perhaps due to our selection of sporadic cases. If all strains isolated from outbreaks were included, then the percentage of non-typable strains will be reduced.

We are most grateful to Professor Y. Aoki of the Nagasaki University Medical School for kindly supplying the whole set of indicator strains for our

study and to the Director of Medical and Health Services for permission to publish the data of the Institute of Pathology.

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