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Shigella Species Isolated in Hongkong with Special Reference to Drug-Resistance of Strains

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ABSTRACT : One hundred and seventy-one *Shigella* strain isolated in Hongkong during a period from 1959 July, to June, 1964 were classified serologically. All of the strains, 105 belonged to *Sh. flexneri*, 2 to *Sh. boydii* and 64 to *Sh. sonnei*. These strains were mainly originated from patients who had clinically been diagnosed as bacillary dysentery and from patients having some or other gastro-intestinal complaints, but it worth special mention that there were 3 cases of extra-intestinal shigellosis. Sensitivity tests to 8 kinds of antibiotics or chemotherapeutic drugs were carried out every year. Sensitive strains to sulfafurazole could hardly be found, and emergence of resistant strains to streptomycin showed a yearly increase. Since 1962 the multiple resistant strains of CTS pattern have increased particularly.

HUANG and CHAN-TEOH (1964) reported the *Salmonella* serotypes isolated from clinical specimens in Hongkong and pointed out that salmonellosis was still endemic in that area. Since the epidemiologies of salmonellosis and shigellosis are closely related, a survey of the serotypes of the latter may be useful for comparison of the incidence of the two in the same area. The data presented

in this report are the results of bacteriological examination of clinical specimens derived from the Queen Mary Hospital mentioned in our previous report and the investigation covers the period from July, 1959 to June, 1964. In view of the rapid development of resistance and cross-resistance of *Shigella* to drugs, special reference is given to this aspect.

Materials and Methods

All, except a few, of the specimens were stools or rectal swabs. They were inoculated into S.S. agar, MacConkey agar and Selenite F broth media for primary isolation of *Shigella* organisms. Suspicious colonies were tested by the usual procedures for identification of *Salmonella-Shigella* groups. Slide-agglutination tests with diagnostic sera supplied by the Wellcome Research Laboratories (England) were performed for confirmation. *Sh. flexneri* and *Sh. boydii* strains were identified with polyvalent subgroup sera without further typing. However, a test for drug sensitivity was carried out for most of the strains. "Multodisks" (Oxoid Division, Oxo

Co., London) containing respectively chloramphenicol (100 μ g), nitrofurantoin (50 μ g), sulphafurazole (100 μ g), polymyxin B(10 μ g), streptomycin (10 μ g) and tetracycline (10 μ g) were employed. Discs of neomycin (10 μ g) or kanamycin (10 μ g) were used for testing some strains. The sensitivity test was done by spreading evenly a loopful of an 18 hr-culture from an agar slope over the surface of an infusion agar plate, then a disc was immediately superimposed over the surface of the inoculated medium. After 18-24-hr incubation at 37° C, the culture was read for presence or absence of zones of inhibition.

Results

During the above-mentioned period, we had examined 7,440 faecal specimens. Of these, 361 were clinically labelled bacillary dysentery while the others varied from diarrhoea for investigation or gastro-enteritis to enteric fever etc. In all, 171 strains of *Shigella* serotypes were isolated. Positive cultures repeatedly isolated from the same patient during admission were considered as a single strain. Of the 171 strains, 105 belonged to *Sh. flexneri* subgroup (61.4%), 2 belonged to *Sh. boydii* subgroup (1.2%) and 64 belonged to *Sh. sonnei* (37.4%).

Table 1. Age distribution of shigellosis

Age group	No. of cases	Percentage
Under 10	61	35.7
11 - 20	16	9.4
21 - 30	36	21.1
31 - 40	34	19.9
41 - 50	6	3.5
51 - 60	7	4.0
over 60	11	6.4
Total	171	100.0

Table 2. Seasonal incidence of shigellosis for a five-year period

Month	No. of cases	Percentage
January	14	8.2
February	12	7.0
March	11	6.4
April	16	9.4
May	19	11.1
June	12	7.0
July	14	8.2
August	20	11.8
September	13	7.6
October	18	10.5
November	12	7.0
December	10	5.8
Total	171	100.0

We had not encountered a single strain of *Shigella* subgroup A during this period of investigation.

Of the positive cultures, 94 (54.9%) were derived from male patients and 77 (45.1%) from female patients. The age and seasonal incidence are presented in Tables 1 and 2. Although shigellosis occurred more often in those under ten years, still it was found in all ages. It occurred throughout the year but was more prevalent in the warmer months.

Shigella has been found in-wound swabs twice and once from eyeswabs of a case of acute conjunctivitis. One strain of *Sh. flexneri* was isolated from an infected wound after surgical drainage of a case of appendicular abscess in a patient whose stool-culture persistently yielded negative results. From the second case, a strain of *Sh. sonnei* was recovered from the peritoneal swab of a patient during appendectomy and drainage of the peritoneal cavity. This strain was sensitive to nitrofurantoin and polymyxin B but not to the other drugs. The same organism was later isolated from the stools of the patient. The faecal strain had the same drug-resistance pattern as the strain isolated from the peritoneum.

The third demonstration of extra-intestinal shigellosis was the isolation of *Sh. sonnei* from eye-swabs of a female patient, aged 7, who had acute conjunctivitis of the right eye. *Sh. sonnei* was isolated from two eye-swabs taken at 5-day intervals. The strain was sensitive to nitrofurantoin, polymyxin B and neomycin but not to the other drugs. The case was successfully treated by topical application of neomycin as proved by subsequent negative cultures. Repeated faecal cultures of the patient were negative. Rectal swab or faecal cultures of her parents, nursing staff and attendants of the hospital ward where she was admitted all gave negative findings.

The results of sensitivity tests are tabulated in

Table 3. Results of sensitivity test

Antibiotic		Nitrofurantoin	Polymyxin B	Sulphafurazole	Chloramphenicol	Tetracycline	Streptomycin	Neomycin	Kanamycin
<i>Sh. flexneri</i>	No. of strains tested	95	95	99	99	99	99	33	6
	No. of sensitive strains	93	92	7	67	58	51	26	6
	Percentage of sensitive strains	97.7	96.7	7.1	67.6	57.5	50.4	78.7	100
<i>Sh. sonnei</i>	No. of strains tested	55	55	60	62	62	62	14	3
	No. of sensitive strains	53	51	1	44	45	38	14	3
	Percentage of sensitive strains	96.3	92.7	1.6	70.9	72.1	61.1	100	100

Table 3. Less than 10% of the strains were resistant to both nitrofurantoin and polymyxin B. There was some significant difference in the behaviour to tetracycline, streptomycin and neomycin between *Sh. flexneri* and *Sh. sonnei*. More strains of the former were resistant to those antibiotics. One of the four nitrofurantoin-resistant strains (*Sh. sonnei*) was also resistant to the other drugs except neomycin. The remainders were,

however, sensitive to chloramphenicol alone. The percentage of resistant strains to sulphafurazole was extremely high in our series. It is generally known that the composition of the medium and the size of the inoculum might well influence the result. Because of that, we had used horse-blood agar (MACKIE and McCARTENEY, 1953) and sensitivity-test-agar (Oxoid Division, Oxo Co., London) to duplicate the test for a number of

Table 4. Emergence of resistant strains to chloramphenicol (C), tetracycline (T) and streptomycin (S)

Year	No. of strains isolated	No. of strains resistant to							Total No. of resistant strains	Percentage of resistant strains
		C	T	S	CT	CS	TS	CTS		
<i>Sh. flexneri</i>	1959(Jul-Dec)	6	0	0	0	0	0	0	0	0
	1960	19	1	0	0	0	0	5	0	31.5
	1961	12	0	0	3	0	1	1	1	50.0
	1962	31	0	1	3	0	1	2	11	58.0
	1963	21	0	0	0	0	0	1	11	57.1
	1964(Jan-Jun)	10	0	0	0	0	0	0	8	80.0
<i>Sh. sonnei</i>	1959(Jul-Dec)	5	0	0	0	0	0	0	0	0
	1960	4	0	0	0	0	0	1	2	75.0
	1961	9	0	0	1	0	1	2	2	66.0
	1962	18	0	0	0	0	2	0	5	38.8
	1963	10	0	0	1	0	1	0	2	40.0
	1964(Jan-Jun)	14	0	0	1	0	1	1	3	42.8

strains. The result showed that 21/22 strains of *Sh. flexneri*, 2/2 strain of *Sh. boydii* and 12/12 strains of *Sh. sonnei* showed resistance to sulphafurazole when tested in these two media specially designed for the purpose. Control test with sensitive organisms such as *Pneumococcus*, *Streptococcus pyogenes* and *Escherichia coli* revealed that there was no loss of potency of the discs employed.

Table 4 shows the change of drug-resistance-pattern of the *Shigella* strains in our series and the emergence of multiple resistant strains for chloramphenicol, tetracycline and streptomycin.

We have encountered more of these multiple resistant strains in recent years than before. However, 31/31 strains of *Sh. flexneri* and 13/14 strains of *Sh. sonnei* which showed multiple resistance to chloramphenicol, tetracycline and streptomycin were sensitive to nitrofurantoin and polymyxin B. The singular strain of *Sh. sonnei* resistant to the latter two drugs was also sensitive to neomycin. Despite the small numbers of strains tested against neomycin and kanamycin, there were indications of their in vitro efficacy especially in the control of *Sh. sonnei*.

Discussion

Salmonellosis and shigellosis are equally prevalent in Hongkong. According to the Hongkong Government Report (1963), there were 4,376 cases of enteric fever as against 3,680 cases of bacillary dysentery during 1959-63. The numbers of *Shigella* strains reported here are very limited but all were bacteriologically proved cases. Except for two strains of *Sh. boydii*, the rest were either *Sh. flexneri* or *Sh. sonnei*. We had not encoun-

tered a strain of *Shigella* subgroup A during the period of our investigation. Table 5 outlines the results provided by the Hongkong Government Pathological Institute. This laboratory handles most of the clinical specimens derived from various government clinics and hospitals in Hongkong. As is seen from Table 5, *Shigella* subgroup A infection occurred sporadically and only 3 strains of *Sh. dysenteriae* type 1 (Shiga's bacillus) was found in 1963 as against 1,228 strains of *Shigella* serotypes isolated in that same year. Hou (1962) reported that the percentage of *Sh. dysenteriae* type 1 gradually decreased from 34.4% to less than 1% and *Sh. sonnei* increased from 0.5% to 26% in the past 30 years in Peking. CHUN (1964) showed that *Shigella* subgroup A constituted only 6.3% of the total number of strains of *Shigella* serotypes found in Korea and HSU (1964) described that 90% of *Shigella* strains in Taiwan belonged to *Sh. flexneri* subgroup and 10% to *Sh. sonnei* and that *Shigella* subgroups A and C had not been found there during the period 1952-63. NAKAYA (1963) gave evidence that there were far more subgroups B and D strains isolated in Japan than subgroups A and C. The incidence of *Shigella* species occurring in Hongkong is quite in accord with

Table 5. Incidence of *Shigella* serotypes isolated from Pathological Institute of Hongkong

Year	1960	1961	1962	1963
Total no. of stools * examined for culture	20,259	25,617	28,443	29,241
<i>Sh. dysenteriae</i>	0	0	0	3
<i>Sh. schmitzi</i>	6	8	2	7
<i>Sh. flexneri</i> subgroup	1083	1085	854	905
<i>Sh. boydii</i> subgroup	10	9	8	28
<i>Sh. sonnei</i>	161	247	404	285

* This does not include those examined for *V. cholerae* only.

those in other locations in the Far East.

The isolation of *Shigella* species from extra-intestinal lesions was accidental. As far as we are aware, the case of acute conjunctivitis due to *Sh. sonnei* in our series is the only record of such an infection by *Shigella* in man in Hongkong. The source of the infection is not known since the stool-cultures of the patient and of those in close contact with her were all negative. GEEKER et al. (1957) had successfully produced keratoconjunctivitis in guinea pigs by inoculating freshly isolated strains of *Shigella* cultures into the conjunctival sacs of the animals. SZTURM-RUBINSTEIN et al. (1957) found that 22 out of 89 strains of *Shigella* serotypes which could produce purulent conjunctivitis in guinea pigs also concurrently caused fatal illness in them. In areas where shigellosis is endemic, *Shigella* conjunctivitis might be more common than it should be. In view of the grave consequence in guinea pigs, wider attention must be attached to their isolation during bacteriological examination.

OLARTE and De La Torre (1959) revealed the fact that there was a disturbing rapid adaptation of *Shigella* population to changes in prevailing therapy. NAKAYA et al. (1960) mentioned that approximately 10% of the *Shigella* strains isolated in Japan since 1958 had been recognised to have a high degree of resistance to chloramphenicol, the tetracyclines and/or streptomycin at the time of their isolation from human faecal specimens and that the resistance-pattern denoted cross-resistance to these drugs. The high frequency of strains with multiple resistance in our series also indicates the development of cross-resistance of the strains iso-

lated and the extensive use of these drugs in recent years in Hongkong. TURK (1960) reviewed the ready development of sulphonamide-resistance by *Shigella* of all types in many parts of the world. He showed that 22/24 strains of *Sh. sonnei* tested by him were sulphonamide-resistant but all 16 strains of *Sh. flexneri* were sensitive. He, therefore, implied that the time was approaching when sulphonamides would cease to be effective against *Shigella* strains. Virtually all strains of *Shigella* in our series were sulphonamide-resistant and we had not seen any alteration of the pattern in the past few years.

According to BARBER and GARROD (1963), a logical combination of drugs for bacillary dysentery would be a fully absorbed drug to act on the organisms in the tissues together with an unabsorbed one to deal with those on the bowel surface and in the lumen. Our results show that one of the nitrofurans compounds (e.g. furazolidine) or polymyxin B given orally should have a place in the therapeutic armoury for shigellosis. The numbers of strains tested for sensitiveness to neomycin and kanamycin in our case are too small to warrant comment. In view of the possibility of transference of resistance from one member of *Shigella* to another and from *Shigella* to *E. coli* and vice versa AKIBA et al., 1960; NAKAYA et al., 1960), the development of multiple resistant strains has become a problem, especially when antibiotics are widely used in recent years. The sensitivity pattern of the infecting organism should, therefore, be predetermined in order to achieve successful therapeutic value.

Summary

The incidence of shigellosis in Hongkong for a five-year period is reported. Three instances of extra-intestinal infection by *Shigella* species are

recorded and the frequency of development of drug-resistance of the strains is described.

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香港で分離された赤痢菌株，特にその薬剤抵抗性。黄啟鐸，張陳静嫻，香港大学病理部細菌学教室

1959年7月から1964年6月に至る間香港で分離された赤痢菌171株の分類成績が先ず述べられている。*Sh. dysenteriae* は検出されず，最も多いのは*Sh. flexneri* で105株，61.4%，次いで*Sh. sonnei* 64株37.4%，残りの2株は，*Sh. boydii* であった。主として臨床上赤痢と診断されたもの及び下痢患者よりの検出であるが，虫垂炎手術後腹膜炎を起したもののその腹腔中から2株，急性結膜炎患者の患部から1株赤痢菌を分離し得たことは特記すべきことと思う。通常の抗生物質CP, TC, SMのほか，Nitrofurantoin, Polymyxin B, Sulfafurazole, Neomycin及びKanamycinに対する抵抗性を逐年調べたが，Sulfafurazole感性菌は殆どなく，SMに対する耐性獲得もかなり進んでいた。これらの事情は*Sh. flexneri*と*Sh. sonnei*において多少異なるが，1962年以降*Sh. flexneri*

のいわゆる三剤耐性菌 (CP, TC, SM) が急激に増えたことは注目に値する

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