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Ryukyu Med. J., 7(3): 124～128, 1984.

Characterization of *Shigella Flexneri* Type 4 Isolated in Kenya

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Key words : *Shigella flexneri* Type 4

Abstract

During the period between October 1980 and July 1982, diarrheal cases were examined for the enteropathogens in Kenya. Shigella was isolated from 275 cases including 198*Shigella flexneri* out of 1,199 cases examined. Twenty-nine strains out of the 198 *Shigella flexneri* belonged to serotype 4. Among these 29 type 4 strains, only 3 could be subtyped into 4a and 4b in accordance with the established serotype. The other 26 strains exhibited no group antigens 3,4, or 6. And some strains of these 26 lacked all group antigens but the others had group antigens 7,8. The agglutination pattern of these organisms against anti *Shigella flexneri* type and group antigens sera was not changed by heat treatment of the organisms. The majority of the strains having no group antigens did not ferment mannitol.

Shigella flexneri type 4 with group antigens 7,8 (IV : 7,8) has rarely been reported, but this kind of strains were the most frequent isolates in Kenya as far as this species is concerned. This fact suggested that a new subtype can be established.

Introduction

Genus Shigella is classified into 4 species mainly by their O-antigen structures. Biological behaviours which usually determine the bacterial species, such as fermentation of mannitol by species other than *Shigella dysenteriae* or slow fermentation of lactose by *Shigella sonnei*, are not the most important criteria for identifying the species of Shigella. Although the ability to reduce selenite may differ between *Shigella flexneri* and *Shigella boydii* (4), the differentiation of the 2 species depends almost completely on their antigen structures. The established antigenic formula of *Shigella flexneri* has been accepted for the past few decades without the addition of a new serotype. According to this traditional classification method, *Shigella flexneri* type 4 is subtyped into 4a and 4b, with respective antigenic formulas of IV : 3,4 and IV : 6,3,4, (1). However, the author isolated many strains of *Shigella flexneri* type 4 that lack group antigens 3,4 or 6. Their antigenic formulas were IV:

7,8 or IV : –. The present paper described these organisms.

Materials and Methods

Bacterial strains : *Shigella flexneri* type 4 isolated from diarrheal patients in Kenya during the period between October 1980 and July 1982 were used in this study. Twenty-nine strains were originally isolated but 10 of them were lost during stock and transprtation, therefore, the remains 19 strains were examined in detail. The organisms have been stocked in the butt of nutrient agar (Eiken) at room temprature and subcultured every 4 to 6 months. All strains examined were confirmed to be *Shigella flexneri* on the bases of the following findings : Gram negative non-motile rods, glucose fermentation without gas production, negative cytochrome oxydase, no growth on Simmos-and Christensen citrate agar (Eiken), negative lysin decarboxylase, no fermentation of lactose and sucrose, and agglutination to the polyvalent anti *Shigella flexneri* sera.

Biological test methods : Sugar fermentation tests were carried out according to the method of Hugh and Leifson (3) except for glucose and lactose which were examined with Kligler iron agar (Eiken). Cytochrome oxdase activity was examined with commercially prepared filter paper containing tetramethyl-para-phenylendiamine (Nissui). Catalase production was tested by pouring 3 % hydrogen peroxide over the colonies grown on the nutrient agar plate and examining air bubble formation. Indol production was evaluated in the SIM media (Eiken) using Ehrlich's reagent.

Serological test method : Antigen structures were determined for live organisms grown on the nutrient agar plate and for heat-killed organisms (121°C 40 minutes). The agglutination tests were carried out on the slide glass using commercially prepared specific anti sera (Denkaseiken).

Results

When the 29 strains of *Shigella flexneri* type 4 were originally isolated in the field study, the antigenic formula of the strains were IV : 3,4 in 2 strains, IV : 6 in 1 strain and others (IV : 7,8 or IV : –) in 26 strains. The strains with the formula of IV : 7,8 were isolated not only in one epidemic but all 3 areas examined (Table 1). Some of these strains were lost during stock and transportation, and we finally (September 1982) obtained 19 strains including the antigenic formula of IV : 3,4 in 2 strains, IV : 7,8 in 10 strains and IV : – in 7 strains. The next subcultures were carried out in Feburuary 1983, at that time the antigenic formula was not altered but the agglutination for the type antigen IV became very weak in 2 strains which had the antigenic formula of IV : 3,4. When the biological behaviours and antigen structures were examined again in August 1983, the above 2 strains had lost their type antigen IV but kept the group antigens 3,4. This was regarded as a serotype conversion from type 4a to Variant Y. Another strain that had the antigenic formula of IV : 7,8 also lost the type antigen IV but

Table 1 Isolation rates of Genus Shigella, Sh. flexneri, and Sh. flexneri type 4.

	Places of Isolation (Kenya)			
	Nyeri	Nairobi	South Coast	Total
No. of cases examined	100	100	999	1,199
No. of cases from whom Shigella was isolated	22	25	228	275
No. of Sh. flexneri	11	19	168	198
No of Sh. Flex. type 4		5	19	29
Antigenic structure of the above 29 strains				
IV : 3,4	0	0	2	2
IV : 6	0	0	1	1
IV : 7,8	2	3	16	26
IV : —	3	2		

kept the group antigens 7,8. This was a serotype conversion from type 4 to Variant X. The other 16 strains were antigenically stable. The agglutination patterns were not changed after heat-killing the organisms.

The relationship between antigenic structure and biological behaviours is shown in Table 2. The remarkable findings involved the fermentation of mannitol and D-xylose.

The strains that had group antigens 7,8 fermented mannitol but not D-xylose. Seven strains out of 9 without group antigens 7,8 did not ferment mannitol but fermented D-xylose. Indole production was positive in mannitol non-fermentative strains but variable in the others. Catalase production was positive in all strains.

For mannitol non-fermentative strains, the agglutination tests for anti *Shigella dysenteriae* sera were all negative.

Discussion

Although the antigen structure of *Shigella flexneri* type 4 is complicated, the subtype 4a and 4b exhibiting the antigenic formulas of IV : 3,4 and IV : 6,3,4 respectively are generally accepted.

In the present study, the group antigens 7,8 were stably present in the type 4 strains without co-existing with the group antigens 3,4,6. And the agglutination pattern was not altered by heat treatment of the organisms. The author would like to emphasize the presence of group antigens 7,8 in *Shigella flexneri* type 4.

Shigella flexneri is characterized by interrelated group antigens within the species. In the present study, however, 7 strains of *Shigella flexneri* type 4 did not have any group antigens.

Table 2. Characterization of *Shigella flexneri* type 4
——Results of the Final Examination[a]——

Strains	Antigen Structure				Fermentation of[b]			Production of	
	IV	3,4	6	7,8	Man.	Xyl.	Sal.	Indole	Catalase
1	+	−	−	+	+	−	−	+	+
2	+	−	−	+	+	−	−	+	+
3	+	−	−	+	+	−	−	−	+
4	+	−	−	+	+	−	−	−	+
5	+	−	−	+	+	−	−	−	+
6	+	−	−	+	+	−	−	−	+
7	+	−	−	+	+	−	−	+	+
8	+	−	−	+	+	−	−	−	+
9	+	−	−	+	+	−	−	−	+
10[c]	−	−	−	+	+	−	−	−	+
11	+	−	−	−	−	+	−	+	+
12	+	−	−	−	−	+	−	+	+
13	+	−	−	−	−	+	−	+	+
14	+	−	−	−	−	+	−	+	+
15	+	−	−	−	−	+	−	+	+
16	+	−	−	−	+	−	−	−	+
17	+	−	−	−	+	−	−	−	+
18[c]	−	+	−	−	−	+	−	+	+
19[c]	−	+	−	−	−	+	−	+	+

(a) : Examined in August 1983. (b) : Man = mannitol, Xyl = D-xylose, Sal = salicin. (c) : Type antigen IV was present until March 1983.

In addition, 5 strains out of the 7 did not ferment mannitol. Although these 5 strains were not agglutinable for anti-*Shigella dysenteriae* sera, the distinction may be of taxonomical importance.

During this study, 3 strains out of 19 lost their type antigen IV, and exhibited a conversion of serotypes to Variant X and Variant Y. This conversion rate appears very high in view of the observation that 30 randomly selected strains of *Shigella flexneri*, except for type 4, obtained during the same period in Kenya, showed no antigenical changes since the isolation.

All strains examined in this study were catalase positive. Carpenter and Lachowicz (2) reported that about 40 % of *Shigella flexneri* type 4a did not produce catalase. That report provides further evidence that the strains with the antigenic formula of IV : 7,8 or IV : - differ from the so-called type 4a.

A strain of *Shigella flexneri* with the antigenic formula of IV : 7,8 was also reported by Sato et al (5). The strain was isolated from a diarrheal patient returning from South-East Asia. They reported in succession another isolation from a traveller returning from South America (6). These findings suggest that incidence of these kinds of organisms throughout the world might increase if subserotyping was routinely performed.

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