

## DISTRIBUTION OF PHAGE TYPES AND TRANSFERABLE DRUG RESISTANCE IN SHIGELLAE (1962-73)\*

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### ABSTRACT

A total of 610 strains of Shigellae isolated from cases of diarrhea in Iran during 1962-73 were studied with respect to their phage type, as well as antibiotic resistance and transferable drug resistance along with serotyping.

It was shown that there was some relation between serotypes and phage types but no association could be found between phage types and resistance pattern.

### INTRODUCTION

Shigellae were previously the microorganisms most frequently isolated from cases of diarrhea in Iran. The patients were of all ages, but children constituted high percentage of cases. During the period of 1962-73, 610 strains of Shigellae were isolated in Tehran and the Caspian Littoral at the School of Public Health and Institute of Public Health Research.

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After 1973 we observed a sharp decrease of Shigellae infections.

This higher frequency of isolation of Shigellae in the past decade and sudden decrease in the isolation of Shigellae has drawn our attention to the need of performing phage typing as well as antibiotyping along with serotyping.

In this study we are including the shigellae isolated upto 1974, and the results of isolations after 1973 will be discussed and compared with this paper in a future presentation.

## MATERIAL AND METHODS

Strains: 610 strains of Shigella isolated from stools or rectal swabs of patients with sporadic bacillary dysentery were studied. Only a few strains came from healthy individuals.

572 out of these 610 strains have been phage typed.

## BACTERIOPHAGES AND TECHNIQUE

a) → Shigella flexneri phages were obtained from Dr. Slopek. 14 bacteriophages were used for Shigella flexneri phage typing the Slopek (8) technique was used for this purpose.

b) Shigella sonnei bacteriophages were obtained from Hammarstroms, and the Szturm-Rubinsten (9) technique was used. Phage typing for Shigella sonnei was done at the Pasteur Institute of Paris.

## DRUG SENSITIVITY TEST

The sensitivity of the strains was determined by two following methods:

1. The disc method described by Bauer-Kirby (3) and Ericson *et al* (6) and the following 16 antimicrobial agents were used; streptomycin (Sm) sulfonamides (Su), tetracyclin (Tc), chloramphenicol (Cm),

ampicillin (Am), carbencillin (Cb), colimycine (Cl), trimethoprim sulphamethoxazole (Sxt), kanamycin (K), cephalothin (C), nalidixic acid (NA), paromycin (PAR), furoxon (Fx), Polymycin - B (PB), gentamycin (Gm), and neomycin (N).

2. Minimal inhibitory concentrations (MIC) were determined, according to Daquet *et al* (5).

## TRANSFER OF ANTIBIOTIC RESISTANCE

Transfer of the R-factor was performed by the following methods:

- a) Farrar *et al* (7); culture and conjugation in liquid media.
- b) Chabert and Patte (4) mixed culture on Cellophane.

## RECIPIENT STRAIN

A mutant of *E. coli* K<sub>12</sub>F<sup>-</sup> ( $\lambda$ ) "sawage" (54-117 Kindly provided by Pasteur Institute, Paris), sensitive to all antimicrobial agents but resistant to 500 ug/ml of sodium azide was used for transfer of the R-factor.

## RESULTS

181 out of 610 strains (29,7%) were *Sh. flexneri* type 2, 161 (26,4%) *Sh. sonnei*, 230 all other types of *Shigella flexneri* such as, *Sh. flexneri* type 1, 58 strains (9.5%) type 3, 65 strains (10.7%) type 4, 1 strains (0.2%) type 5, 8 strains (1.3%), type 6, 98 strains (16.1%) 20 strains were *Shigella boydii* and 18 strains *Sh. dysenteriae*.

## PHAGE TYPES OF SHIGELLA FLEXNERI

Table 1 and 2 shows that there is some relation between serotypes and phage types. For example from 152 serotype 2 (9 Phage types) 67% belonged to phage type 12, followed by phagetype 1 with a frequency of 18.4%. A comparison of the flexneri 2 isolated in the two geographical area (Tehran and Caspian Littoral) also show a difference. Thus while in Tehran phagetype 12 is the predominant type 67% in the Caspian Area the priority belongs to phage type 2(48.4%). *Shigella flexneri* serotype 6 in both areas mostly belong to phage type 53 with almost no differences in the two areas Tehran 80% and Caspian Littoral 78.6%.

## PHAGE TYPES OF SHIGELLA SONNEI

The 108 *Shigella sonnei* isolated in Tehran were classified in 22 phage types with type 36 (23%) and type 6 (22%) being the predominant types, while the 53 strains isolated from Caspian Littoral belonged to 12 different phage types; phage types 93(54.7%) and 75(13%) showing the higher frequency as indicated in table 3 and 4.

With regards to the relation between phage types and resistance certain phage types were found to be 100% resistant: *Shigella flexneri* type 2 phage types 1a and 26 were 100% resistant, but in the case of *Shigella Sonnei*, only phage type 23 showed 88.9% resistance.

We attempted, but were not able to find a precise pattern of resistance associated with certain phage types.

The *Shigella flexneri* type 2 strains, after losing their plasmides spontaneously, were phage typed again and compared with the original resistant strains. No difference were observed in the lysotype patterns.

Transfer of resistance factor was successful in *Shigella* strains. The most frequent patterns which showed a high percentage of transfer are shown in tables 5, 6 and 7.

## DISCUSSION

The results reported in this paper indicate that *Shigella flexneri* and *Shigella sonnei* strains isolated from sporadic cases of diarrhea in Iran belonged to a number of various phage types. This could possibly be explained by the fact that these strains had been isolated and kept at the laboratory from a number of years.

In this study the predominant lysotype for *Shigella sonnei* isolated in Tehran was type 36, followed by type 6. In the Caspian Littoral the most frequent lysotype was found to be type 93. According to Szturm-Rubinsten (10) lysotype 65 has been found to be predominant in all countries. In our cases however this lysotype presented only 9.4% of the isolates in the Caspian Littoral.

While it has been shown by other investigators that in a certain geographical area the resistance of *Shigella* strains is associated with lysotype and biotype (10), we could not find such an association in studying phage types and resistance pattern. On the other hand many authors have shown the influence of the transfer factor on phage sensitivity of *Salmonella* (1, 11, 12).

Our resistant *Shigella flexneri* 2 strains after spontaneous loss of their plasmid did not show any difference in their lysotype pattern, as has been reported in our previous paper (2).

**Table 1**  
**DISTRIBUTION OF PHAGE TYPES OF SHIGELLA FLEXNERI**  
**(TEHRAN 1962-1973)**

Sero- types	1	2	3	4	5	6	Total
Phage types	Number & Percent						
1	12(26.7)	28(18.4)	4(11.4)	—	—	1(1.4)	45(14.5)
1a	—	10(6.6)	—	—	—	—	10(3.2)
1b	—	1(0.7)	—	—	—	—	1(0.3)
2	8(17.8)	—	20(57.1)	—	—	—	28(9.0)
7	1(2.2)	—	—	—	1(12.5)	—	2(0.6)
8	7(15.6)	—	1(2.9)	—	3(37.5)	—	11(3.6)
9	10(22.2)	—	1(2.9)	—	—	—	11(3.6)
10	3(6.7)	—	—	—	1(12.5)	—	4(1.3)
11	1(2.2)	—	—	—	—	—	1(0.3)
12	—	102(67)	2(5.7)	—	—	—	104(33.4)
12b	—	1(0.7)	—	—	—	—	1(0.3)
15	—	4(2.6)	—	—	—	—	4(1.3)
21	—	—	—	—	1(12.5)	—	1(0.3)
26	—	4(2.6)	—	—	—	—	4(1.3)
28	—	—	—	—	2(25.0)	—	2(0.6)
31	1(2.2)	—	—	—	—	—	1(0.3)
32	—	1(0.7)	1(2.9)	—	—	—	2(0.6)
43	—	1(0.7)	—	—	—	—	1(0.3)
44	—	—	1(2.9)	—	—	—	1(0.3)
52	—	—	—	—	—	11(15.7)	11(3.5)
53	—	—	—	—	—	56(80.)	56(18.2)
un- typed	2(4.4)	—	5(14.2)	1(100)	—	2(2.9)	10(3.2)
<b>Total</b>	<b>45</b>	<b>152</b>	<b>35</b>	<b>1</b>	<b>8</b>	<b>70</b>	<b>311</b>

**Table 2**  
**DISTRIBUTION OF PHAGE TYPES OF SHIGELLA FLEXNERI**  
**(CASPIAN LITTORAL 1966-1972)**

Sero- types	1	2	3	4	5	6	Total
Phage types	Number & %	Number & %	Number & %	No. & %	No. & %	Number & %	
1	2(15.4)	7(24.1)	19(63.3)	—	—	2(7.1)	30(30)
2	1(7.7)	14(48.4)	4(13.3)	—	—	—	19(19)
4	—	2(6.9)	—	—	—	—	2(2)
8	3(23.1)	—	1(3.4)	—	—	—	4(4)
9	5(33.4)	1(3.4)	1(3.4)	—	—	—	7(7)
12	—	—	3(10)	—	—	1(3.6)	4(4)
28	—	1(3.4)	—	—	—	—	1(1)
46	—	1(3.4)	—	—	—	—	1(1)
53	—	—	—	—	—	22(78.6)	22(22)
Un- typed	2(15.4)	3(10.4)	2(6.6)	—	—	3(10.7)	10(10)
<b>Total</b>	<b>13</b>	<b>29</b>	<b>30</b>	<b>—</b>	<b>—</b>	<b>28</b>	<b>100</b>

Table 3

DISTRIBUTION OF PHAGE TYPES OF  
SHIGELLA SONNEI (TEHRAN 1962-1973)

Phage Types	Number of Strains	%
36	25	23.1
6	24	22.2
93	12	11.1
23	9	9.3
12	7	6.5
21a	6	5.7
Other	25	23.1
Total	108	100

Table 4

DISTRIBUTION OF PHAGE TYPES OF  
SHIGELLA SONNEI (CASPIAN LITTORAL 1966-1972)

Phage Types	Number of Strains	%
93	29	54.7
75	7	13.2
65	5	9.4
Other	12	22.7
Total	53	100



Table 5

THE MOST FREQUENT TRANSFER OF RESISTANCE IN  
SHIGELLA FLEXNERI (TEHERAN 1962-1973)

Patterin of Resistance	Number	Number of Transfer	% Transfer
Tc Su	46	28	60.9
Tc Cm	7	7	100.0
Sm Su Tc Cm	98	95	96.9
Sm Su Tc Cm Am Cb	23	23	100.0
Total	174	153	87.3

Tc = Teteraculcin

Su = Triple Sulfa

Sm = Streptomycin

Cm = Chloramphenicol

Am = Ampicin

Cb = Carbencillin

Table 6

THE MOST FREQUENT TRANSFER OF RESISTANCE  
IN SHIGELLA SONNEI (1962-1973)

Pattern of Resistance	Number	Number of Transfer	% Transfer
Sm Su	13	3	23.1
Cm Tc	10	10	100.0
Sm Su Re	4	1	25.0
Sm Su Tc Cm	50	27	54.0
Sm Su Tc Cm Am	4	4	100.0

Sm = Streptomycin

Su = Triple Sulfa

Tc = Teteracuclin

Sm = Chloramphenicol

Am = Ampicilin

Table 7

THE MOST FREQUENT TRANSFER OF RESISTANCE  
IN SHIGELLA BOYDII AND SHIGELLA DYSENTRIAE

Pattern of Resistance	Number	Number of Transfer	% Transfer
Sm Su Cm Tc	8	5	62.5
Sm Su Tc Cm Am Cb	4	4	100.0

Sm = Streptomycin

Su = Triple Sulfa

Tc = Teteracyclin

Cm = Chloramphenicol

Am = Ampiciline

Cb = Carbenicilin

## REFERENCES

1. Anderson, E.S., Influence of the transfer factor on the phage sensitivity of Salmonellae. *Nature (Lond.)*, 1966, 212, 795-799.
2. Badalian K., Parsapour B. et Ghorachi, B., Repartition de lysotype et etude de la sensibilite aux antibiotiques des *Shigella flexneri* 2 Isolees en Iran. *Bulletin de la societe de Pathologie Exotique* 69 No.3, Mai-Juin, 1976.
3. Bauer A.W., Kirby W.M.M., Sherris J.C., and Turck M., Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Path.*, 1966, 45, 493-496.
4. Chabbert Y.A., Et Patte J.C., Cellophane transfer: application to the study of activity of combinaison of antibiotics, *Appl. Microb.*, 1960, 8, 193-197.
5. Daguet G.H., et Chabbert Y.A., *Technique on Bacteriologie, Serologic bacterienne, Antibiotiques en Bacteriologie medicale.* Flammarion, 1972.
6. Ericson M.H. et Sherris J., Antibiotic sensitivity testing. *Acta Pathologica et Microbiologica Scandinavica*, 1971, B. 217/
7. Farrar W.E., and Dekle L.C., Transferable antibiotic resistance associated with an outbreak of Shigellosis. *Ann. Intern. Med.*, 1967; 67, 1208-1215.
8. Slopek S., Durlakowa I., Kucharewicz-Krukowska A. et Kszywy T., 3rd Meeting of International Working Group for *Shigella* Phage Typing Wernigerode, 1971.
9. Szturm-Rubinsten S., Piechaud D., Gasser A., et D'hauteville H., Type de resistance aux antibiotues et aux sulfamides de 590 souches de *Sh. sonnei*. Repartition géographique; Rapport avec le biotype et lysotype. *Bulletin de la Societe de Pathologie Exotique*, 1974, 67, No.6, 564-573.
10. Szturm-Rubinsten, S. 1964, Repartition géographique des biotypes et lysotypes 743 souches de *Sh. sonnei*. *Annales de*

l'Institute Pasteur, 106, 114-122.

11. Toucas. M., Relations entre le changement de lysotype et les transferts de facteurs de resistance de *S. typhi* d'origine mexicaine. C.R. Acad. Sc., Serio D, Paris, 1974, 278, 397.
12. Toucas M. et Vieu J.F., Incidences sur la lysotypie de *S. typhi* de l'acquisition de resistance transferables aux antibiotiques. Ann. Institut Pasteur, 1973, 124A, 477- 487.