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COLICINE TYPES, BIOCHEMICAL TYPES AND DRUG RESISTANCE PATTERN OF 154 STRAINS OF SHIGELLA SONNEI ISOLATED IN IRAN*

S. Mehrabian**
K. Badalian**
M. Sigaroudinia**

ABSTRACT

The colicine types, biotypes and resistance pattern to antibacterial drugs of 154 strains of Shigella sonnei isolated in two different geographical areas of Iran, are presented. While a difference in the predominant biotype was observed in the two areas, the resistance pattern and the colicine types were found to be almost identical.

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** Department of Epidemiology and Pathobiology, School of Public Health and Institute of Public Health Research, University of Tehran, P.O.Box 1310, Tehran, Iran.

INTRODUCTION

Infection with Shigella sonnei is the common cause of bacillary dysentery in Iran.

Recent reports in the literature have drawn attention to mass outbreaks of this infection in many countries, e.g. France (Szturm-Rubinsten, 1968), Eastern Europe (Kostrzewsky et al., 1968), Japan (Aokoi, 1968), Hong Kong (Chan-Teoh et al., 1971), the United Kingdom (Gillis, 1964) and the United States (Reller, 1971).

Because of the increased incidence of this infection over other Shigella species in Tehran (Badalian et al., 1973), the colicine types, biotypes and drug resistance pattern of 154 Shigella sonnei strains isolated during the period 1963-1973 on the Central Plateau and Caspian littoral of Iran were studied.

MATERIAL AND METHODS

Sources of Strains

The strains of Shigella sonnei were isolated from stools or rectal swabs of patients having sporadic bacillary dysentery. Only a few strains were derived from healthy individuals. All cultures were confirmed to possess the characteristics of Shigella sonnei by biochemical reactions and slide aggutination according to Le Minor (1972).

Strains used for Colicine Typing

The 16 standard strains producing colicine were A, B, C, D, E, F, G, H, I, J, K, S, S_3, S_4, S_5; V and the indicator strain Row were kindly provided by Professor Frederique from Belgium. All Shigella sonnei strains were tested for colicine sensitivity by the method described by Frederique (1957).

Biotype Determination

For identification of biotypes, the Szturm-Rubinsten (1964) method was used as follows:

1- B-galactosidase discs were used to determine lactose fermentation and tests were performed according to Le Minor (1972).

2- Xylose and Rhamnose fermentation tests were checked from 24 hours to 21 days (Le Minor, 1972).

Biotypes were determined according to the following schedule:

Biotype	B-galactosidase	Xylose	Rhamnose
"a"	+++	–	ferm. 24 hrs.
"g"	+++	–	late ferm.
"d"	–	ferm. 24 hrs.	ferm. 24 hrs.
"e"	+++	" or late	" 24 Hrs. or Late
"f"	–	–	

Drug Sensitivity Test

The sensitivity test was done by two methods:

1. The disc method described by Bauer et al. (1966) was employed to test the sensitivity of Sh. sonnei on Muller-Hinton agar against 14 antimicrobial agents, namely: ampicillin (A), 10 μg; streptomycin (Sm), 10 μ g; colimycin (Cl), 10 μ g; bactrim (SXT), 25 μg; chloramphenicol (C), 30 μ g; kanamycin (K), 30 μ g; tetracycline (Tc), 30 μg; neomycin (N), 30 μ g; cephalothin (CF), 30 μ g; nalidixic-acid (NA), 30 μg; paromomycine (PAR), 30 μ g; furoxon (FX), 100 μ g; triple-sulfa (SSS), 300 μg; and polymyxin-B (PB), 300 units.

2. Minimal Inhibitory Concentrations (MIC) were determined by tube dilution tests in trypticase soy broth over the range of 2-2000 μ g/ml according to Chabbert (1963). Sulfonamide tube sensitivity tests were carried out in a broth with special preparation described by Buttiaux et al. (1966), and dilution tests over the range of 2-2500 μg/ml were performed.

RESULTS

The results of colicine typing of 154 strains of Shigella sonnei are presented in Table 1 and 2.

Strains isolated on the Central Plateau and in the Caspian littoral were sensitive to colicine producing strains by the following orders of frequency, respectively: K, D, V, S_3+I, F, E+I, J+I, A, H, G, C, S, S_5, B, S_4 and I; K, F, S+I, V, D, A, C, S, S_3, G, H, J+I, S_5, S_4, I and B.

The biotypes of Shigella sonnei examined are presented in Table 3. The most predominant biotype on the Central Plateau was found to be biotype "g", while in the Caspian littoral it was biotype "a". Biotypes "e" and "f" were not encountered on the Central Plateau during the period of our study, and were encountered only rarely in the Caspian littoral.

The results of sensitivity tests to single drugs showed that in both areas almost 100% of the strains were sensitive to Colimycin (Cl), Furoxon (FX), Plymyxin-B (PB), Ampicillin (Am), Kanamycin (Km), Cephalothin (CF), Neomycin (Nm), Paromomycine (PAR), Nalidixic-acid (NA) and Bactrim (SXT).

Sensitivity to Tetracycline (Tc), Chloramphenicol (Cm), Streptomycin (Sm) and Triple-Sulfa (Su) was higher in the strains from the Caspian littoral (71.7%) than in the strains from the Central Plateau (58.4-53.5%). However, the difference was not found to be satistically significant.*

* Chi Square Test.

In regard to the drug resistance pattern, the predominant pattern in both areas was found to be the same (Tc, Cm, Sm, Su),(Tables 4 & 5)

MIC for all resistant Shigella sonnei strains in both areas was:

Sm	$\geqslant$	15.6 –		1000 ηg/ml
Cm	$\geqslant$	125 –	$\leqslant$	500
Tc	$\geqslant$	62.5 –	$\leqslant$	250
Su	$\geqslant$	750 –		2500
Nm	$\geqslant$	250 –	$\leqslant$	1000
Km		1500 –	$\leqslant$	1700
Am	$\geqslant$	625 –	$\geqslant$	1000

DISCUSSION

Many investigators have successfully used type differentiation by colicine of Shigella sonnei to identify the sources of epidemics and outbreaks (Chan-Teoh et. al., 1971; Reller, 1971; Gillics et. al., 1964; Abbott and Shannon, 1958; McGeachie et. al., 1967). On the other hand, Shigella sonnei strains isolated from cases of endemic diarrhoea in this study have shown more than 40 types,

thus creating some difficulty in the classification of this organism. Moreover, these findings are in complete accord with the results of Papavassiliou (1964).

No difference in the colicine types was observed between the strains isolated in the two different geographical areas.

Type differentiation by biochemical reactions (Szturm-Rubinsten, 1968) seems to be an easy, practical and reliable method for use in the first stages of epidemiologic investigations.

In the study, the predominant biotype on the Central Plateau was "g" followed by "a". This situation was reversed in the Caspian littoral. It should be noted that biotype "d", which is rarely encountered in Iran, is the most frequent biotype in France, England, Sweden and Russia (Szturm-Rubinsten, 1968).

The resistance pattern of these strains was not found to be different in the two areas. However, although the resistance pattern was the same in both areas, the percentage of resistant strains was higher on the Central Plateau.

No correlation was observed between colicine type and resistance pattern of the strains, or between colicine type and biotypes of Shigella sonnei examined. The predominant biotypes were more resistant than were the other ones.

Since all of our strains were isolated from sporadic cases of diarrhoea, the results of this study do not imply that colicine types, biochemical types and drug resistance patterns are not useful for epidemiological study of strains isolated from outbreaks.

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TABLE No.2
NUMBER AND PERCENT OF SHIGELLA SONNEI STRAINS SENSITIVE TO VARIOUS COLICINES

NUMBER OF ACTIVE COLICINES	STUDIED AREA			
	CENTRAL PLATEAU		CASPIAN LITTORAL	
	NUMBER OF SENSITIVE STRAINS	PERCENT OF SENSITIVE STRAINS	NUMBER OF SENSITIVE STRAINS	PERCENT OF SENSITIVE STRAINS
0	0	0.0	0	0.0
1	10	9.9	1	1.9
2	19	18.8	0	0.0
3	25	24.8	1	1.9
4	13	12.9	6	11.3
5	11	10.9	9	17.0
6	8	7.9	13	24.5
7	4	4.0	2	3.8
8	1	1.0	7	13.2
9	1	1.0	0	0.0
10	1	1.0	2	3.8
11	4	4.0	0	0.0
12	1	1.0	1	1.9
13	2	2.0	1	1.9
14	0	0.0	6	11.3
15	0	0.0	3	5.7
16	1	1.0	1	1.9

TABLE No. 3
BIOTYPES OF SHIGELLA SONNEI ISOLATED ON THE CENTRAL PLATEAU AND IN THE CASPIAN LITTORAL OF IRAN (1963-1973)

AREA / BIOTYPE	CENTRAL PLATEAU		CASPIAN LITTORAL	
	NUMBER	%	NUMBER	%
"a"	12	11·9	39	73·6
"g"	87	86·1	8	15·1
"e"	2	2·0	3	5·7
"d"	-	-	2	3·7
"f"	-	-	1	1·9
TOTAL	101	100·0	53	100·0

TABLE No. 4
RESISTANCE PATTERN OF SHIGELLA SONNEI BIOTYPES ISOLATED ON THE CENTRAL PLATEAU, IRAN

BIOTYPES / PATTERN OF RESISTANCE	"g"		"a"		"e"		TOTAL	
	No	%	No	%	No	%	No	%
Tc,Cm,Sm,Su,Km,Nm,Par	1	1.1	-	-	-	-	1	1.0
Tc,Cm,Sm,Su,Am	3	3.5	-	-	-	-	3	3.0
Tc,Cm,Sm,Su	25	28.8	1	8.3	-	-	26	25.6
Tc ,Sm,Su	2	2.3	-	-	-	-	2	2.0
Sm,Su,Am	2	2.3	-	-	-	-	2	2.0
Cm,Sm,Su	1	1.1	-	-	-	-	1	1.0
Nm,Km,Par	1	1.1	-	-	-	-	1	1.0
Tc,Cm	10	11.5	-	-	-	-	10	9.9
Sm,Su	10	11.5	-	-	-	-	10	9.9
TOTAL RESISTANT	55	63.2	1	8.3	-	-	56	55.4
TOTAL SENSITIVE	32	36.8	11	91.7	2	100.0	45	44.6
TOTAL	87	100.0	12	100.0	2	100.0	101	100.0

Tc=TETRACYCLINE Cm=CHLORAMPHENICOL Sm=STREPTOMYCYN
Su=TRIPLESULFA Am=AMPICILLIN Km=KANAMYCIN
Nm=NEOMYCIN Par=PAROMOMYCINE

TABLE No. 5
RESISTANCE PATTERN OF SHIGELLA SONNEI BIOTYPES
ISOLATED IN THE CASPIAN LITTORAL, IRAN

PATTERN OF RESISTANCE \ BIOTYPES	"a"		"g"		"d"		"e"		"f"		TOTAL	
	No	%	No	%	No	%	No	%	No	%	No	%
Tc, Cm, Sm, Su, Am	-	-	1	12.5	-	-	-	-	-	-	1	1.9
Tc, Cm, Sm, Su	13	33.3	1	12.5	-	-	1	33.3	-	-	15	28.2
Cm, Sm, Su	1	2.6	-	-	-	-	-	-	-	-	1	1.9
Sm, Su	-	-	1	12.5	-	-	-	-	-	-	1	1.9
Tc	-	-	1	12.5	-	-	-	-	-	-	1	1.9
TOTAL RESISTANT	14	39.9	4	50.0	-	-	1	33.3	-	-	19	35.8
TOTAL SENSITIVE	25	64.1	4	50.0	2	100.0	2	66.7	1	100.0	34	64.2
TOTAL	39	100.0	8		2	100.0	3		1	100.0	53	100.0

Tc=TETRACYCLINE Cm=CHLORAMPHENICOL Sm=STREPTOMYCYN
Su=TRIPLESULFA Am=AMPICILLIN