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Incidence of R-factors in Food-Borne *Shigella sonnei*

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Abstract The pattern of drug resistance and incidence of R-factors were studied in *Shigella sonnei* as food-borne pathogen strains isolated from chicken meat in Iran. In this study we examined for transferring R-factors of *S. sonnei* to sensitive *Escherichia coli* $k_{12}\bar{F}(\lambda)$. The results showed that 19 out of 57 strains (33.3%) were resistant to one or more drugs and multiple drug resistance was more common than single drug resistance. The most predominant pattern of resistance observed was Tetracycline (Tc), Chloramphenicol (Cm), Streptomycin (Sm), and Sulfonamide (Su). 100% of the strains from the Caspian littoral transferred at least a part of their resistance pattern to sensitive *E.coli* $k_{12}\bar{F}(\lambda)$.

Key words: drug resistance, R-factor, *Shigella sonnei*, Iran, Caspian littoral

Introduction

After Japanese investigators succeeded in discovering the transfer of multiple drug resistance *in vitro*, they attempted unsuccessfully to transfer the resistance with cell-free filtrates of resistant donor cultures. Accordingly, they concluded that cell-to-cell contact (or conjugation) was essential for the transfer. Their results also indicated that neither the serological nor the biochemical markers which were studied routinely in diagnostic bacteriology were changed by the transfer of multiple drug resistance (1).

It was found by other investigators that multiple drug resistance could easily be transferred between *Shigella* and *Escherichia coli* by mixed cultivation. This discovery led many workers to the genetic study of multiple drug resistance. They have found that multiple drug resistance factors were carried and transferred by episomes (extra chromosomal elements) called drug-resistant plasmids, R-factors or R-plasmids. Therefore multiple drug resistance is an example of 'infective heredity' (2, 11).

Since there are many immigrated chicken in the Caspian littoral region of Iran, we guess that the risk of infected bacteria will increased in them, then we examined for transferring R-factors of *Shigella sonnei* to sensitive *E. coli* $K_{12}\bar{F}(\lambda)$. It is very important to have some idea of how common resistant strains are in the alimentary tract of normal, healthy people so that a rough estimate of the probability of infective resistance can be made in the population (3).

Materials and Methods

The strains of *S. sonnei* examined in the study were 57 strains isolated from meat of chicken obtained from the Caspian littoral region of Iran. All cultures demonstrated the characteristics of *S. sonnei* as described by Finegold and Baron (4), and were kept in deep agar until 2003. Strains were isolated in two separate periods spanning from 1997 to 2003. Strains isolated during the earlier period (1997-1999) were subcultured a maximum of two times while the strains isolated from 1999 to 2003 have never been subcultured. The sensitivity test was done in two ways:

1. The disc method described by National Committee of Clinical Laboratory Standards (NCCLS) sub committee's recommendation (5) was employed to test the sensitivity of *S. sonnei* on Muller-Hinton agar against 14 antimicrobial agents which obtained from Hejrat Company in Iran, namely: Ampicillin (A), 10 μg; Streptomycin (Sm), 10 μg; Colimycin (C1), 10 μg; Bactrim (SXT), 25 μg; Chloramphenicol (C), 30 μg; Kanamycin (K), 30 μg; Tetracycline (Tc), 30 μg; Neomycin (N), 30 μg; Cephalothine (CF), 30 μg; Nalidixic acid (NA), 30 μg; Paromomycine (PAR), 30 μg; Furoxon (FX), 100 μg; Triple-sulfa (SSS), 300 μg; and Polymyxin-B (PB), 300 units.

The strains *of S. sonnei* that were resistant to one or more of the above- mentioned sulfonamides and antibiotics were considered drug resistant.

2. Minimal Inhibitory concentrations (MIC) were determined by tube dilution tests in trypticase soy broth over the range 2-2000 μg/mL, according to Finegold and Baron (4). The transfer of antibiotic resistance was performed by modifying and combining the Gaikwad and Deodthar methods (6) for transferring of the R-factor. The recipient strain was a mutant of drug-sensitive *E. coli* $K_{12}\bar{F}(\lambda)$: 'sauvage' (54-117 kindly provided by Pasteur Institute, Paris, France), which was resistant to 300 μg/mL sodium azide (Difco Laboratory, Detroit, MI, USA).

Results and Discussion

Our observations show that the incidence of antibiotic resistance among 57 *S. sonnei* strains isolated from meat of chicken in the Caspian littoral region of Iran during (1997-2003) was 33.3%. Probably due to a rather short period of observation, we did not observe any significant changes in the sensitivity of *S. sonnei* strains during the study period (Table 1).

Table 2 shows the patterns of resistance to antibiotics observed during the study. Resistance to two or more drugs was more commonly observed than resistance to a single drug. Resistance to Tc, Cm, Sm, and Su was the most frequent pattern (state exactly how many strains of

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Table 1. Frequency of *Shigella sonnei* strain resistance to one or more antibiotics isolated in Caspian littoral, Iran

Dates	Number of strains			Percent of resistant (%)
	Total	Sensitive	Resistant	
March 1997 –Feb 1999	24	15	9	37.5
March 1999 –Feb 2001	14	10	4	28.6
March 2001 –Feb 2003	19	13	6	31.6
Total	57	38	19	33.3

Table 2. Transfer of resistance pattern to *Escherichia Coli* by strains of *Shigella Sonnei* isolated in Caspian littoral, Iran

Pattern of resistance	N. Test	Transfer of resistance pattern		
		Complete	partial	none
Tc[1], Cm[2], Sm[3], Su[4], Am[5]	1	1	0	0
Tc, Cm, Sm, Su	16	14	2	0
Cm, Sm, Su	1	1	0	0
Tc	1	1	0	0
Total	19	17	2	0

[1]Tetracycline; [2]Chloramphenicol; [3]Streptomycin; [4]Triplesulfa; [5]Ampicillin.

the 19 had this pattern of resistance... i.e., 16 of 19 strains). The predominant pattern of resistance to (Tc, Cm, Sm, Su) is easily transferred to a sensitive *E. coli* strain. Resistance factors from 17 of 19 *S. sonnei* strains investigated were completely transferred to *E. coli* $K_{12}\overline{F}(\lambda)$. Two strains resistant to (Tc, Cm, Sm, Su) demonstrated only partial transfer of resistance as only the R-factors related to (Tc, Cm) were transferred. The transfer rate of the R-factor of the strains in the Caspian littoral is shown in Table 3. Hundred % of the strains isolated during the years 1997-2003 transferred their factors successfully to *E. coli* K_{12}.

The prevalence of *Shigella* resistant to multiple drugs is quite a serious problem from the standpoint of epidemiology and public health as resistance is transferred to pathogenic enteric organisms both directly and by way of non-pathogenic enteric bacteria such as *E. coli* and *Klebsiella* (7). Furthermore, the problem of multiple drug resistance is not limited to *Shigella* and *Salmonellae*, but is also a problem with all *Entrobacteriaceae* (8). Five percent of contaminated chicken or chicken products caused food-borne disease outbreaks in the United States in recent years (9).

Apart from this medical importance, multiple drug resistance offers interesting material for bacterial genetics. As mentioned in the introduction, the 'resistance transfer factor' is a new episome (extra chromosomal elements). Fifty-seven strains of *S. sonnei* isolated in the Caspian littoral were resistant to Triple-sulfa, Tetracycline, and Chloramphenicol (31.5%) and 1.7% to Ampicillin. This high percentage of resistance to antibiotics observed in our country may be related to the extensive usage of these drugs during the past decade as feed additives for growth promotion (10).

In other studies which examined on *E. coli* isolates showed high resistance to drug used, i.e., many workers showed drug resistance with *E. coli* isolated from poultry. Such development of multiple antibiotic-resistance strains in poultry could be due to the use of antibiotics as feed additives for growth promotion and prevention of disease, as confirmed by the present study. (11-13)

References

1. Yamuda M, Muto J, Kitazume JI, Suzuki M. The molecular epidemiological analysis of *Shigella sonnei* isolates in from outbreak cases in Yokohama. Kansenshogaka Jasshi. 73: 923-929 (1999)
2. Delappe NO, Halloran F, Fannings C-FG, Cheasty T, Cormican M. Antimicrobial resistance and genetic diversity of *Shigella sonnei* isolates in western Ireland, an area of low incidence of infection. J. Clin. Microbiol. 41:1919-1924 (2003)
3. Bograd A, Vanden E, London N, Driessen C, Stobberingh E. Antibiotic resistance of fecal *Escherichia coli* in poultry, poultry farmers, and poultry slaughterers J. Antimicrob. Chemoth. 47: 763-771 (2001)
4. Finegold SM, Baron EJ. Diagnostic Microbiology. 8th ed. The C. V. Mobsy. pp.120-289 (1990)
5. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 11th information supplement. M100-s11 NCCLS, Villanova, PA, USA (2001)
6. Gaikwad SS, Deodther LP. Shigellosis (antibiotic resistance and transfer of R-factor). J. Postgraduate Medicine 31: 24-27 (1985)
7. Yu HS, Lee JC, Kang HY, Jeong YS, Lee EY, Chui CH, Tea SH, Lee Y, Seol SY, Taekcho D. Prevalence of dfr genes associated with dfr Ah among urinary isolated of *Escherichia coli* in Korea. J. Antimicrob. Chemoth. 53: 445-450 (2004)
8. Quendnau M, Ahrne S, Petersson AC, Molin G. Antibiotic resistant strains of enterococcus isolated from Swedish and Danish retailed chicken and pork. J. Appl. Microbiol. 84: 1163-1170 (1990)
9. Turtura GC, Massa S, Ghazvinizadeh H. Antibiotic resistance among coliform bacteria isolated from carcusses of commercially slaughtered chicken. Int. J. Food Microbiol. 11: 351-354 (1990)
10. Ranjbar R, Soltan DM, Aslani M. Study of antimicrobial resistance of *Shigella* SPP. Isolated from patients referring to the five hospitals in Tehran during the last year. In: 6th Iranian Congress of Microbiology. Feb. 16-18, Tehran, Iran (2004)
11. Rafiiei TR, Nasirian A. Comparison of plasmid profile analysis, serotyping and antimicrobial resistance patterns of E. coli isolated from poultry in Tehran. Food Sci. Biotechnol. 13: 100-103 (2004)
12. Panigrahy B, Ling YS. Differentiation of pathogenic and non-pathogenic *Escherichia coli* isolated from poultry. Avian Dis. 34: 941-943 (1990)
13. Geomaras I, Hasting JW, Holy A. Genotypic analysis and their susceptibilities to antimicrobial agents. Appl. Environ. Microbiol. 67: 1940-1944 (2001)

Table 3. The transfer rate of R-factor of the strains in Caspian littoral

Dates	Number of			Percent of transfer
	Resistance rate	Non transfer rate	Transfer rate	
March 1997 - Feburary 1999	9	0	9	100%
March 1999 - Feburary 2001	4	0	4	100%
March 2001 - Feburary 2003	6	0	6	100%
Total	19	0	19	100%