

MULTIDRUG RESISTANT *SALMONELLA TYPHI* IN ASYMPTOMATIC TYPHOID CARRIERS AMONG FOOD HANDLERS IN NAMAKKAL DISTRICT, TAMIL NADU

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Abstract

Purpose: To screen *Salmonella typhi* in asymptomatic typhoid carriers and to find out drug resistance and ability of the strains to transmit drug resistance to other bacteria. **Methods:** Cultural characters, biochemical tests, antibiotic sensitivity test (disc diffusion), agarose gel electrophoresis, and conjugation protocols were done. Thirty five stool samples were collected from the suspected food handlers for the study. **Results:** Among 35 samples, (17.14%) yielded a positive result. Out of these 4 (20.0%) were women and 2 (13.33%) were men. The isolates were tested with a number of conventional antibiotics viz, amikacin, amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, co-trimoxazole, rifampicin, gentamicin, nalidixic acid, ofloxacin and tetracycline. Five isolates were having the multidrug resistant character. Four (66.66%) multidrug resistant isolates were found to have plasmids, while one (16.66%) multidrug resistant isolate had no plasmid and the chromosome encoded the resistance. Only one strain (16.66%) showed single antibiotic resistance in the study and had no plasmid DNA. The molecular weights of the plasmids were determined and found to be 120 kb. The mechanism of spreading of drug resistance through conjugation process was analyzed. In the conjugation studies, the isolates having R⁺ factor showed the transfer of drug resistance through conjugation, which was determined by the development of antibiotic resistance in the recipients. **Conclusion:** This study shows that drug resistant strains are able to transfer genes encoding drug resistance.

Key words: Asymptomatic typhoid carriers, antibiotics, drug resistance, plasmids, conjugation

Typhoid is one of the most wide spread of all bacterial diseases in India. The main source of typhoid is asymptomatic carriers. An individual can asymptotically carry the typhoid germ for days to years without showing any of symptoms of typhoid fever. In such carriers, the typhoid bacillus continues to multiply in the gall bladder. It reaches the intestine through the bile duct. The silent carriers are the source of typhoid germs for the continued episodes of infections. Women exceed men as carriers by a ratio of 3:1.^{1,2}

The rate of resistance development in bacteria has been found to be increasing. Several disease causing bacteria including typhoid causing *Salmonella* species have now become resistant to one or more antibiotics.³ The population of multi drug resistant (MDR) *Salmonella typhi* is steeply increasing in the Indian subcontinent, Southeast Asia and other geographical regions. The emergence of drug resistant pathogens poses a major challenge to the treatment and prevention of typhoid. In particular, the concern is about the spread of these multi drug resistant strains to rural India.⁴ The main focus of this study is to determine the ability of the

isolates to transfer the drug resistance by conjugation.

Materials and Methods

Stool samples (35) were collected from the individuals of food handlers to screen the asymptomatic typhoid carriers. They were processed for the presence of *Salmonella typhi*. The strains were identified by biochemical reactions and by slide agglutination using monospecific antisera.⁵

Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion method. Overnight cultures in peptone water were lawned over the Mueller-Hinton agar (Hi-media). The antibiotic discs were used at the following concentration: ampicillin (A) 30 µg, amikacin (Ak) 30 µg, amoxicillin (Am) 25 µg, chloramphenicol (C) 25 µg, ciprofloxacin (Cf) 30 µg, co-trimoxazole (Co) 25 µg, gentamicin (G) 30 µg, nalidixic acid (Na) 30 µg, ofloxacin (O) 30 µg, rifampicin (R) 30 µg, tetracycline (T) 10 µg (Hi-media).

Plasmid profile analysis was done as described by Kodo and Liu⁶ and chromosomal DNA analysis was done as described by Grimberg *et al.*⁷ Conjugation ability of the isolates was determined by Cappuccino and Shermann procedure.⁸

Results

This study revealed 16.66% (6/35) subjects to be asymptomatic typhoid carriers. Of these 20.0% (4/35) were women and 13.33% (2/35) were men in the age group of 15-

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Table 1: Antimicrobial activity of various antibiotics against isolates

Antibiotics (µg)	Isolates no. and zone of inhibition (mm)					
	SS-1	SS-2	SS-3	SS-4	SS-5	SS-6
A (25)	9 (R)	17 (S)	13 (R)	20 (S)	13 (R)	18 (S)
Ak (30)	17 (S)	18 (S)	17 (S)	12 (R)	10 (R)	12 (R)
Am (25)	18 (S)	13 (R)	13 (R)	19 (S)	19 (S)	18 (R)
C (25)	12 (S)	11 (R)	12 (R)	12 (R)	12 (R)	19 (S)
Cf (30)	21 (S)	22 (S)	21 (S)	21 (S)	21 (S)	15 (R)
Co (25)	16 (S)	17 (S)	16 (S)	10 (R)	10 (R)	18 (S)
G (30)	15 (S)	15 (S)	16 (S)	16 (S)	15 (S)	12 (R)
Na (30)	20 (S)	20 (S)	21 (S)	12 (R)	20 (S)	20 (S)
Of (30)	19 (S)	20 (S)	19(S)	19(S)	14(R)	20(S)
R (30)	21 (S)	16 (S)	15 (R)	15 (R)	14 (R)	15 (R)
T (10)	19 (S)	10 (R)	11 (R)	10 (R)	10 (R)	10 (S)

A – Ampicillin, Ak – Amikacin, Am – Amoxicillin, C – Chloramphenicol, Cf – Ciprofloxacin; Co – Cotrimaxazole, G – Gentamicin, Na – Nalidixic acid, Of – Ofloxacin, R – Rifampicin; T – Tetracycline; S – Sensitive, R – Resistant

Table 2: The percentage of antibiotic resistance pattern shown by isolates

Antibiotics	Total No. of resistant isolates	(%)
A	3	50.00
Ak	3	50.00
Am	2	33.33
C	5	83.33
Cf	1	16.66
Co	2	33.33
G	1	16.66
Na	1	16.66
Of	1	16.66
R	5	83.33
T	5	83.33

A – Ampicillin, Ak – Amikacin, Am – Amoxicillin, C – Chloramphenicol, Cf – Ciprofloxacin; Co – Cotrimaxazole, G – Gentamicin, Na – Nalidixic acid, Of – Ofloxacin, R – Rifampicin; T – Tetracycline

45. Table 1 shows the characteristic antibiotic resistant patterns of the representative strains in antibiotic sensitivity test.

A varying percentage of drug resistance patterns of total isolates against individual antibiotics were observed (table 2).

Out of various *Salmonella typhi* isolates, 83.33% were multidrug resistant and 16.66% were single drug resistant. These multidrug resistant strains were having 120 kb plasmids, which were detected in agarose gel by using Hind III digest of DNA as a marker. The chromosomal DNA alone was encoding for resistance to nalidixic acid and ciprofloxacin that was confirmed in a strain lacking plasmid.

In conjugation studies, T^R C^R strains (isolates) were used as donors against T^S C^S (*E. coli* CSH 57) strain as a recipient,

Table 3: Transfer of drug resistance pattern of isolates through conjugation

Isolates (donor)	Recipient strain	Resistant occurrence	Total %
SS-1 C ^R T ^S	<i>E. coli</i> CSH57 S ^R T ^S C ^S	—	66.6
SS-2 Am ^R C ^R R ^R T ^R	<i>E. coli</i> CSH57 S ^R T ^S C ^S	C ^R T ^R	
SS-3 A ^R Ak ^R Am ^R C ^R R ^R T ^R	<i>E. coli</i> CSH57 S ^R T ^S C ^S	C ^R T ^R	
SS-4 C ^R Co ^R G ^R R ^R	<i>E. coli</i> CSH57 S ^R T ^S C ^S	C ^R	
SS-5 A ^R Ak ^R C ^R G ^R O ^R R ^R	<i>E. coli</i> CSH57 S ^R T ^S C ^S	C ^R	
SS-6 Cf ^R T ^S	<i>E. coli</i> CSH57 S ^R T ^S C ^S	—	

66.66% recipient strains acquired resistance to tetracycline and chloramphenicol (table 3).

Discussion

Poor personal hygiene and inadequate food handling can potentiate the transmission of *Salmonella typhi*. Several food products kept at room temperature were found to favour the growth of *Salmonella* species. The spread of typhoid is associated with 20% of the newly identified carriers and also 9% previously identified carriers.⁹ The food handlers prominently play a role in disseminating typhoid bacilli through different food products and water.

The susceptibility to typhoid fever lies between the age 15-25 years.¹⁰ Carrier state of typhoid was observed in the age group 15-45 years in this present investigation.

In various *Salmonella typhi* isolates, 78.4% were resistant to the antibiotics such as ampicillin, chloramphenicol and trimethoprim which are conventionally used to treat typhoid fever.¹¹ There was a gradual increase in the time period in mean minimum inhibitory concentration of ciprofloxacin.¹² In this study, isolates were least resistant to ofloxacin, ciprofloxacin, gentamicin and nalidixic acid, while high resistance to tetracycline, rifampicin and chloramphenicol was also observed.

The isolates were not identical at developing antibiotic resistance. Some isolates of *Salmonella typhi* showed the resistance to chloramphenicol, ampicillin, streptomycin and tetracycline, while sensitive to gentamicin. Mirza and Hart had also observed similar results.¹³

Drug resistance is due to acquisition of plasmid encoded inactivating β -lactamase and chloramphenicol acetyl transferase. Ceftriaxone or fluoroquinolone is highly effective. Ciprofloxacin is effective in treatment of carrier state.¹⁴ However, one of the isolates in this study showed resistance to ciprofloxacin.

Plasmid encoded resistance to chloramphenicol, amikacin, ampicillin and chromosome encoded resistance to nalidixic acid and ciprofloxacin have been reported.¹⁵ Plasmid (120 kb) bearing strains were found similar to the study of Karmaker *et al.*¹⁶ In the present study, 33% of strains showing chromosomal DNA encoded resistance to ciprofloxacin and nalidixic acid were observed.

Transferable plasmids have been found in fourteen strains and six of them expressed multiple drug resistance.¹⁵ The plasmids were transferred by 66.66% strains during conjugative recombination in the present study which showed multidrug resistance plasmid. In contrast, the strain-SS-1 showing single antibiotic resistance had no plasmid. The strain SS- 6 has also no plasmid, but its resistance to ciprofloxacin was due to chromosomal DNA.

Indiscriminate usage of chemotherapeutic agents in the treatment of various diseases leads to occurrence of drug resistance. From this study it was concluded that drug resistant was found in the isolates from asymptomatic typhoid carrier. *In vivo* transfer of resistant genes to normal microbial flora pose hazard to human kind.

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