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Screening of Asymptomatic Typhoid Carriers from Nail Samples from Roadside Food Handlers

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Abstract: In the present study, to screen the asymptomatic typhoid carriers and find out the emergency of drug resistant by antimicrobial susceptibility test. The 25 nail bits samples were collected from the food handlers (Roadside hotels) from the different age group people. Among 25 nail samples, 5 samples show a positive result. Positive results were identified by growing on selective media such as Bismuth Sulphite Agar media and it was further conformed by various biochemical tests such as Triple Sugar Iron Agar (TSI) test, Indole test, Methyl Red (MR) test and Voges-Proskeur test (VP) etc. In this 4 were women and 1 was men. These isolates were further tested with a number of conventional antibiotics viz: Amikacin, Amoxicillin, Ampicillin, Chloramphenical, Ciprofloxacin, Co-trimazole, Rifamycin, Gentamycin, Nalidixic acid and Tetracycline. Among these 4 isolates show MDRS and 1 isolate show resistance to Chloramphenicol and Gentamycin towards Amikacin, Amoxicillin, Ampicillin, Rifamycin, Nalidixic acid.

Key words: Salmonella carriers, biochemical characterization, multiple drug resistance strains, antibiotic drug resistance

INTRODUCTION

Most of this burden occurs among citizens of low-income countries, particular those in Southeast Asia, Africa and Latin America. In South Sulawesi, Indonesia, typhoid fever is one of the most important infectious diseases. The disease is endemic throughout the region and is the fourth most frequently reported infectious disease in most of its 24 districts. In South-Sulawesi, typhoid is the most important cause of community-acquired septicemia, with a reported incidence rate exceeding 2,500/100,000 in many districts (Hatta *et al*., 2007). Current statistics for food borne illness in various countries may be caused by poor food handling techniques and by contaminated food served in food service establishment (Sen *et al*., 2007).

Typhoid is one of the most wide spread of all bacterial diseases in world. The main source of typhoid is asymptomatic carriers. An individual can asymptomatically 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 rate of resistance development in bacteria has been found to be

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increasing. Several disease causing bacteria including typhoid causing *Salmonella* species have now become resistant to one or more antibiotics. The population of Multi Drug Resistant (MDR) *Salmonella typhi* is steeply increasing in the Indian subcontinent, Southeast Asia and other geographical regions (Senthil-Kumar and Prabakaran, 2005).

Ciprofloxacin is the drug of choice for treating typhoid fever in areas where, Multidrug-Resistant (MDR) *Salmonella typhi* strains are prevalent. In India, this trend started in 1991 when the resistance of *Salmonella typhi* strains to Chloramphenical and other antimicrobial agents reached its peak. Recently, however, there have ben reports of Ciprofloxacin resistance being detected among *Salmonella typhi* strains, leading many clinicians to question the efficacy of this drug. These reports have been based on limited numbers of strains, representing small geographical areas. In an effort to obtain a more comprehensive view of the situation in India (Mehta *et al*., 2001).

Salmonella typhi is noteworthy in the etiology of outbreaks and sporadic cases of typhoid fever, which remains as an important public health problem, causing 16 million cases of the disease and 600,000 deaths, annually all over the world (Ivanoff, 1995). Paratyphoid fever is also an endemic disease in developing countries, but its incidence is lower than that of typhoid fever (Arya and Sharma, 1995). These bacteria can continue to grow in gall bladder and reach the intestine through the bile duct in his case of carriers. The silent carrier's contribute to continued episodes of infection.

The infection is acquired by the infection of *Salmonella typhi* through faecal contamination of water and food (Egoz *et al*., 1998). Carriers such as food handlers are an important source of transmission (Cote *et al*., 1995). An individual can asymptomatically carry the typhoid bacilli for days to years without slowing any of the symptoms of typhoid fever. Food borne diseases continue to be a major public health problem in the developed and developing worlds alike.

Typhoid fever caused 21,650,974 illness and 216,510 deaths annually. Several reports indicated Multiple Drug Resistance Strains (MDRS). *Salmonella typhi* with plasmid mediated resistance to conventional antibiotics like chloamphenicol, ampicillin and cotrimoxazole thriving in different part of the world (Wain *et al*., 1997). Since 1997, infection with nalidixic acid resistant *Salmonella typhi* and (*Salmonela enterica serovar typhi)* with decreased susceptibility to ciprofloxacin has been reported from Vietnam, Tajikistan, the screen the asymptomatic carriers who can act as a source of infection and also find out the emergency of drug resistance by Antimicrobial Susceptibility test (Sridhar *et al.,* 1983).

MATERIALS AND METHODS

Nail bits were collected as a sample around Namakkal, Tamilnadu, India, from individual of different age group of people ranges from (18 to 40 years) to screen the asymptomatic typhoid carrier with the period of 2007-2008. The samples were transported to the laboratory by using screw-capped tubes containing enrichment medium as Selenite F broth. The broth was incubated at 37°C for 24 h. After 24 h, the sample was processed and identified the presence of *salmonella typhi* by growing on selective media such as Bismuth Sulphite Agar and it was further conformed by various Biochemical test such as Indole test, MRVP test, Citrate Utilization test and Triple Sugar Iron test etc. to identify an entrobacteriace.

Antimicrobial Susceptibility Patterns

Antimicrobial susceptibilities were determined by Kirby Bauer Disc diffusion method described by Bauer *et al*. (1966). The isolates were dichtornized as either susceptible or

non-susceptible based on minimum inhibitory concentration values with isolates determined to be intermediate or resistant categories as non-susceptible. The antibiotic disc were used at the following as Ampicillin, Amphotericin, Amikacin, Cifrofloxacin, Chloramphenicol, Gentamicin, Nalidixic acid, Rifampicin and tetracycline.

RESULTS

In this study, 25 samples were processed and 5 samples from this shows positive results. The results were shown in the Table 1, which indicates the presence of typhoid bacilli in the nail sample or the individual can asymptomatically harbor the typhoid bacilli without showing any symptoms of typhoid fever. Present study revealed that out of 25 samples of food handlers, four women samples and one male sample in the age group of 18-40 shows the presence of typhoid bacilli. The positive isolates were identified by streaking on selective media such as Bismuth sulphite agar (Fig. 1). MDRS of *Salmonella typhi* has been reported in India since 1960 and outbreaks by these strains occur at intervals in various parts of India. In this study, 4 isolated shows MDRS to Ampicillin, Amoxicillin, Amphotericin, Amikacin, Nalidixic acid and Rifampicin and 1 isolates show resistance to Gentamicin and Chloramphenicol (Fig. 2). Antimicrobial susceptibility pattern of *Salmonella typhi* of various isolates showed the zone of inhibition represented in Table 2 followed by this, it indicates the isolates were either sensitive or resistance or intermediate to various

Table 1: Characterization of *Salmonella typhi* from nail sample

Test	Result
Gram's staining	Negative
Methyl red	Positive
Indole test	Negative
Vogus-proskauer	Negative
Triple sugar iron test	Alkaline slant, Acid butt, H_2S production
Citrate utilization test	Positive

Fig. 1: Growth of *Salmonella typhi* on Bismuth sulphite agar

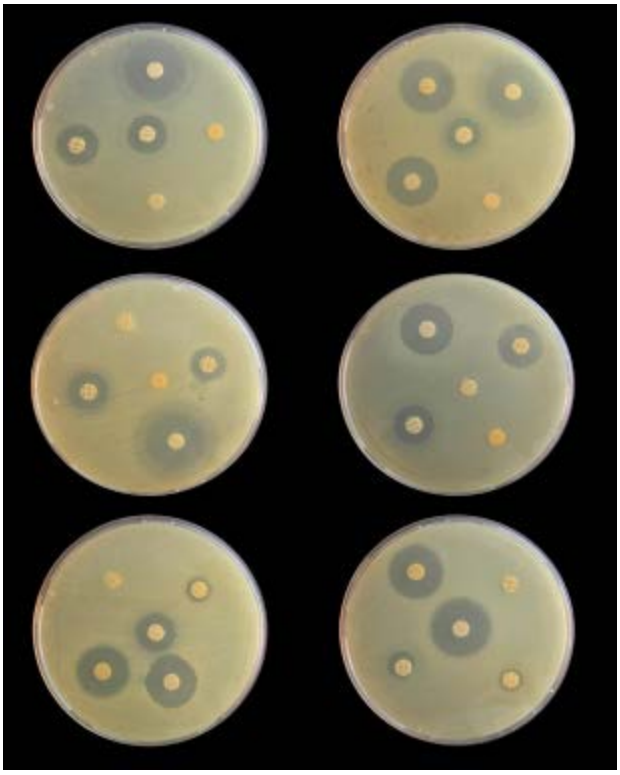

Fig. 2: Antimicrobial susceptibility pattern of *Salmonella typhi* of various isolates

Table 2: Antimicrobial susceptibility pattern of *Salmonella typhi* of various isolates

	Isolates No. and zone of inhibition (mm)				
Antibiotics	S-1	S-2	S-3	S-4	S-5
Ampicillin (A)	R(16)	R(14)	R(15)	R(10)	R(18)
Amphotericin (Am)	R(15)	R(15)	R(13)	R(10)	S(17)
Amikacin (Ak)	R(16)	R(14)	R(16)	R(16)	S(18)
Amoxycilin (Am)	R(17)	R(14)	R(18)	R(18)	S(15)
Cifrofloxacin (Cf)	S(24)	S(21)	S(23)	S(28)	S(25)
Chloramphenicol (C)	S(24)	S(21)	S(22)	R(16)	S(22)
Gentamicin (G)	S(11)	S(16)	S(18)	S(17)	S(16)
Nalidixic acid (Nal)	R	R	R	R(10)	R(9)
Rafampicin (R)	R(10)	R(10)	R(10)	R(10)	R(10)
Tetracycline (T)	S(28)	S(28)	S(25)	R(10)	S(22)

Table 3: The percentage of antibiotic resistant pattern of *Salmonella typhi* of various isolates

Antibiotics	Total No. of resistant isolates	Percentage
Ampicillin (A)	5	100
Amphotericin (Am)	4	80
Amikacin (Ak)	4	80
Amoxycilin (Am)	4	80
Cifrofloxacin (Cf)	No	-
Chloramphenicol (C)	1	20
Gentamicin (G)	1	20
Nalidixic acid (Nal)	5	100
Rafampicin (R)	5	100
Tetracyclin (T)	1	20

antibiotics disc. These were identified by measuring the zone of inhibition and it was compared with a standard chart. The percentage of antibiotics resistance against *Salmonella typhi* measured at statistics results were represented in the Table 3.

DISCUSSION

Typhoid fever is endemic in all parts of India. Poor sanitation can act as a potential source for the transmission of *Salmonella typhi.* Poor personal hygiene and inadequate food handling can act as a potential source for the transmission of *Salmonella typhi.* Several food products kept at room temperature were found to favour the growth of *Salmonella species* (Senthil-Kumar and Prabakaran, 2005).

Gibson (1987) described a Selenite F broth for the detection of *Salmonella* in foods by automated conductance measurement and showed that positive results could usually be obtained within 24 h. In the present study, 25 samples were collected in different places from different food handlers. Normally these food handlers are very poor in hygienic condition, when compared with first class hotel worker; there is more probability to spread the antibiotic resistance bacteria to the susceptible person.

Carrier people serve as the reservoir of typhoid fever, when comparing women with men, the women as a carrier by a ratio of 3:1 (Hornick *et al*., 1970). In the present study revealed among food handlers, women are highly served as a carrier for typhoid fever. In this study we collected nail bits samples to screen the carriers. From this, all the positive isolates were shows susceptible to ciprofloxacin. There are several reports has revealed to screen the ciprofloxacin resistant strain from the blood sample in the report 24 isolates shows resistant to ciprofloxacin only one strain sensitive to ciprofloxacin (Raverndran *et al*., 2008).

In present report, all the strains showed multi drug resistant to ampicillin, amikacin, nalidixic acid. Low ever these strains susceptible to Gentamicin, Chloramphenical (Onyango *et al*., 2008). They isolated *Salmonella typhi* form blood samples it showed resistant to Streptomycin, Ampicillin, Chloramphenical and Cotrimoxazole.

Food handlers are susceptible for the spreading of bacilli due to improper sanitation. Unless washing with disinfectants they cannot be eliminated easily from the body surface. Moreover nail bits can act as a favorable environment for their growth. These food handlers could easily contaminate the water and food stuffs while handling them. So, they can act as a source of infection. In present results shows that, one isolate were resistant to Ciprofloxacin. When we compared with as Musgrove *et al*. (2009), 2.6% were resistant to Ceftriaxone and Cipofloxacin and one isolate was resistant to Ciprofloxacin.

All the isolates in this study were sensitive to Ciprofloxacin but one strain resistant to Ciprofloxacin. However, Ciprofloxacin suggests no longer is considered the drug of choice in treating salmonella infections due to its high level resistance. In this present study, the positive isolates showing resistance to Nalidixic acid and Ciprofloxacin. One strain showing resistant to Chloramphenical. The Chloramphenical resistance in *salmonella typhi* is plasmid mediated where as Nalidixic acid resistance is chromosomal. Hence this association is likely to be coincidental (Ray *et al*., 2006).

In this study, out of the 5 *Salmonella typhi* isolates tested, all the strains shows Multiple Drug Resistant to ampicillin, Nalidixic acid and Rifampicin. However our results are compared with predictive efficacy of nalidixic acid resistance as a marker of fluroquinolone resistance is a matter of concern (Ray *et al*., 2006). Detection of nalidixic acid resistance as a predictor for decreased fluroquinolone susceptibility in *Salmonella* has been reported (Butt *et al*., 2006).

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