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Characterization of Multidrug Resistant Patterns of *Salmonella* sp.

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Abstract: Twenty fecal samples have been collected from various clinical laboratories in Viruthunagar district, Tamilnadu. Among this, 19 samples were positive for *Salmonella* sp. and they were identified after enrichment by standard microscopic, cultural characteristic and biochemical characterization. Among this; six, seven and six isolates were characterized as *S. typhi*, *S. paratyphi* A and S. *paratyphi* B, respectively. They were tested against 13 different antibiotics by Kirby Bauer method. Among the 19 isolates high rate of resistance was noticed.

Key words: Antibiotic · *Salmonella* sp · Kirby Bauer · MDR

INTRODUCTION

Salmonella is a Gram negative facultative rod shaped bacterium, trivially known as "enteric bacteria". *Salmonella typhi*, the etiologic agent of typhoid fever, is a human restricted pathogen. Typhoid fever is still a major disease in endemic areas of the world. There are over sixteen million of cases of typhoid fever reported in the 1990's into 600,000 deaths annually worldwide [1].

The molecular mechanism of Salmonella pathogenicity is complex. The investigations of the molecular mechanisms of Salmonella virulence factors have shown that pathogenic *Salmonella* sp. are distinguished from their non-pathogenic relatives by the presence of specific pathogenicity genes, often organized in so-called pathogenicity islands (PIs) [1, 2]. The genus Salmonella infect human beings leading to enteric fever, gastroenteritis and septicemia and parasite the underline of a large number of vertebrate species. These infections are the major causes of morbidity and mortality in developing world resulting in over quarter of all childhood deaths [3].

Globally Salmonella are the major causative agents associated with acute enteric infection. Environmental source of organism includes water, soil factor, kitchen, animal feces, raw milk and meat. All age groups are susceptible to salmonellosis, but it is more severe in elderly infants. In Japan according to statistics of food poisoning by the Ministry of Health, Labour and Welfare, cases of bacterial food poisoning totally reach 27741 in 1993, 32417 in 2000, 15753 in 2001 and 17533 in 2002. Salmonellosis accounted for 43% in 1999, 21% in 2000, 31% in 2001 and 33% in 2002 [4].

In recent years, the prevalence of antimicrobial resistant bacterial pathogens has become a major public health concern and increasing antimicrobial resistance in *Salmonella enterica* is a serious clinical problem worldwide. The majority of the *Salmonella* sp. has acquired multidrug resistance which means that they have outdated to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines [5]. The expression of CTX-M mediated resistance gene present in *Salmonella* sp. there by limiting the therapeutic option for typhoid fever [6].

Non-typhoid salmonellosis is one of the most frequently-reported bacterial foodborne diseases and is a major economic and public health issue worldwide. In the United States, Salmonella serotypes cause an estimated 1.4 million cases of foodborne disease each year and the primary reservoirs of Salmonella are food-producing animals, the three main sources being poultry, cattle and pigs [7]. Of the numerous different serotypes, only a few are frequently isolated from human and animal sources. Serotypes enteritidis and typhimurium are the most frequently encountered in human and animal sources [8]. In above background information, the present work was mainly focused on the assessment of the effect of various antibiotics against *Salmonella* sp.

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MATERIALS AND METHODS

Sample Collection: From January 2007 to December 2009, 20 fecal samples were collected from children hospitalized in a Paediatric ward and adults in Sivakasi, Tamilnadu, India.

Bacteriological Analysis: Collected samples were transported immediately to the laboratory for analysis. They were manipulated according to conventional microbiological methods and *Salmonella* were identified based on biochemical characteristics [9].

Serological Identification: Strains with *Salmonella* biochemical characteristics were confirmed with polyvalent anti-*Salmonella* serum and the positives were sent to the lady hardinge medical hospital, New Delhi, for serotyping.

Antibiotic Susceptibility Testing: Kirby-Bauer method [4] was carried out. An overnight growth broth of each Salmonella strain (OD-0.6) was spread on the surface of Muller Hinton agar plates. The antibiotic discs (amikacin 30mcg, cephotaxime 30mcg, chloramphenicol 30mcg, ciprofloxacin 5mcg, gentamicin 10mcg, kanamycin 30mcg, nalidixic acid 30mcg, neomycin 30mcg, norfloxacin 10mcg, ofloxacin 5mcg, piperacillin 100mcg, streptomycin 10mcg and co-trimazine 1.25/23.75mcg) were placed on the surface of the inoculated plates in such a way that each disc was made to adhere perfectly to the surface of the agar by gently pressing. The plates were incubated at 37°C for 24 hours and after incubation the zones of inhibition were measured with standard chart [4]. The diameters of zones of inhibition of MDR were compared with the critical zones diameters in the published tables (ATCC).

RESULTS

Biochemical Characterization: Out of 20 samples analyzed 19 *Salmonella* species were obtained including 6 *S. typhi*, 7 *S. paratyphi* A and 6 *S. paratyphi*.

Antibiotic Sensitivity Patterns of *Salmonella* Spp.: Antibiotic susceptibility patterns of these isolates were studied against 13 different antibiotics by Kirby Bauer method (Table 1).

DISCUSSION

The major reason for the presence of MDR among *Salmonella* spp. is the inappropriate use of antibiotics due to lack of uniform policy and disregard to hospital infection control practices [10]. Among the 19 isolates, high rate of resistance was noticed against all the tested antibiotics.

In recent years, the prevalence of antimicrobial resistant bacterial pathogens has become a major public health concern and increasing antimicrobial resistance in *Salmonella enterica* is a serious clinical problem worldwide [11]. During the last decade, a single clone of multi resistant *S. enterica* serovar *typhimurium* of phage type DT104 has spread worldwide. This *Salmonella* type has emerged within a few years as one of the most common causes of human salmonellosis in several countries. The majority of the DT104 isolates have a multidrug resistance phenotype called ACSSUT; which means that they are resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline [11].

In the present study, the isolates showing resistance against greater than 3 antibiotics were considered as MDR strains. All the 19 isolates had MDR; 6 *S. typhi*, 7 *S. paratyphi* A and 6 *S. paratyphi* B. The isolate 1 (*S. paratyphi* B) showed resistance towards amikacin, cephotaxime, gentamicin, neomycin, norfloxacin, piperacillin, chloramphenicol, ciprofloxacin, kanamycin, nalidixic acid, ofloxacin, streptromycin and Co-Trimazine.

The isolate 2 (*S. paratyphi* B) was resistant to amikacin, ciprofloxacin, ofloxacin, piperacillin, cephotaxime, chloramphenicol, gentamicin, kanamycin, nalidixic acid, neomycin, norfloxacin, streptomycin and co-trimazine and sensitive to piperacillin. The isolate 3 (*S. paratyphi* A) conferred resistance against amikacin, cephotaxime, ciprofloxacin, gentamicin, nalidixic acid,

Table 1: Drug resistant and sensitive patterns of the 19 tested *Salmonella* isolates

	Ak	Ce	C	Cf	G	K	Na	N	Nx	Of	Pc	S	Cm
Isolates	30 mcg	30 mcg	30 mcg	5 mcg	10 mcg	30 mcg	30 mcg	30 mcg	10 mcg	5 mcg	100 mcg	10 mcg	1.25/23.5 mcg
Resistant No. (%)	13(68.4)	18(94.7)	12(63.2	11(57.9)	12(63.2)	15(78.9)	14(73.7)	13(68.4)	11(57.9)	12(63.2)	15(78.9)	15(78.9)	13(68.4)
Sensitive No. (%)	6(31.6)	1(5.3)	7(36.8)	8(42.1)	7(36.8)	4(21.1)	5(263)	6(31.6)	8(42.1)	7(36.8)	4(21.1)	4(21.1)	6(31.6)

Ak-Amikacin; Ce-Cephotaxime; C-Chloramphenicol; Cf-Ciprofloxacin; G-Gentamicin; K-Kanamycin; Na-Nalidixic acid; N-Neomycin; Nx-Norfloxacin; Of-Ofloxacin; Pc-Piperacillin; S-Streptomycin; Cm-Co-trimazine

neomycin, ofloxacin, piperacillin, chloramphenicol, kanamycin, streptomycin and co-traimazine. Therlfall *et al*. [12] reported that 2% of *S. typhi* isolates are resistant to ciprofloxacin among the 13% multidrug resistance strains of *S. typhi*. In this work, highest level of resistance was observed against cephotaxime (94.73%), kanamycin (78.94%), piperacillin (78.94%) and streptomycin (78.94%). In 2003, 80% of strains from Ahamadabad were found resistant to chloramphenicol, ampicillin and trimethoprim but sensitive to ciprofloxacin and ceftriaxone whereas, 86.5% of the strains from Rourkela were found sensitive to chloramphenicol and 100% to ceftriaxone. Resistance to nalidixic acid and ciprofloxacin has also begun to emerge a national survey found that, among the 4008, *Salmonella* isolates tested, 21 (0.5%) were resistant to ciprofloxacin, by 2000, the rate of nalidixic acid resistance had increased 5 fold to 25% [13].

The emergence of antimicrobial resistant pathogens now threatens the discovery of potent antimicrobial agents. Antimicrobial resistance has resulted in increased morbidity and mortality as well as health care costs. Yearly expenditures arising from drug resistance in the United States are $4 billion and are rising [14]. The isolate 11 (*S. paratyphi* B) showed resistance against chloramphenicol, ciprofloxacin, gentamycin, neomycin, norfloxacin, cephotaxime, kanamycin, nalidixic acid, ofloxacin, piperacillin, streptomycin, co-trimazine and Amikacin. These results are similar to those of several workers [15-18].

The isolate 12 (*S. paratyphi* A) showed resistance towards amikacin, ciprofloxacin, ofloxacin, cephotaxime, chloramphenicol, nalidixic acid, neomycin, norfloxacin, streptomycin and co-trimazine and sensitivity to gentamycin, kanamycin and piperacillin. The isolate 13 (*S. typhi*) was resistant against chloramphenicol, gentamicin, nalidixic acid, piperacillin, amikacin, cephotaxime, ciprofloxacin, kanamycin, neomycin, norfloxacin, oflaxacin, streptomycin and co-trimazine. The results are similar to the results of Archibald *et al.* [19].

In the present work 100% of *S. typhi* isolates showed multidrug resistance. Randall *et al.* [20] found that 55% of *S. typhi* isolates are susceptible to all antibiotics and 45% of *S. typhi* isolates are resistant to more than three antibiotics. Thi *et al.* [21] found that *S. enterica* serovar *typhi* strains isolated in Vietnam are resistant to ampicillin, chloramphenicol, tetracycline, streptomycin and cotrimoxazole. Sandvang *et al*. [22] reported that 78.7% of *Salmonella* sp. are MDR during 1995 to 1997, 89% are MDR during 1998 to 2002 and 18% of *S. typhi* are susceptible. These reports slightly varied from present work.

These studies revealed the complex set of pathogenic interferences between intracellular *Salmonella* and its host cells. The understanding of how *Salmonella* evade the host defense system and establish pathogenesis will be important for proper disease management.

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