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Phenotypic and genotypic characterization of multidrug resistant Salmonella enterica serovar Typhi isolated from a tertiary care centre.

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Introduction

Typhoid fever is one of the most common bacterial diseases in Indian sub continent. Carriers are found to be one of the cause of typhoid. A person may hold the typhoid germ asymptomatically for days to years without showing any signs or symptoms of typhoid fever. The typhoid bacillus continues to multiply in the gall bladder of such carriers. The bile duct transports it to the intestine. The silent carriers are the source of typhoid germs that cause infections to recur[1]. With over 21.6 million cases and at least 250,000 deaths per year, it remains a global public health problem. Asia accounts for almost 80% of cases and deaths, with Africa and Latin America accounting for the remainder. The disease has an incidence ranging from 102 to 2,219 per 100,000 of the population in developing countries like India[2].

Enteric fever, septicaemia without localization, focal disease gastroenteritis, and the carrier condition are the five clinical types of Salmonella infection in humans. Salmonella typhimurium, Salmonella choleraesuis, and Salmonella typhi are all capable of causing any of these syndromes, although some serotypes are linked to specific symptoms, such as gastroenteritis, septicaemia, and enteric fever in the carrier state. The typhoid salmonellas and S. choleraesuis are the most invasive in the immune-competent host, so this behavior represents their intrinsic virulence. Several other factors play a role in salmonellosis pathogenesis, and changes in locally protective mechanisms can increase susceptibility to invasive disease. The increasingly common association of multiple drug resistance with non-typhoid salmonella strains is of therapeutic significance in this situation[3].

The advent of S. Typhi strains resistant to antibiotics prescribed for treatment has exacerbated the high incidence rates of typhoid fever. Antibiotic-resistant S. Typhi is a significant concern,

according to the Centers for Disease Control which Prevention (CDC), and needs constant surveillance and prevention to prevent the spread of resistant strains. Chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole were once the first-line antibiotics for treating typhoid. In the late 1980s, however, multidrug-resistant S. Typhi, identified as strains resistant to these first-line antibiotics, appeared[4].

S. Typhi is genetically monomorphic, which has traditionally limited S. Typhi molecular surveillance until the advent of high-throughput whole genome sequencing (WGS). This global genomic framework for S. Typhi revealed that the majority of MDR S. Typhi infections worldwide are linked to a single phylogenetic lineage, genotype 4.3.1 (H58 in the legacy scheme), that has spread from South Asia to East Africa since the 1990s. In West Africa, however, MDR S. Typhi is linked to a particular genotype, 3.1.1. The MDR phenotype is encoded by a hybrid transposon carrying genes conferring resistance to five drug groups, including chloramphenicol, penicillins, and co-trimoxazole, and the determinants are usually found on IncH1 plasmids in both S. Typhi 4.3.1 and 3.1.1. (plasmid sequence type PST6 in 4.3.1, and PST2 in 3.1.1). In S. Typhi, resistance to newer agents is also on the rise. Reduced fluoroquinolone susceptibility caused by QRDR mutations is common in S. Typhi 4.3.1, particularly lineage II, but rare in 3.1.1 and other genotypes. In South Asia, the prevalence of S. Typhi 4.3.1 QRDR triple mutants (carrying two mutations in gyrA and one in parC) showing total resistance to ciprofloxacin is increasing. WGS research revealed that recent gatifloxacin treatment failure in Nepal was caused by the introduction of a 4.3.1 lineage II triple mutant from India, triggering a rethinking of the region's dependence on fluoroquinolones. In Pakistan, an outbreak of S. Typhi 4.3.1 was recorded, with resistance to ceftriaxone, ciprofloxacin, and all three first-line antibiotics. The outbreak strain had the MDR composite transposon inserted in the chromosome at yidA, as well as an E. coli IncY plasmid containing the ESBL gene blaCTX-M-15 (which confers resistance to ceftriaxone and other third-generation cephalosporins) and the quinolone resistance gene qnrS. This strain has been classified as XDR (extensively drug resistant, characterized as resistance to chloramphenicol, penicillins, co-trimoxazole, ceftriaxone, and ciprofloxacin) and has severely limited treatment options, with azithromycin being the only oral antibiotic left[5].Hence the present study is planned to elucidate the prevalence and antimicrobial susceptibility pattern of Salmonella Typhi from patients attending tertiary care centre in Centrel India.

Materials and Method

The present study was conducted at Department of Microbiology, Index Medical college hospital & Research centre, Indore. Ethical clearance was obtained for the study. The period of study was 3 years and the study population consisted of 140 patients of different age groups attending outpatient department and the patients admitted in different wards. Blood samples were collected from the study subjects for culture.Study subjects yielded other than S.enterica serovar Typhi are excluded from the study.

Identification of Salmonella Typhi:

Blood samples were collected from patients with fever of unknown origin and transferred to sterile brain heart infusion broth (Himedia Ltd,Mumbai), with the specifics of the cultures being registered. The culture bottle were incubated at 37^0C for 18-24 hours and subcultures were made on sterile Mac Conkey's agar and Deoxy citrate agar plates, which were then incubated overnight at 37°C. Non lactose fermenting colonies on Mac Conkeys agar were further processed with standard procedures to identify the bacteria till species level.

Slide agglutination tests:

Anti-Salmonella agglutinating sera containing Salmonella Typhi and Para typhi A & B, which are used for rapid serotyping of Salmonella species, were used in the slide agglutination experiments. The agglutination process was observed with the naked eye[6,7].

Antibiotic susceptibility testing[8]

Antibiotic susceptibility testing was performed on the Salmonella typhi isolate using the Kirby-Bauer disc diffusion technique in accordance with CLSI guidelines[8]. As a control, Salmonella typhi ATCC 13311 was used.

Agar dilution tests:

Following the determination of isolate susceptibility to different antibiotics, the concentration of antibiotic that will inhibit bacterial growth was calculated using the agar dilution method according to the Clinical and Laboratory Standards Institute's guidelines (CLSI, 2007).

Plasmid DNA isolation by alkaline lysis method[9]

Plasmids are circular extrachromosomal bacterial replicons that are non-obligate. Plasmid DNA isolates necessitate the isolation of DNA from the bacterial cell's chromosomal DNA, as well as the polysaccharides, lipids, and proteins that make up the cell. The plasmid DNA must be free of impurities for subsequent manipulation, especially enzymatic modification.

Transformation of the resistant plasmids into competent cells (E.coli)[10] 500 µl of plasmid DNA was gently combined with 500 l of one-shot chemically competent E.coli (Invitrogen, CA). For 5 to 30 minutes, the mixture was incubated on ice. (This approach causes E.coli to shrink.) The cells were then expanded by placing the mixture in a water bath at 42 to 42.5oC for 30 to 50 seconds without shaking. The tubes were then immediately moved to the ice. To the mixture, 250 liters of LB broth (Lysogenic broth) were added (to enhance cell growth at room temperature). The tubes were tightly capped and shaken horizontally (200 rpm) for 1 hour at 37 degrees Celsius. The qualified cells' transformed mixture was kept separate.

Electrophoresis

1 x TAE buffer was used to clean the apparatus, and 50 ml of TAE buffer was used to make 1 percent agarose. 3 l ethidium bromide was applied to the agarose solution (wear gloves when doing this) and poured onto the comb electrophoresis plate. After that, the agarose slab was placed in an electrophoresis apparatus. The plasmid was mixed with 6x loading dye (fermentas) and a control, then run at 100 V for 60 minutes, with the bands read using a gel-doc device.

Results

The study was conducted over a period of 3 years and 140 samples were collected. All the samples were sub cultured on Nutrient agar, Blood agar, Macconkey agar, confirmed by biochemical tests and slide agglutination test.

Phage typing

Table 7 - Different Phage types among *Salmonella enterica* serovar Typhi strains

S.No.	Phage type	No. of isolates*	Percentage (%) of isolate
1	El	107	76.42
2	UVS	6	4.28
3	A	4	2.85
4	E9	4	2.85
5	D8	2	1.42
6	D2	2	1.42

(*) - 9 (7.03%) isolates were found to be Vi-negative

UVS - Untypable Vi-strains

The most common phage form was El (76.42%), followed by UVS (4.28 %), A (2.85 %), E9 (2.85%), D8 (1.42%), and D2 (2.85%) (1.42%). Vi-negative isolates made up 8.03% of the total isolates. The use of phage typing to provide epidemiological information is limited due to the prevalence of El or A phage types as predominant types.

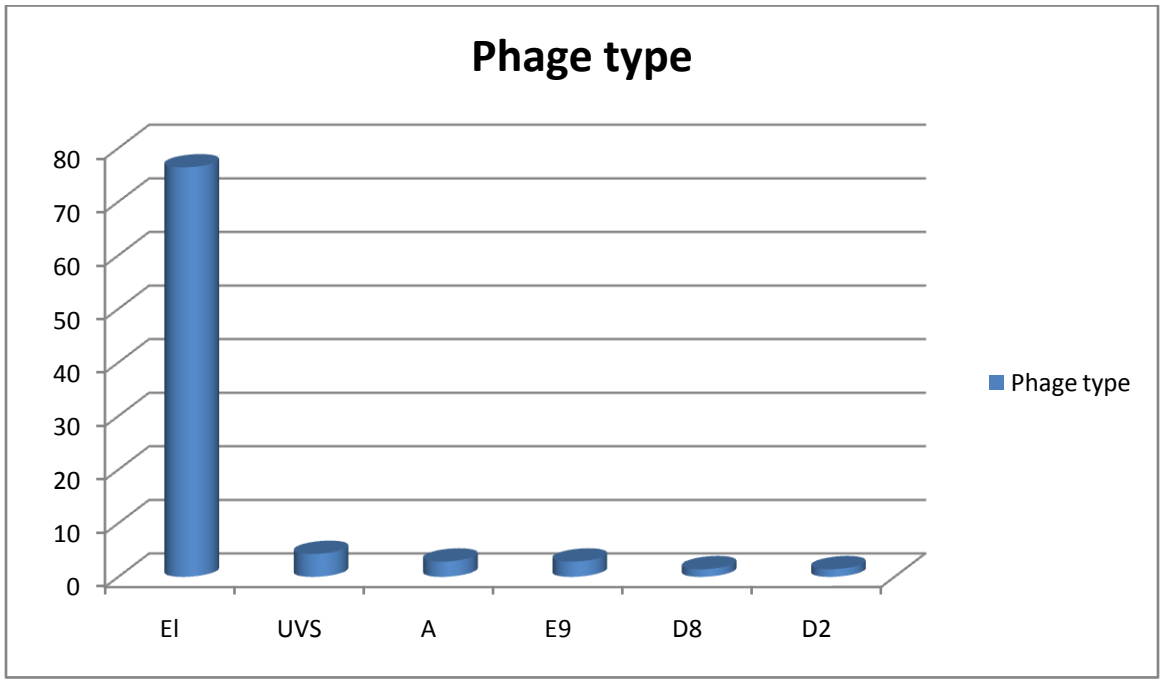

Figure - Different Phage types among *Salmonella enterica* serovar Typhi strains

Antibiogram patterns

Table 1 - Antibiogram patterns of Salmonella enterica serovar Typhi strains

S.No.	Antibiotic	% of isolates		
		Sensitive	Intermediate	Resistant
1	Cephotaxime	88.46	7.03	0
2	Chloramphenicol	98.72	0	1.28
3	Ceftazidime	100	0	0
4	Ciprofloxacin	100	0	0
5	Tetracycline	92.31	7.69	0
6	Ampicillin	98.72	0	1.28
7	Streptomycin	96.15	2.56	1.28
8	Gentamicin	97.44	2.56	0
9	Kanamycin	87.18	12.82	0

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10	Nalidixixc acid	6.41	0	93.59
11	Trimethoprim	98.72	0	1.28

100% of the isolates tested positive for Ceftazidime, Ciprofloxacin, and Gentamicin. The highest resistance to Nalidixic acid was discovered. During the study era, no resistance to Cephotaxime, Tetracycline, Gentamicin, or Kanamycin was observed. Chloramphenicol, Ampicillin, Streptomycin, and Trimethoprim resistance was observed in 1.28 percent of the strains.

Gentamicin, Chloramphenicol, Ampicillin, and Trimethoprim were found to be effective against 100 percent of the strains, followed by Ceftazidime and Ciprofloxacin. Nalidixic acid resistance was found in 93.75 percent of the strains, followed by Chloramphenicol, Ampicillin, Streptomycin, and Trimethoprim resistance in 4.69 percent of the isolates for each antibiotic.

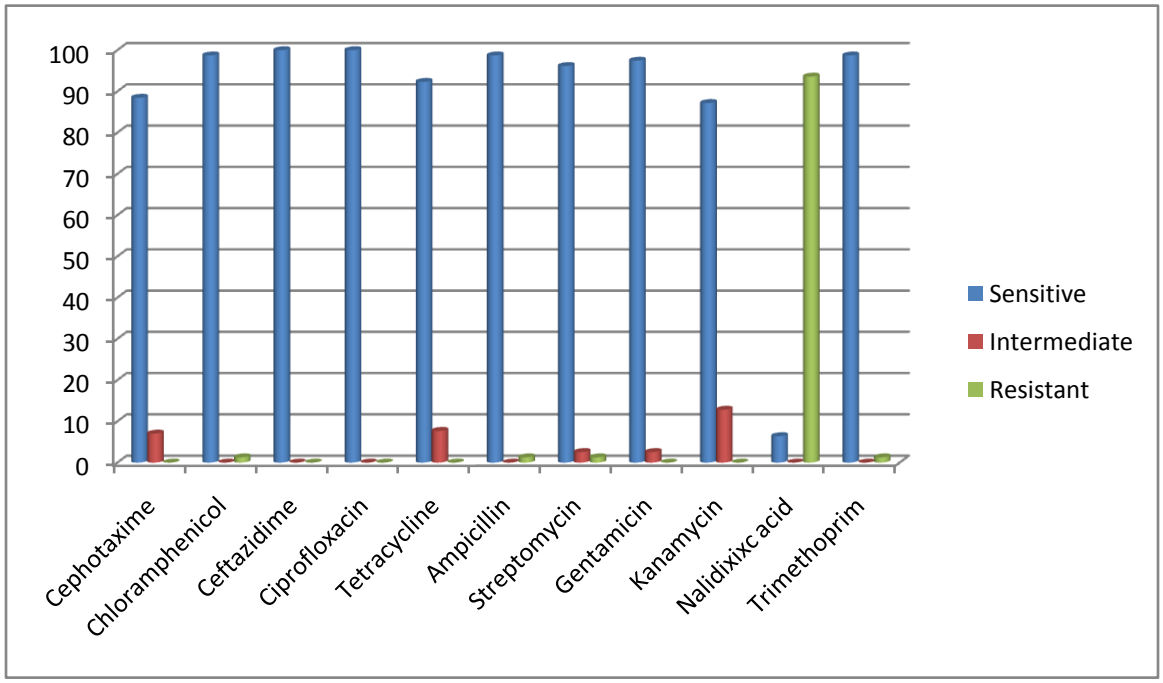

Figure 1 - Antibiogram Pattern of *Salmonella enterica* serovar Typhi strains

Discussion

In this study the most common phage form was El (76.42 percent), followed by UVS (4.28 percent), A (2.85 percent), E9 (2.85 percent), D8 (1.42 percent), and D2 (2.85 percent) (1.42 percent). Vi-negative isolates made up 8.03 percent of the total. The use of phage typing to provide epidemiological information is limited due to the prevalence of El or A phage types as predominant types.

In this study Chloramphenicol, Ampicillin, and Trimethoprim resistance has been observed in 95.3 percent of Salmonella enterica serovar Typhi isolates. The present study found re-emergence of susceptibility to historically used drugs in higher proportions than those recorded from different parts of the country (Dutta et al., (2014), Sen et al., (2007), Achia et al., (2005), Gautam et al., (2002)[11,12,13,14], with the exception of one study from northern India which reported 96% sensitivity to chloramphenicol (Madhulika et al., 2004)[15]. The current findings are consistent with those of other studies from the country's central west, which found susceptibility to chloramphenicol, ampicillin, and trimethoprim (Krishnan et al., (2009), Nagshetty et al., (2010)[16,17], with the exception of one that found 100 percent tolerance to these drugs (Tankhiwale et al., 2003)[18].

The results may be extremely useful in assisting health officials in rationalizing the strategy of scientific typhoid fever care. Furthermore, there are benefits of returning to the use of these medications, such as their availability in developing countries, lower costs, and well-established clinical efficacy (Kumar et al., 2009)[19]. It should be noted that patients treated with ampicillin have a greater risk of relapse and the formation of a carrier state than those treated with chloramphenicol.

Conclusion

The study found that the use of phage typing to provide epidemiological information is limited due to the prevalence of El or A phage types as predominant types.

The study found that Salmonella enterica serovar Typhi has a high prevalence of Nalidixic acid tolerance (94.5%), which is higher than other research from around the world. During the research, the resistance to commonly used antibiotics (chloramphenicol, ampicillin, and trimethoprim) resurfaced. Furthermore, the analysis found a lower prevalence of multidrug-resistant Salmonella enterica serovar Typhi strains.

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