STUDY OF MINIMAL INHIBITORY CONCENTRATIONS AND MULTIPLE DRUG RESISTANCE PLASMIDS IN *SALMONELLA TYPHI*

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ABSTRACT

S. typhi isolated from blood cultures were tested for minimal inhibitory concentrations of antimicrobial drugs using an simple easily fabricated multiple inoculator. Multiple drug resistant *S. typhi* were inhibited by high concentrations of ampicillin, sulphamethoxazole, trimethoprim and chloramphenicol (> 128 mg/L) suggestive of R factor mediated resistance. They showed presence of a single large plasmid with molecular weight ranging from 85 to 110 megadaltons. Sensitive *S. typhi* isolates were inhibited by lower concentrations of antimicrobial and did not show evidence of any plasmid. All isolates were equally sensitive to extremely low concentrations of ceftazidime, cefuroxime, ciprofloxacin, norfloxacin and gentamicin (< 0.5 mg/L).

MJAFI 1995; 51 : 151-154

KEY WORDS : *Salmonella typhi*; Multiple drug resistance; Plasmids.

Introduction

Chloramphenicol has been the gold-standard in the treatment of typhoid fever [1]. With the emergence of chloramphenicol resistance, ampicillin and cotrimoxazole have been used in the therapy of typhoid fever [2]. Resistance to chloramphenicol, streptomycin, sulphonamide and tetracycline in *S. typhi* was first reported in Mexico [3]. In India enteric fever due to multiple drug resistance *S. typhi* (MDRST) is widespread and has been a major cause for increased morbidity and mortality [4,5].

Enteric fever due to MDRST is fulminant with a higher incidence of organomegaly, hyperpyrexia, greater duration of hospitalisation and longer defervescence after appropriate antimicrobial therapy [6]. The MDRST cause severe infections characterised by hyperpyrexia, shock, gastrointestinal haemorrhage, loose motions and hepatitis [5]. MDRST are resistant to ampicillin, chloramphenicol, sulphamethoxazole and trimethoprim. However, they are uniformly sensitive to flouroquinolones, newer cephalosporins,

and gentamicin [6]. In this study *S. typhi* isolates were analyzed for minimal inhibitory concentrations (MIC) against various antimicrobial and presence of plasmids.

Material and Methods

Sixty isolates of *S. typhi* isolated from cultures of patients presenting with enteric fever from Jan 1991 to Dec 1992 at Air Force Hospital, Bangalore were used in this study. The blood cultures were processed routinely and the organisms identified biochemically by sugar reactions and serologically by appropriate antisera [7]. Antimicrobial susceptibility testing was done by the Stokes method [8]. MICs of ampicillin, chloramphenicol, sulphamethoxazole, trimethoprim, ciprofloxacin, ceftazidime, cefuroxime and gentamicin against *S. typhi* were determined using a multipoint replicating inoculator. MIC was the lowest concentration of the drug which prevented visible growth.

The multiple inoculator used was fabricated using four-inch polypropylene petri dishes, twelve 1.5 ml Eppendorf tubes (with their lids removed) and 2 cm long aluminum

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rivets (thickness 0.40 cm). Using a punch of 1.2 cm diameter, holes were drilled equidistant from each other into the cover of the petridish into which the tubes snugly fit. Corresponding smaller holes were made in the base of the petri dish into which the aluminium rivets could be loosely inserted. The apparatus so designed could be autoclaved. The aluminium rivets were heat sterilised separately. The inoculum consisted of broth cultures diluted with sterile saline to a turbidity slightly greater than that of a Macfarland 0.5 standard [7]. 1.3 ml of the inoculum was deposited into each of the Eppendorf tubes. Aluminum rivets were inserted into holes in the base of petri dish cover. Impressions were taken on antimicrobial drug containing agar plates prepared by conventional technique [8]. Care was taken to see that the amount of fluid carried over on the rivet foot was approximately equal since the inoculum size per spot would influence the growth. Results were read after overnight incubation disregarding minutely small colonies. One plate without antimicrobial drug was used as the viability control. Each set plated contained *E. coli* NCTC strain 10418 as the antibiotic sensitivity control. Strains of *S. typhi* showing MIC values more than 128 mg/L were analyzed for their pattern of resistance to various antimicrobials.

The 60 isolates were examined for plasmids by the alkaline lysis technique as described by Birnboim and Doly [9]. The plasmids were characterised by agarose gel electrophoresis as described by Meyers et al [10]. Plasmids from *E. coli* V517 which ranged from 1.4 to 35.8 megadaltons were used as standards [11]. Molecular weights of *S. typhi* plasmids were obtained by extending the graph of relative mobility of plasmids from *E. coli* V517 strain and extrapolating the negative logarithm of the relative mobility.

Results

The susceptible *S. typhi* (SST) were susceptible to all antimicrobials while MDRST were resistant to ampicillin (100%), chloramphenicol (100%), sulphamethoxazole (95%),

trimethoprim (100%) and tetracycline (95%). All isolates were sensitive to the newer aminoglycosides, norfloxacin and ofloxacin and to the cephalosporins, cephalixin and ceftazidime.

The multiple inoculator designed is shown in Fig 1. The pattern of resistance to various antimicrobial in MDRST in concentrations of more than 128 mg/L are shown in Table 1. SST showed low MIC values for chloramphenicol (< 4 mg/L), ampicillin (< 2 mg/L), sulphamethoxazole (< 8 mg/L) and trimethoprim (< 4 mg/L). All the isolates were equally sensitive to ceftazidime (< 0.25 mg/L), cefuroxime (< 0.25 mg/L), ciprofloxacin (< 0.125 mg/L), norfloxacin (< 0.125 mg/L), and gentamicin (< 0.5 mg/L).

TABLE 1

Antimicrobial resistance pattern in *S. typhi* (n=40)
(MIC value of 128 mg/L and above)

Resistance pattern	No. of strains
A, C, Su, Tmp	25
A, C, Su	2
C, Su, Tmp	2
Su, Tmp	5
A, C	1
A, Su	1
Su	2
Tmp	2

A - ampicillin; C - chloramphenicol;
Su - sulphamethoxazole; Tmp - trimethoprim

Fig. 1 : The simple replicating inoculator indigenously made for the study.

Fig. 2 : Ethidium bromide stained agarose gels as seen under UV fluorescence. Lanes 1,3,4,6 show MDRST plasmid. Lane 5 shows plasmid extracted from *E.coli* V 517. Lane 2 is an extract of SST and does not show any plasmid.

The 40 MDRST isolates showed the presence of a single large plasmid with molecular weights ranging from 85 to 110 megadaltons (Fig 2). The 20 SST did not show the presence of any plasmid DNA.

Discussion

Two populations of *S. typhi* can be identified on the basis of ABST patterns. MDRST show a resistance to ampicillin, chloramphenicol, trimethoprim, sulphonamide and tetracycline, while the SST were uniformly sensitive to all antibiotics. These findings have been reported earlier [12,13].

The use of a replicating inoculator in the determination of MICs helps in screening a large number of samples for a variety of antimicrobial over a wide range of concentrations which is not possible by the conventional broth dilution technique. Prefabricated replicating inoculator devices (e.g. the Steers-Foltz replicator [7]) are not easily available in India. The indigenous replicating inoculator described in this article is cheap, easily fabricated and brings routine MIC testing within the capability of a small laboratory.

Anderson and Smith [14] in 1972 reported MICs of chloramphenicol against sensitive *S. typhi* in the range of 1 to 3 mg/L. MDRST from Kuwait showed transferable resistances with high MIC values (chloramphenicol 250 mg/L, ampicillin 250 mg/L and tetracycline 62.5

mg/L). Strains from the outbreak of MDRST infection in Mexico reported resistance to chloramphenicol, streptomycin, sulphonamide and tetracycline (MIC of chloramphenicol was 150 mg/L). Paramasivan et al [15] reported transmittable resistance to ampicillin (MIC > 100 mg/L), chloramphenicol (MIC > 200 mg/L), streptomycin (MIC > 100 mg/L), and tetracycline (MIC > 200 mg/L). Bryan et al [16] reported MICs for sensitive *S. typhi* isolates as ampicillin 0.28 to 0.5 mg/L, chloramphenicol 2.5 mg/L, cotrimoxazole 2.5 mg/L, gentamicin 3.1 mg/L, cefuroxime 2.7 mg/L, cefotaxime 0.1 to 0.025 mg/L, ceftazidime 0.25 mg/L, ciprofloxacin 0.02 mg/L and norfloxacin 0.05 mg/L.

Compared to the earlier reported MICs against *S. typhi* of various antimicrobial [14-16], recent studies [17-18], have shown higher values. Dar et al [17] reported MICs against MDRST for chloramphenicol to be greater than 1024 mg/L and trimethoprim greater than 128 mg/L. Rao et al reported MIC values of chloramphenicol in the range of 500-1000 mg/L [18]. In this study, MDRST showed antimicrobial resistance above 128 mg/L to one of the antimicrobial tested. 63% of the MDRST tested had MIC values of 128 mg/L and above for 4 antimicrobials (ampicillin, chloramphenicol, sulphamethoxazole and trimethoprim), while 13% had high MICs for sulphamethoxazole and trimethoprim. Only SST isolates tested showed MIC values similar to those reported in the earlier studies. All isolates were uniformly sensitive to ciprofloxacin, ceftazidime, cefuroxime, and gentamicin with extremely low MIC values.

Resistance conferred on a microorganism against antimicrobials by R factors is of a high order and MIC values often exceed 128 mg/L [8]. The high incidence of resistance to high level of antimicrobial drugs is a pointer to a wide prevalence of R factor mediated resistances. Murray et al [19] found plasmids in 8% and 6% of SST isolates from Chile and Thailand respectively. Maher et al [20] examined plasmids from *S. typhi* in Chile and Peru. Plasmids were present in 17% and 7%

of the isolates respectively but they did not confer any drug resistance. Threfall et al [21] have reported the presence of MDR plasmids of H1 incompatibility group with molecular weights ranging from 110 to 120 megadaltons in MDRST. H1 incompatibility group plasmids have a conjugation transfer efficiency optimal at 22 to 30°C. They have also a capacity to encode for multiple antibiotic resistance [22]. These traits implicate H1 incompatibility group plasmids in spread of multidrug resistance within indigenous bacterial flora of the soil and sewage. In our study, we have found plasmids with molecular weights ranging from 85 to 110 megadaltons conferring resistance against antibiotics.

This study indicates that high MICs in MDRST are due to presence of R plasmids. Previous studies have shown that MDRST cause a more severe clinical syndrome as compared to SST [5,6]. It is thus possible that R factor conferring antibiotic resistance may range from plasmid mediated changes in outer membrane protein and lipopolysaccharide, resistance to complement killing, epithelial cell invasion and adhesion or intramacrophage survival or replication. Further studies are being carried out to elucidate this aspect of infection by MDRST.

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