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MULTIPLE ANTIBIOTIC RESISTANCE AMONG *SHIGELLA* SPECIES ISOLATED FROM PATIENTS OF SHIGELLOSIS IN PAKISTAN

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Abstract: Twenty-four Shigellae strains were isolated from different clinical sources in Pakistan. These isolates were screened for their resistance to the antibiotics, ampicillin, chloramphenicol, enoxacin, gentamicin, septran, tetracycline, urixin and velosef. Minimum inhibitory concentrations were determined and the isolates showed the highest frequency of resistance against Septran at 50 and 100 μg/ml whereas at 250 and 500 μg/ml, the highest frequency of resistance was against Urixin. The lowest frequency of resistance was against Enoxacin, followed by gentamicin and Chloramphenicol. At 250 μg/ml level, the isolates showed a considerable decrease in the resistance frequency of almost all antibiotics. In the present study, 95.8% of these Shigellae were multi-drug resistant strains. Our results emphasize the urgent need to exercise restraint in the use of oral antibiotics in Shigellosis. The overall incidence of *Shigella* infections can be decreased by improvement in the levels of environmental and personal hygiene.

Key words: Antibiotic resistance, shigellosis, Pakistan

INTRODUCTION

Shigellosis is problem in both developed and under developed countries. This is one of the most prevalent and important diseases in the developing countries (Arora *et al.*, 1982; Albert *et al.*, 1990). Shigellosis is one of the major causes of morbidity and mortality among children less than 5 years of age in developing countries. Shigellosis is usually transmitted from person to person in households (Wilson *et al.*, 1981; Makintubee *et al.*, 1987), however outbreaks due to contaminated food (Weissman *et al.*, 1974; Black *et al.*, 1978) or water (Baine *et al.*, 1975; Makintubee *et al.*, 1987) are not unusual.

Resistance of Shigellae to antibacterial drugs has been reported from different parts of the world and is increasing (Gedebou and Tassew, 1982). Resistant *S. flexneri* was first reported in Japan (Suzuki *et al.*, 1986). The development of transferable multi-drug resistance was soon reported from various parts of the world (Wang *et al.*, 1976; Chugh *et al.*, 1985; Panigrahi *et al.*, 1987; Lin and Chang, 1992; Ling *et al.*, 1993; Bratoeva and John, 1994; Casalino *et al.*, 1994; Ries *et al.*, 1994; Samonis *et al.*, 1994).

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In Pakistan, like other developing countries, there is also a general increase in drug-resistance especially to all commonly used antibiotics. Furthermore, a great worldwide proportion of Shigellae strains of various serotypes (especially, *S. dysenteriae* type I and *S. flexneri*) are now multiple drug resistant and new, simple and effective treatments of Shigellosis have not yet been developed (WHO, 1990; Hale, 1991).

In the present study the screening for antibiotic resistance was performed to study plasmid associated nature of these resistances. A number of R-plasmids were isolated during these studies and will be reported elsewhere.

MATERIALS AND METHODS

Bacterial isolates Shigellae strains were isolated from stools of patients suffering from diarrhoea or related symptoms at National Institute of Health, Islamabad, Armed Forces Institute of Pathology, Rawalpindi, and Shaikh Zayed Hospital, Lahore, Pakistan. The isolates were identified on the basis of routine biochemical and serological tests. The identification numbers used in this study are our own. Bacterial cultures were maintained in freezing glycerol LB media at -20 °C. For routine experiments, the cultures were maintained on LB agar plates at 4 °C and subcultured bimonthly.

Chemicals and media

Chemicals and antibiotics used in this study were obtained from Sigma Chemicals Co., and were of molecular biology grade. Culture media were purchased from Difco Laboratories. Difco (USA) LB broth and agar were used for the screening of cultures for antibiotic resistance.

Antibiotics used in these studies were, Ampicillin (A), Chloramphenicol (C), Enoxacin (E), Gentamycin (G), Septran (S), Tetracycline (T), Urixin (U) and Velosef (V). Stock solutions (10 mg/ml) of antibiotics were made in distilled water. Chloramphenicol was dissolved in ethanol. All solutions were sterilized by millipore (0.45 mμ) filters and refrigerated.

Antibiotic resistance/sensitivity tests

Antibiotic sensitivity was performed using diffusion method of Bauer *et al.* (1966). Minimum inhibitory concentration (MIC) was determined by an agar dilution technique using lactose neutral red peptone (LNRP) as the basal medium with 2% peptone, 1.5% Difco agar, 3.5 ml/l neutral red solution and 1% lactose. Serial dilution of antibiotics *i.e.*, ranging from 500 μg/ml to 25 μg/ml were prepared in LNRP and used in the resistance tests. The MICs of the antibiotics were tested for 24 strains. A loopful (2 mm size) of 4 h bacterial culture was inoculated on different antibiotic plates. The inoculated plates were incubated for 24 h at 37 °C before recording MICs.

RESULTS

Of the 24 strains isolated, 45.8% isolates were *S. flexneri*, 33.3% *S. boydii*, 12.5% *S. sonnei* and 8.3% *S. dysenteriae*. The antibiotic resistance was screened against these Shigellae isolates. Overall 69% of strains were resistant to Urixin (U), 68% to Septran (S), 60% to Velosef (V), 58% to Ampicillin (A), 50% to Tetracycline (T), 36% to Chloramphenicol (C), 34% to Gentamycin (G) and 29% to Enoxacin (E).

The MICs of 8 antibiotics against 24 strains of Shigellae are shown in a comparative account of the antibiotic resistance of cultures, at different levels in Fig.1. Generally, the cultures, showed the highest frequency of resistance against Septran at 50 and 100 μg/ml whereas at 250 and 500 μg/ml, the highest frequency of resistance was against Urixin. The lowest frequency of resistance was against Enoxacin at all the levels of antibiotics screened, followed by Chloramphenicol and Gentamycin. There was a slight decrease in the number of Septran resistant cultures at the levels of 50-500 μg/ml compared with the level of 25 μg/ml. At 250 μg/ml level, the cultures showed a considerable decrease in the resistance frequency of almost all antibiotics.

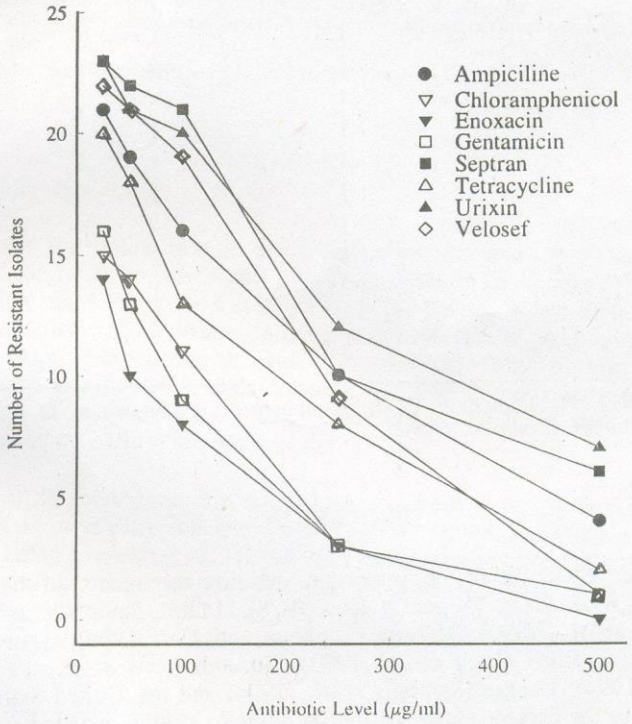

Fig. 1: Antibiotic resistance of bacterial isolates at different levels of antibiotic.

Multiple antibiotic resistance among Shigellae isolated from clinical sources is not uncommon. Out of the 24 strains screened for resistance 60.9% were resistant to one or more antibiotics at 100 μg/ml, 30.2% were resistant to one or more antibiotics at 250 μg/ml and 11.4% were resistant to one or more antibiotics at 500 μg/ml. The resistant cultures showed different patterns of antibiotics resistance at three levels of antibiotics (100 μg/ml, 250 μg/ml and 500 μg/ml) (Table I). The most common pattern was ASUV at 100 μg/ml.

Table 1: Antibiotic resistance pattern of Shigellae isolated from various clinical sources.

Resistance pattern*	Number of isolates at		
	100 μg/ml	250 μg/ml	500 μg/ml
ACEGSTUV	1	-	-
AEGSTUV	1	1	-
ACGSTU	1	-	-
ACESTU	1	1	-
AEGSUV	1	-	1
CESTUV	1	-	-
CGSTUV	1	-	-
CEGSUV	1	1	1
CGTUV	1	1	-
ACEUV	1	1	-
ASTUV	1	-	1
AGSUV	1	2	-
ASTU	2	1	1
ASUV	6	3	2
CSTV	3	2	1

* Abbreviations used: A, ampicillin; C, chloramphenicol; E, enoxacin; G, gentamycin; S, septran; T, tetracyclin; U, urixin; V, velosef.

DISCUSSION

The Shigellae continue to be important aetiological agents of dysentery and gastroenteritis in Bangladesh (Nigar *et al.*, 1978), India (Panigrahi *et al.*, 1987), Australia (Albert *et al.*, 1990), Nigeria (Olukoya and Oni, 1990), Taiwan (Lin and Chang, 1992), Hong Kong (Ling *et al.*, 1993), Burundi (Ries *et al.*, 1994), Somalia (Casalino *et al.*, 1994), Greece (Samonis *et al.*, 1994) and the United States (Bratoeva and John, 1994). In the present study, of the 24 *Shigella* strains, isolated from different Pakistani clinical sources, *S. flexeneri* is the most commonly found species (45.8%) and is followed by *S. boydii* (33.3%), *S. sonnei* (12.5%) and *S. dysenteriae* (8.3%).

The results indicate that antibiotic resistance among indigenous clinical Shigellae is very common. Out of a total 24 isolates, 23 were found to be resistant to single or multiple antibiotics. Different resistance patterns were observed among the resistant isolates. Resistance to Septran was shown to be the most common. Maximum number of the resistant isolates were found at the level of 25 μg/ml. Resistance to Enoxacin was found to be the lowest at all levels (25-500 μg/ml), whereas Gentamicin and Chloramphenicol are more effective at higher concentrations (100-500 μg/ml). Velosef and Tetracycline are effective only at 500 μg/ml. Analogous results have been reported by Shang-Yuan *et al.* (1992).

Maximum strains (69%) were found to be resistant to Urixin. This was followed by Septran (68%), Velosef (60%), Ampicillin (58%), Tetracyclin (50%), Chloramphenicol (36%), Gentamicin (34%) and Enoxacin (29%). These findings were similar to those reported from India and Taiwan, especially in case of Tetracycline and Ampicillin (Arora *et al.*, 1982; Lin and Chang, 1992) and may reflect the widespread overuse of these antibiotics especially, Tetracycline.

Multi-drug resistant Shigellae has been reported to be increasing in incidence in many countries by several workers. Kaliyugaperumal *et al.* (1978) reported multi-drug resistant in 79.4% of strains while Panikar *et al.* (1978), Arora *et al.* (1982) and Panigrahi *et al.* (1987) reported their number to be 94%, 76% and 81%, respectively. In the present study, 95.8% of the Shigellae isolates were multi-drug resistant strains. Only 4.1% of isolates were sensitive to all eight antibiotics tested. We also observed very high resistance levels in 23 strains for which MICs of the antibiotics were tested.

The prevalence of drug resistance in Shigellae observed here and elsewhere in the world indicates the futility of routine antibiotic therapy in bacillary dysentery, which is a self-limiting disease in majority of cases. Our results emphasize the urgent need to exercise a restraint in the use of oral antibiotics in Shigellosis. Samonis *et al.* (1994) also supported the view that Shigellosis can be controlled without the use of antibiotics. The overall incidence of *Shigella* infections can be decreased by improvements in the levels of environmental and personal hygiene.

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