Multi Drug Resistant *Shigella* Species in Nepal, a Retrospective Study Conducted at National Public Health Laboratory (NPHL), 1999 to 2002.

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Abstract

Introduction Antimicrobial susceptibility patterns of most of the organisms especially the gram-negative organisms in the Enterobacteriace family and the related ones has been changing very rapidly. Plasmid mediated resistance transfer and the emergence of the multidrug resistant *Shigella* (MDRS) has been well recognized. We have encountered several such strains of Shigella in our study which showed varied resistance pattern.

Objectives This study was carried out to isolate different species of *Shigella* and to know the antibiotic susceptibility patterns of these isolates.

Methods Culture of stool specimen for Shigella isolation by standard microbiological techniques at NPHL as well as collection of Shigella isolates from different hospitals. Antibiotic sensitivity was studied by Kirby Baur's standard disc diffusion technique.

Results A total of 53 Shigella isolates were studied which included *S. dysenteriae* (42 %), *S. flexnerii* (38%), *S. sonnei* (15 %) and *S. boydii* (4%). Shigella isolates were resistant to more than three commonly used antimicrobial agents such as Ampicillin, Cotrimoxazole and Nalidixic acid but susceptible to second line of drugs such as Ciprofloxacin and Mecillinam. Among all the isolated species of Shigella only *S. flexnerii* showed resistance to Ciprofloxacin (20%). *Shigella sonnei* and *Shigella boydii* showed cent percent resistance to Cotrimoxazole. Ciprofloxacin was the drug of choice as it showed highest sensitivity.

Conclusion Specific antibiotics should be given only after the laboratory results are available. Culture, isolation and sensitivity testing for Shigella species should be done regularly. Monitoring of emergence of resistance is recommended .A regular feed back should be given to the clinicians

Key Words Multi drug resistant Shigella, Antibiotic susceptibility pattern, Retrospective study, NPHL

Introduction

As reported by several scientists, antimicrobial susceptibility patterns of most of the organisms especially the gram-negative organisms in the Enterobacteriace family and the related ones have been changing very rapidly. It has also been found that the commensal flora present in the intestinal tract of the human beings is becoming resistant to antibiotics taken for some other infections. Plasmid mediated resistance transfer thereby the emergence of the multiple drug resistant *Shigella* (MDRS) have been well recognized. We have encountered several such strains of Shigella in our study. These organisms are gram negative rods, nonmotile, catalase positive (with some exception *S. dysenteriae*), oxidase negative, facultative anaerobic organisms which ferment glucose but not Lactose (with some exception), some ferment mannitol (with some exception), others produce indole (with some exception) but *S. sonnie* is a late lactose fermentor. These organisms do not break down citrate, urea and and do not form hydrogen sulphide on triple sugar iron Agar (TSI) slants. These organisms are easy to cultivate in the ordinary laboratory media and easily

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recognized over the surface of Mac-conkey (MA) and Desoxycholate citrate (DCA) agar plates. The serological typings is based upon the presence of the type specific antigens on the cell wall of the organisms called somatic antigens (O antigens) as these organisms have no flagellae.

Materials and Methods

All the isolates from three different hospital laboratories such as Kanti Children Hospital, Sukra Raj Tropical Hospital and National Public Health Laboratory were collected and susceptibility pattern was studied using the Kirby Bauer technique of simple disc diffusion method on Mueller Hinton agar (MHA) plates (Oxoid). A set of antibiotic discs (Oxoid) used were Ampicillin (25µg/disc), Cotrimoxazole (300 µg/disc), Nalidixic acid (300µg/disc), Mecillinam (10µg/disc), and Ciprofloxacin (5µg/disc) as a second line of drugs.

The stool specimens were plated over MA and DCA plates and incubated over night at 37° C and the plates were examined for the presence of non-lactose fermenting colonies (NLF). NLF colonies appeared pale or slight pink, small, round or slightly irregular colonies that were moist. The isolated colonies were further tested by a set of biochemical tests such as Indole, Citrate, Urea, Semisolid indole motility medium (SIM) and Triple sugar iron (TSI) agar slants. *Shigella* were Indole negative, Citrate negative, Urea negative and Hydrogen Sulphide negative in TSIs slant and these were non motile. Such organisms were further tested by type specific antisera of group A, B, C and D *Shigella* antisera (Welcome Laboratories). After the identification, susceptibility testing was done. The zone of inhibition was measured using a scale and compared with that of the standard organisms, American Type Culture (ATCC) strain *Escherichia coli* 25922.The results were interpreted as per standard charts from NCCLS (National committee for Clinical Laboratory Sciences, Document M100-56: Sixth informational Supplement).

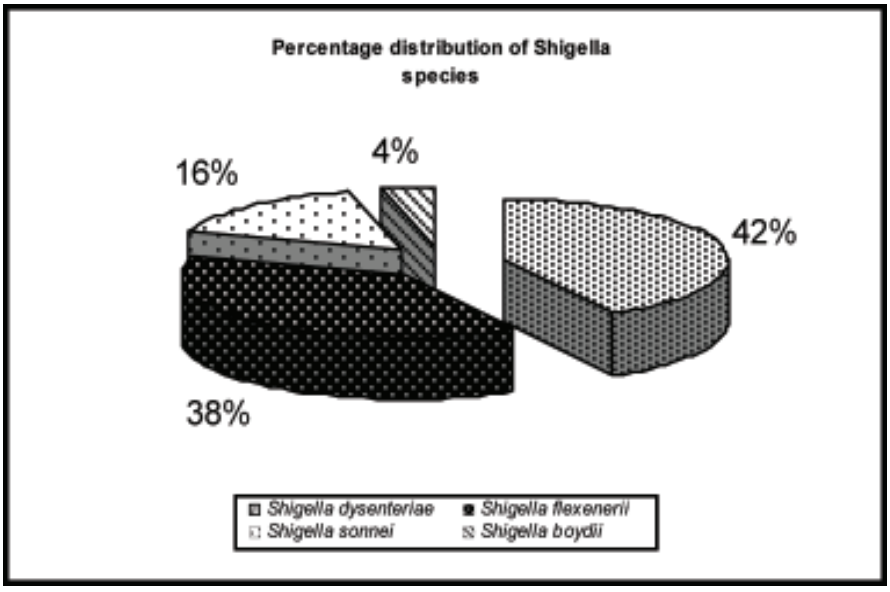
Results

A total of 53 Shigella isolates were studied and S. dysenteriae accounted the highest percentage while S. boydii was the lowest.

Table 1: Percentage of distribution total *Shigella* isolates

Shigella dysenteriae	42%
Shigella flexenerii	38%
Shigella sonnei	16%
Shigella boydii	4%

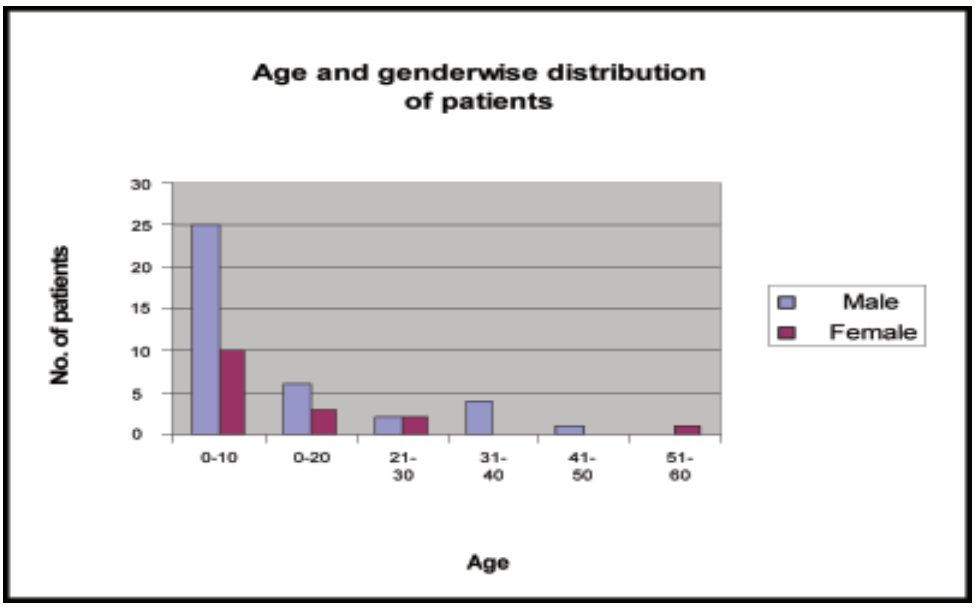
Fig 1: Chart showing percentage prevalence of *Shigella* species



Age and genderwise distribution of patients

Out of total 53 *Shigella* isolates, the prevalence was high in the male patients and within the age group 0-10 years.

Fig 2: Age and genderwise distribution of patients



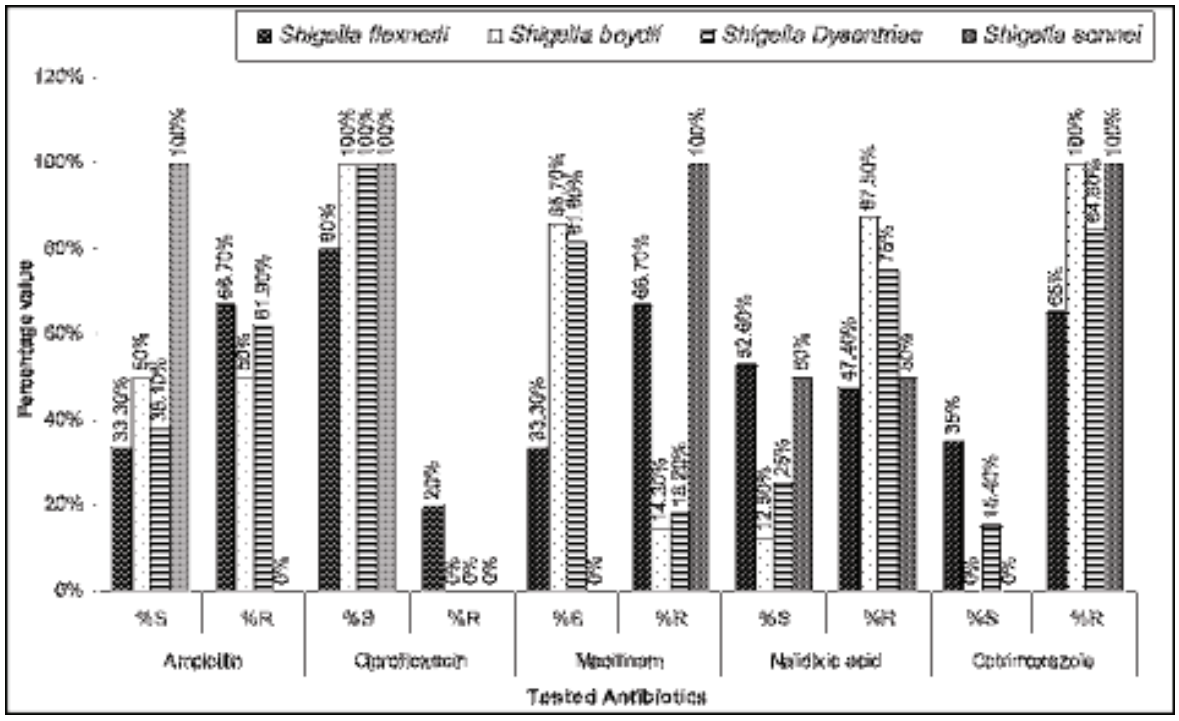
Antibiotic sensitivity pattern of total Shigella isolates

The antibiotic sensitivity profile studied revealed that *Shigella* sp. Were resistant to almost all the common antibacterial agents such as Ampicillin(59%R), Cotrimoxazole (77%R), Nalidixic acid (56%R), Mecillinam (39%R),and Ciprofloxacin (8%R).

Table 2: Antibiotic susceptibility pattern of *Shigella* isolates

Isolates	Ampicillin		Ciprofloxacin		Mecillinam		Nalidixic acid		Cotrimoxazole	
	% S	% R	% S	% R	% S	% R	% S	% R	% S	% R
Shigella Dysentriae	38.1	61.9	**100**	0	81.8	18.2	25	75	15.4	84.6
Shigella flexnerii	33.3	66.7	80	**20**	33.3	66.7	52.6	47.4	35	65
Shigella boydii	50	50	**100**	0	85.7	14.3	12.5	87.5	0	**100**
Shigella sonnei	**100**	0	**100**	0	0	**100**	50	50	0	**100**

Fig 3: Bar diagram showing susceptibility pattern of *Shigella* islotes



Discussion

The present study showed *Shigella* species with different susceptibility pattern to a wide range of antimicrobial agents. It has been found that these organisms are continually developing resistance to commonly used antimicrobials.

It is evident from the table 1 and 2 that majority of *Shigella* isolates were resistant to more than three commonly used antimicrobial agents such as Ampicillin, Cotrimoxazole and Nalidixic acid but were susceptible to second line of drugs such as Ciprofloxacin and mecillinam. By referring to table 2 *Shigella dysenteriae* showed susceptibility to Ciprofloxacin (100%), Mecillinam (82%), Ampicillin (38%), Nalidixic acid (25%) and Cotrimoxazole (15%). We came across many cases of multidrug resistant strains of Shigella. Since 1999 we have followed the susceptibility pattern of *Shigella* species isolated at our laboratories and found this changing pattern.

Conclusion

Specific antibacterials / antibiotics should be given only after the laboratory results are available. Culture / isolation and sensitivity testing for *Shigella* species should be done regularly. Monitoring of emergence of resistance is highly recommended. A regular feed back and antibiogram should be given to the clinicians for effective management of disease caused by *Shigella* species.

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