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Original Article

Shigella isolates of Nepal: Changes in the incidence of shigella subgroups and trends of antimicrobial susceptibility pattern

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Abstract

Objectives: Shigellosis is an important cause of bloody diarrhoea in all age groups, especially in children. A retrospective study was done to analyse the pattern of shigella isolates and the antimicrobial susceptibility trend of these shigella isolated at different hospitals of Nepal from Jan, 2003- Dec, 2005.

Materials and methods: A total of 118 Shigella species isolated at nine different hospital laboratories of Nepal were reported to National Public Health Laboratory during January, 2003- December 2005 .The isolates were tested for the confirmation of identification and antimicrobial susceptibility pattern by standard bacteriological techniques.

Results: Of the 118 Shigella isolates reported, *Shigella flexneri* 51 (43.22%) was the predominant of the four species followed by *Shigella dysenteriae* 49(41.52 %) , *Shigella boydii* (7.62%) and *Shigella sonnei* (7.62%).But the yearly distribution of the Shigella isolates in 2003 and 2004 showed that *Shigella dysenteriae* was the most common of the four species. In 2005, a shift in the species was noted as Sh flexneri replaced Sh dysenteriae and became the most prevalent species. The percentage of *Shigella dysenteriae type-1* among all *Shigella dysenteriae* were 66.66 % in 2003, 44.44 % in 2004 and 60 % in 2005. Individual or multiple resistances to Ampicillin, Nalidixic acid, and/or Cotrimoxazole was seen in all the four species of Shigella. 33% of the total Shigella isolates reported were multi drug resistant (showing resistance to 3 or more antibiotics at a time). *Shigella dysenteriae type-1(Sd* 1) isolates resistant to ciprofloxacin were also encountered in the present study .Of the total 25 *Shigella dysenteriae type 1* isolates reported, 18(72%) were ciprofloxacin resistant . All the Shigella isolates were however sensitive to Ceftriaxone and Azithromycin.

Conclusion: Distribution of different species of Shigella and their antibiotic susceptibility profile may vary from one geographical location to another and may also change with time. Systematic monitoring of the species and serotypes of Shigellae and their antimicrobial susceptibility can help to guide therapy and reveal periodic epidemics due to *Sd* 1, which may have acquired resistance to antibiotics that have previously been effective.

Key words: Dysentery, Shigella, *Shigella dysenteriae type-1*, Antimicrobial resistance.

Shigella belongs to the family *Enterobacteriacae*. It is a small, non-capsulated, non-motile Gram negative rod and is the most common cause of bacillary dysentery. Shigella were discovered by a Japanese scientist Shiga, for whom they are named. The four species in this genus are referred to by a letter designation based on their serological antigen : Serotype A- *Shigella dysenteriae* , Serotype B- *Shigella flexneri* , Serotype C -*Shigella boydii* and Serotype D-*Shigella sonnei*. The first three are common in developing countries while *Shigella sonnei* is common in USA. Unlike other secretory diarrhoeas, shigellosis is the result of invasion of the distal small bowel and/or colon by these bacteria and as dehydration is not as severe as in secretory diarrhoeas, oral rehydration therapy does not significantly reduce the case fatality rates for shigellosis [(1,2)].Most hospital-based studies suggest that the case-fatality rate due to shigellosis is highest among children less than 5 years of age, particularly if there is malnutrition .In epidemic situations, a mortality rate as high as 3.9% in children under age 1 and 19.3 % for infants under 4 months of age has been reported. The case fatality rate declines with increasing age [(2, 3)]. An unusual finding from recent studies of epidemic shigellosis in Central Africa was high mortality rates in young adults. This region also has high HIV rates, so an interaction between the two infections is possible [(4)].

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Of all the serotypes of shigellae, *Shigella dysenteriae* type 1 (Shiga's bacillus) attracts special attention for its epidemic-causing potential and its association with most serious dysentery cases, with a high attack rate, high case-fatality rate, and various complications [2] . *Shigella* species show a pattern of steadily increasing resistance to antibiotics. *Shigella dysenteriae* type-1 is an endemic human pathogen causing outbreaks of acute bacillary dysentery in high-density populations, and recent clones are reported to be resistant to a wide spectrum of antimicrobials [5]. Among the four species, *S. dysenteriae type- 1* is generally the first to develop resistance to a new antibiotic, but then the other *Shigella* species follow. Rarely does susceptibility reappear once resistant strains have become endemic in a region [4].

Shigella species have managed to survive the antibiotic era via ingenious mechanisms of resistance. Shigellosis may occur in epidemic form, causing considerable morbidity and mortality in developing nations [6, 7]. Shigellosis is primarily a disease of resource-poor, crowded communities that do not have adequate sanitation or safe water, and where disease rates may be high. Antimicrobial therapy is usually recommended for treatment of shigellosis [4]. However, antimicrobial resistance in enteric pathogens, including *Shigella* isolates, complicates the situation in developing countries, where shigellosis is endemic and indiscriminate use of antimicrobial agents is common. In order to ensure appropriate treatment, continual surveillance is required to determine which antibiotics are still active. This strategy of "trying to keep one step ahead" implicates the continual development and testing of new antibiotics, which inevitably are more expensive. After extensive use of these new antibiotics, their prices do fall, but not to the level of the older, previously effective antibiotics. In the last two years, *Shigella dysenteriae* type-1 resistant to quinolone- ciprofloxacin have been reported in India , Bangladesh and Canada [8 ,9,10], presumably following the established trend of this organism to rapidly develop resistance to the current therapeutics. Moreover, this resistance profile leaves few reliable and economical therapeutic options for *Sh. dysenteriae* type-1.

Materials and methods

A total of 118 Shigella species isolated from patients of all age group at nine different hospital laboratories of Nepal during 2003-2005 were reported to National Public Health Laboratory (NPHL).These included Shigella isolates from different hospitals/laboratories of Kathmandu (NPHL, Bir Hospital, Patan Hospital, Kanti Children Hospital), Dharan (BPKIHS), Pokhara(Western Regional Hospital and Manipal Teaching Hospital) , Butawal (AMDA- Siddarth Children Hospital and Lumbini Zonal Hospital).

The reported isolates were tested at NPHL for confirmation of their identity by standard bacteriological techniques. The biochemical tests used for identification were Catalase, Oxidase , Methyl Red-Voges Praskauer(MRVP), Triple sugar iron(TSI), Sulphide Indol Motility(SIM), Citrate utilization and Urease. The biochemically confirmed isolates were further reconfirmed by serotyping using commercially available antisera from Denka Seiken , Japan.

Antibiotic susceptibility test was performed for all the identified Shigella isolates by Standard Kirby Bauer's Disc Diffusion technique. The antibiotics used for analysis were Ampicillin (Amp-10mcg), Cotrimoxazole(Sxt-25mcg), Nalidixic acid(NA-30mcg), Ciprofloxacin(Cip-5mcg), Azithromycin (Azm-5mcg) and Ceftriaxone(CRO-5 mcg).

Results

An analysis of 118 Shigella isolates reported in the three year period from 2003 to 2005 showed that *Shigella dysenteriae was the prevalent species in Nepal in the years 2002 and 2003 .But a* change in serotype in *Shigella* species was noticed during 2005. *Sh. flexneri* (58%) replaced *Sh. dysenteriae* (20%) and became the most prevalent species in 2005, followed by *Sh. sonnei* (12%) and *S. boydii* (10%).A marked increase in the number of Shigella sonnei(N=6) was also noticed in the year 2005(Table:1). During 2003 to 2005; the number of *Sh dysenteriae* type 1 strains were 8, 12 and 6 respectively. (Table :2).

The antimicrobial susceptibility profile of Shigella isolates in the years 2003, 2004 and 2005 is shown in the tables 3, 4, 5 and 6.On the basis of their pattern of antimicrobial resistance to the six antibiotics used in the study, the reported Shigellae were grouped into 9 phenotypes (Table:7). 33% of the total Shigella studied were multi drug resistant(showed resistance to 3 or more antibiotics). None of the *Shigella* were resistant to Ceftriaxone and Azithromycin. Resistance to Ciprofloxacin was seen only among *Shigella dysenteriae*-1 strains.

Table 1: Yearly distribution of different species of Shigella

Year	*Sh dysenteriae*	*Sh flexneri*	*Sh boydii*	*Sh sonnei*	**Total**
2003	12	7	3	2	**24**
2004	27	13	2	1	**43**
2005	10	31	4	6	**51**
Total	**49(41.52%)**	**51(43.22%)**	**9(7.62%)**	**9(7.62%)**	**118**

Table 2: Yearly breakdown of *Shigella dysenteriae* isolates

Year	*Shigella dysenteriae*			*Sh dysenteriae type (2-14)*	Total *Sh dysenteriae*
	Cip-S	Cip-R	Total		
2003	2	6	8	4	12
2004	3	9	12	15	27
2005	3	3	6	4	10
Total	**8**	**18**	**26**	**23**	**49**

Table 3: Antimicrobial sensitivity profile of *Shigella dysenteriae* .

Year	*Antibiogram results for Shigella dysenteriae (Total, N =49)*					
	Ampicillin	***Cotrimoxazole***	***Nalidixic***	***Ciprofloxacin***	***Azithromycin***	***Ceftiaxone***
2003	*91%R*	*75%R*	*83.3%R*	*50%R*	*100%S*	*100%S*
2004	77.77%R	*66.66%R*	*55.55%r*	*33.33%R*	*100%S*	*100%S*
2005	*80%R*	*70%R*	*70%R*	*30%R*	*100%S*	*100%S*
3yrs	*82.9%R*	*70.3%R*	*69.6%R*	*37.7%R*	*100%S*	*100%S*

Table 4: Antimicrobial sensitivity profile of *Shigella flexneri*

Year	*Antibiogram results for Shigella flexneri (Total, N=51)*					
	Ampicillin	***Cotrimoxazole***	***Nalidixic***	***Ciprofloxacin***	***Azithromycin***	***Ceftriaxone***
2003	*100%R*	*85.8%R*	*100%R*	*100%S*	*100%S*	*100%S*
2004	*53.8%R*	*61.5%R*	*38.4%R*	*100%S*	*100%S*	*100%S*
2005	*60%R*	*67.8%R*	*59%R*	*100%S*	*100%S*	*100%S*
3yrs	*71.26%R*	*71.7%R*	*65.8%R*	*100%S*	*100%S*	*100%S*

Table 5: Antimicrobial sensitivity profile of *Shigella boydii*

Year	*Antibiogram results for Shigella boydii(Total, N=9)*					
	Ampicillin	***Cotrimoxazole***	***Nalidixic***	***Ciprofloxacin***	***Azithromycin***	***Ceftriaxone***
2003	*66%R*	*66%R*	*33%R*	*100%S*	*100%S*	*100%S*
2004	*100%R*	*50%R*	*0%R*	*100%S*	*100%S*	*100%S*
2005	*66%R*	*100%R*	*50%R*	*100%S*	*100%S*	*100%S*
3yrs	*77.77%R*	*72%R*	*27.66%R*	*100%S*	*100%S*	*100%S*

Table 6: Antimicrobial sensitivity profile of *Shigella sonnei*

Year	*Antibiogram results for Shigella sonnei(N=6)*					
	Ampicillin	***Cotrimoxazole***	***Nalidixic***	***Ciprofloxacin***	***Azithromycin***	***Ceftriaxone***
2003	*50%R*	*100%S*	*100%S*	*100%S*	*100%S*	*100%S*
2004	*0%r*	*100%R*	*100%R*	*100%S*	*100%S*	*100%S*
2005	*33%R*	*50%R*	*50%R*	*100%S*	*100%S*	*100%S*
3yrs	*37.77%R*	*50%R*	*50%R*	*100%S*	*100%S*	*100%S*

Table 7: Drug resistant phenotypes of Shigella

Pattern of drug resistance	***Shigella dysenteriae*** (No. (%))	***Shigella flexneri*** (No. (%))	***Shigella boydii*** (No. (%))	***Shigella sonnei*** (No. (%))	**Total**
Amp-R, (Sxt,Cip,NA,CRO,Azm-S)	6(12.2%)	6(11.7%)	2(22.22%)	0(0%)	**14(11.8%)**
Sxt-R, (Amp,Cip,NA,CRO,Azm-S)	2(4%)	3(5.8%)	1(11.11%)	0(0%)	**6(5%)**
NA-R, (Amp,Cip,Sxt,CRO,Azm-S)	0(0%)	3(5.8%)	0(0%)	0(0%)	**3(2.5%)**
Amp and Sxt-R, (Cip,NA,CRO,Azm-S)	3(6.1%)	15(29.4%)	1(11.11%)	0(0%)	**19(16.1%)**
Amp and NA-R, (Cip,Sxt,CRO,Azm-S)	1(2%)	5(9.85%)	2(22.22%)	0(0%)	**8(6.77%)**
NA and Sxt-R, (Cip, Amp, CRO, Azm-S)	0(0%)	7(13.7%)	0(0%)	4(44.44%)	**11(9.3%)**
Multi-resistant (**Amp,Sxt & NA–R),** (Cip,CRO,Azm- S)	12(24.5%)	7(13.7%)	1(11.11%)	2(22.22%)	**22(18.6%)**
Multi-resistant (**Amp, Sxt, NA & Cip –R**) , (CRO,Azm- S)	18(36.7%)	0(0%)	0(0%)	0(0%)	**18(15.25%)**
All six antibiotics sensitive strains.	7(14.2%)	5(9.8%)	2(22.2%)	3(33.33%0	**17(14.4%)**
Total	**49**	**51**	**9**	**9**	**118**

Discussion

Emergence of multidrug-resistant *Shigella* strains is of concern to clinicians in treating shigellosis cases. Adequate treatment for shigellosis depends upon the availability of effective antimicrobial agents. A variety of antibiotics are available for treatment of shigellosis, although options are becoming limited due to globally emerging drug resistance. Originally, both sulfonamides and tetracycline were reported to be effective, but *Shigella* strains rapidly developed resistance to these agents. Ampicillin and Cotrimoxazole were then used and continued to be effective in many industrialized countries. Unfortunately, in many parts of the world, *Shigella species* resistant to these low-cost agents have been reported, and neither of these can now be confidently used as empiric therapy for shigellosis [11,12 ,13, 14,15, 16, 17,18].One of the few remaining, relatively inexpensive and effective drugs for shigellosis was the quinolone-Nalidixic Acid, but resistance to this drug has also been reported in high percentage from different parts of the world[7,19,20] The present study also shows high resistance among Shigella species to the first line low cost drugs commonly used in the treatment of shigellosis like Ampicillin, Cotrimoxazole and Nalidixic acid.18.6% of the total isolates tested were found to be resistant to all these three antibiotics.

Shigellae strains are susceptible *in vitro* to other antimicrobial drugs, such as gentamicin and certain cephalosporins, but these drugs have not been useful clinically [20].To add more to this increasing trend towards antimicrobial resistance, multidrug resistant *Sh. dysenteriae* -1 strains (resistant to ampicillin, cotrimoxazole, nalidixic acid) with the emergence of ciprofloxacin resistance have been reported[8,9,10]from various countries. After the emergence of NA and Sxt resistant shigellae, Ciprofloxacin has remained the drug of choice for treatment of shigellosis in adults. However with the emergence of Ciprofloxacin resistance, the drug options have narrowed. Our study also shows that 15.2% of the total Shigella analysed were resistant to Ciprofloxacin. These ciprofloxacin-resistant shigellae were also resistant to Ampicillin, Cotrimoxazole and Nalidixic acid. But fortunately, the Ciprofloxacin resistance in Shigella at present in Nepal, according to our study is, limited to *Sh dysenteriae type*-1 strains only and most cases of shigellosis continue to be caused by strains other than *Sh dysenteriae type*-1, and they remain susceptible to Ciprofloxacin. Out of 8, 12 and 6 Shigella dysenteriae type-1 isolated in 2003 ,2004 and 2005 respectively, 6, 9 and 3 were found to be resistant to Ciprofloxacin.

Plasmid profiles of isolated *Sh dysenteriae* 1 strains and typing the strains by using various molecular tools could provide insight into the origin of these recently isolated *Sh dysenteriae* type1 strains and the relationships among the strains. The challenge for clinical management is identifying which drugs retain their activity and clinical effectiveness. Center for Disease Control and Prevention (CDC) has recommended that sensitivity testing be performed to guide selection of appropriate antimicrobial therapy for shigellosis [4]. However, testing requires several days to complete, resulting in treatment delay, and is generally not feasible in developing countries. Because antimicrobial susceptibility patterns of shigellae may vary greatly in different geographical areas and over time, monitoring resistance patterns is needed to guide selection of appropriate empiric treatment [21, 22, 23].

Search for alternate new drugs should be continued because, although newer antimicrobial drugs can offer hope for treatment of shigellosis, emergence of resistance to the new drugs is also not far in the future. Thus, intensive water and sanitation programs and vaccine development would seem to be critical. It is not realistic to continue relying on the introduction of a new antibiotic every few years. However, accomplishing those objectives and reaching the goal is not an easy task in developing countries. Laboratory detection capabilities also need to be strengthened at all levels to increase the baseline surveillance data for improved isolation of the pathogen. Shigella infection is typically via faecal oral contamination and ingestion and since multidrug resistance is increasing, emphasis should be laid on prevention .The spread of Shigella can be stopped by frequent and careful handwashes, basic food safety precautions(avoiding uncooked and contaminated food), and regular drinking water treatment. Perhaps the more effective way of reducing the impact of the disease and the risk of contracting infection lies in improving poor living condition, disseminating health education, and supplying safe drinking water.

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