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Identification, Characterization and Antibiotic Susceptibility of *Salmonella* and *Shigella* species Isolated from Blood and Stool Samples of Patients Visiting N. I. H, Islamabad

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The study was designed to determine the incidence of *Salmonella* and *Shigella* spp. in blood and stool samples of patients. A total of 150, samples of blood (110) and stool (40) were collected from patients, reporting at NIH (National Institute Health) and six *Salmonella* and two *Shigella* strains were identified by colony identification and biochemical tests and serotyping. Of the 6 *Salmonella* isolates; 2 were of *S. typhi,* 1 of *S. paratyphi* A, 1 of *S. paratyphi* B and 2 of other *Salmonella* spp. The 2 *Shigella* strains from stool samples were *S. flexneri* (Poly B serotype). *Salmonella* isolates were found to be susceptible to gentamicin, ofloxacin, cefotaxime, amikacin, tobramycin, cefaclor, while resistant to ampicillin, cefamendole, chloramphenicol, gentamicin and cefuroxime. *Shigella* strains showed resistance to vibramycin, ampicillin, tetracycline and sensitivity against nalidixic acid, norfloxacin, chloramphenicol, amikacin, and aztreonam. Despite the fact that the overall prevalence of *Salmonella* and *Shigella* reported in this study was low, there is sufficient evidences to indicate that better hygiene and water treatment and management would even further reduce the incidence of these infections.

Key words: *Salmonella*, *Shigella*, blood, stool, antibiotic susceptibility

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Introduction

Diarrheal diseases and enteric infections are the major causes of morbidity and mortality in developing world resulting in over quarter of all childhood deaths. Globally *Salmonella* and *Shigella* remain the major causative agents associated with acute enteric infections, with non-typhoid salmonella isolated in increasing number from diverse geographic regions (Jousilahti *et al*., 1997). *Salmonella* species inhabit the gastrointestinal tracts of animals. Humans acquire the infection by ingesting the organisms in contaminated animal products, or waters. Other *Salmonella* species are found only in humans, and infections are transmitted by human carriers (Mahon and Manuselis, 1995). Environmental source of organism includes water, soil factory, kitchen, animal feces, raw milk and meat. All age groups are susceptible to salmonellosis, but it is more severe in elderly infants (Preston and Borezyk, 1994).

Infection caused by *Shigella* species are associated with human, no animal reservoir has been identified. *Shigella* dysentery usually indicates improper sanitary conditions and poor personal hygiene and is principally a disease – shigellosis – of humans, as well as other primates (Mahon and Manuselis, 1995). Shigellosis symptoms range from abdominal pain, cramps, fever, vomiting to bloody diarrhea, with mucus in stool. Some strains produce enterotoxin and Shiga toxin (very much like the toxin of *E. coli* O157:H7). Infections are associated with mucosal ulceration, rectal bleeding, drastic dehydration; fatality may be as high as 10-15% with some strains (Preston and Borezyk, 1994; Bogaerts *et al*., 1997). Antimicrobial resistance in enteric pathogen is of the great importance in the developing countries, where the rate of diarrheal diseases is the highest and indiscriminate use of antibiotics is a fact. Shigellosis caused by multiple antibiotic resistant shigella are predominant isolates and show resistance to ampicillin, but susceptibility to chloramphenicol, with the exception of *S. flexneri*, which is also susceptible to gentamicin (Urio *et al*., 2001). *S. typhi* and *S. paratyphi* have shown resistance to ampicillin, chloramphenicol and cotrimoxazole is now a recognized problem in many parts of world especially in the sub-continent, which further emphasizes the need to perform antibiotic sensitivity to minimize the hazards of drug resistance (Butt *et al*., 2000).

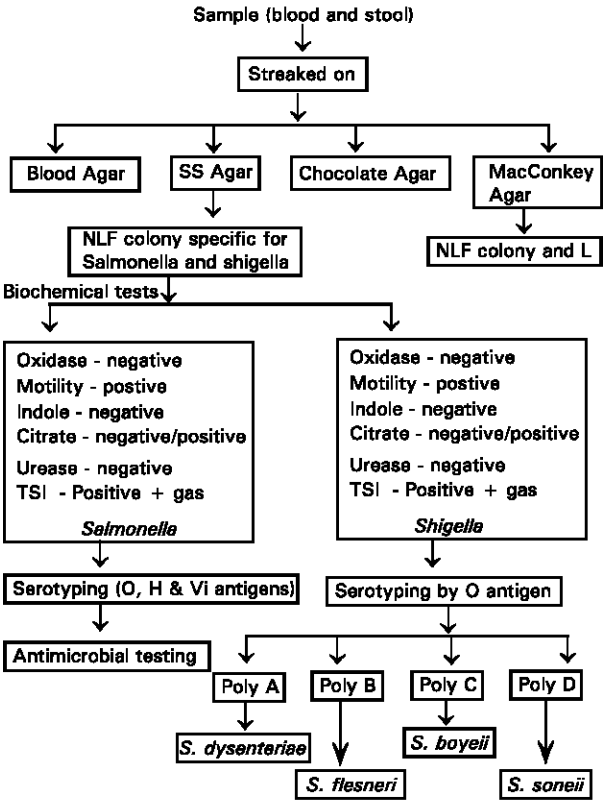
This study was designed to determine the incidence of *Salmonella* and *Shigella* spp. in blood and stool samples of patients suffering from diarrhea, visiting the NIH at Islamabad, and to ascertain the level of susceptibility of these clinical isolates to various antimicrobial drugs so, as to determine the extent of resistance, of our isolates, against commercially available drugs.

Materials and Methods

The study was conducted at the Bacteriology Laboratory, Public Health Division, NIH, Islamabad, between January and June 2001. One hundred and fifty samples, blood (110) and stool (40), were collected from diarrhea patients, residing in Rawalpindi Islamabad, visiting the Bacteriology laboratory at the NIH. These samples were streaked on blood, SS, MacConkey and chocolate agar and stool samples were inoculated in Selenite broth for sub-culturing, while Tryptic Soya Broth is used for sub-culturing blood samples and incubated for 24 to 72 hrs, respectively. The pathogens were identified by colony morphology as they form colorless colonies on all agars. Confirmatory tests included biochemical tests and serotyping (Annexure 1) (Ellen *et al*., 1994).

Serotyping: All the pathogenic forms of *Salmonella* and *Shigella* spp were confirmed up to species level by serotyping. For serological testing O (somatic), H (Flagellar) and Vi (capsular) antigens were used for confirmation. *Shigella* were serotyped by their O (somatic) antigens using polyvalent *S. dysenteriae, S. flexneri, S. boydi* and *S. soneii* antisera. *Salmonella* serotypes were done using O, H and Vi antigens. The groups were designated as A to Z, 51 to 61, 64 – 66. Medically important *Salmonella* belonged to groups A to G. The *Salmonella* was serotyped by their H antigen. They occurred in two antigenic forms referred as phase I and phase II. Vi antigen was mostly used for S. *typhi* and *S. paratyphi C*. (Cheesbrough, 1991). The procedure followed was in accordance with Edward and Ewing (1972).

Antibiogram pattern: Antibiogram pattern of isolated strains were determined on Mueller – Hinton agar. A sterile cotton swab was dipped into a sample from well-mixed colonies in peptone water and applied onto a Mueller – Hinton agar plate. Commercially available anti-microbial disks were placed on the plate by means of a multi-disk dispenser and pressed firmly onto the agar with sterile



Annexure 1: *Salmonella* and *Shigella* isolation and identification

forceps and incubated at 37°C for 24 hours, before measuring the zones of inhibition to determine the susceptibility of the isolates. For comparison, the anti-microbial susceptibilities of isolates from the standard cultures were determined by a standard disk method according to the guidelines of the National Committee for Clinical Laboratory Standard (NCCLS). *E. coli* ATCC 25922 was included for quality control (Johnson *et al*., 1995).

Zones of inhibition were determined with the help of list of break points of antibiotics. If the zone of inhibition was less than the minimum value of break point then it was assumed to be ineffective and if the zone of inhibition was equal to the maximum value or greater than maximum value of break point, then the antibiotics were assumed to be effective against *E. coli* (Cheesbrough, 1991).

Results and Discussion

Of the total blood and stool samples subjected to analysis for *Salmonella* and *Shigella species*, our study showed that 6 *Salmonella* isolates were identified all from the blood samples, whereas the 2 *Shigella* strains isolated were from stool samples (Table 1). *Salmonella species* isolated from blood culture included 2 isolates of *S. typhi*, 1 of *S. paratyphi* A, 1 of *S. paratyphi* B and 2 isolates of other *Salmonella* spp. of the 2 strains isolated from

Table 1: Incidence of *Salmonella* and *Shigella* spp.

Total no of samples	Blood samples	Stool samples	*Salmonella* species	*Shigella* species
150	110	40	6* (4%) *all from blood samples	2 ** (1.33%) ** both from stool samples

Table 2: Incidence of *Salmonella* and *Shigella* species/serotypes isolated from clinical samples

Species/ serotypes	No. of isolates
Salmonella typhi	2
Salmonella paratyphi A	1
Salmonella paratyphi B	1
Salmonella species	2
Sh. Flexneri	2

Table 3: Sex-wise distribution of *Salmonella* and *Shigell*a species

Isolates	Male (%)	Female (%)
Shigella species(6)	4(66.6%)	*2 (33.3%)*
	2 Salmonella typhi	*1 Salmonella*
	1 Salmonella paratyphi A	*Paratyphi B*
	1 Salmonella spp.	*1 Salmonyphi* spp.
Sh. Flexneri	1 (50%)	1 (50%)

Table 4: Antibiotic susceptibility of *Shigella* species

Antibiotics Used	*Shigella flexneri*
Nalidixic acid	S
Norfloxacin	S
Ciprofloxacin	S
Vibramycin	R
Aztreonam	S
Cotrimoxaaole	R
Impenam	S
Amikacin	S
Tetracycline	R
Ampicillin	R
Chloramphenicol	S
Cefotaxime	S
Amikacin	S

S - sensitive R - resistant

Table 5: Antimicrobial susceptibility of *Salmonella* species

Antibiotics used	*S. paratyphi A*	*S. typhi*	*Salmonella* spp.	*S. paratyphi B*
Amikacin	S	S	S	
Tobramycin	S	S	S	
Gentamicin	S	S	S	R
Ciprofloxacin	S			
Cefuroxime	S	R		
Nalidixic acid	S			
Ofloxacin	S	S	S	
Tetracycline	I			
Cefotaxime		S		I
Chloramphenicol		R		
Cefamendole		R	S	S
Cefaclor(cec)		S	S	
SulphaMetoxazole Trimethoprium		S	S	
Ampicillin		S	S	R
Cefoperazone(cfp)		S	S	
Enoxacin			S	S
Orelox(orx)				S

S - sensitive R - resistant I- intermediate

stool samples both were *Shigella flexneri* (Table 2). This low percentage of isolates may be due to the fact that the samples were collected during the cold dry season, whereas *Salmonella* and *Shigella* infections are more common during the rainy season and often occur as outbreaks (Yamashiro *et al.*, 1998). Sex-wise distribution showed that the 2 *Shigella flexneri* isolates were evenly distributed, i.e., one male and one female case. Whereas, the 6 *Salmonella* species showed a distinct distribution pattern in that I. e., 4 were from males and 2 from females (Table 3). These findings are in agreement with the reports of other researchers (Saqib *et al.*, 2000).

Drug susceptibility of *S. flexneri* isolates showed that they were resistant to vibramycin, cotrimoxazole, tetracycline and ampicillin, but sensitive to nalidixic acid, norfloxacin, ciprofloxacin, aztreonam, chloramphenicol, cefotaxime and amikacin (Table 4). Of the total 17 antibiotics used against *Salmonella* spp., all the *S. paratyphi* A strains showed intermediate resistance to tetracycline, and sensitivity to amikacin, tobramycin, gentamicin, ciprofloxacin, cefuroxime, nalidixic acid and ofloxacin. Whereas, *S. typhi* was resistant against cefuroxime, tetracycline, chloramphenicol, and cefamendole, while sensitive to amikacin, tobramycin, gentamicin, ofloxacin, cefotaxime, cefaclor, sulpha metoxazole-trimethoprim and cefoperazone. *S. paratyphi* B showed resistance against gentamicin and ampicillin, intermediate resistance against cefotaxime, but complete sensitivity against orelox, enoxacin, and cefamendole and intermediate against cefotaxime (Table 5). Similar findings have been reported by Binsztein *et al.* (1999) and Cruchaga *et al.* (2001).

Furthermore, the results of this study shows that *Shigella flexneri* resistant to tetracycline, ampicilline and cotrimoxazole, and sensitivity to amikacin, nalidixic acid, norfloxacin, aztreonam and chloramphenicol, which have also been reported by other researchers (Poiata *et al.*, 1996; Urio *et al.*, 2001; Yamashiro *et al.*, 1998).

The study under discussion also revealed that *S. typhi* strains showed greater resistance agaisnt drugs, which is in agreement with the findings of other workers (Farooqi *et al.*, 1990), who reported that *S. typhi* and *S. paratyphi* showed resistance against ampicilline and chloramphenicol, as indeed reflected in this study. As for the sensitivity pattern, our study showed that cefotaxime, cefaclor, SXT, amikacin and ofloxacin were effective against *S. typhi*, which is in conformity with other findings (Binsztein *et al.*, 1999, Butt *et al.*, 2000) and the overall resistance pattern for Sa*lmonella* spp showed that chloramphenicol, ampicilline, gentamicin and cefamendole were ineffective, which has also been reported by Saqib *et al.* (2001).

The findings of this study do reflect that there is *Shigella* infections, although few, were equally in both males and females, while in *Salmonella* infections, especially those caused by *S. typhi* were more common in men.

However, despite the low prevalence of *Salmonella* and *Shigella* infections, the mere outbreak of six and two incidences, over a period of a couple of months, does reflect the poor level of hygiene and water treatment being practiced today in Pakistan. This issue of environmental health is very important and should be addressed by the concerned authorities so as to minimize such outbreaks. Drug susceptibility tests are also essential to ensure effective therapy. In addition, the clinicians must stress patient compliance and over-the-counter sales of antibiotics should be curtailed. Only then will the spread of resistance strains be brought under control.

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