

Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and *Shigella* species associated with acute diarrhea in Tehran, Iran

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A study was performed to determine the prevalence and antimicrobial resistance of *Shigella* species and diarrheagenic *Escherichia coli* isolates cultured from patients with acute diarrhea in Tehran, Iran. Between May 2003 and May 2005, 1120 diarrheal specimens were collected and assayed for bacterial enteropathogens by conventional and molecular methods. Etiological agents were isolated from 564 (50.3%) specimens, and included 305 (54%) *E coli*, 157 (27.8%) *Shigella* species, and 102 (18%) from other genera of bacteria. The predominant *E coli* was Shiga toxin-producing *E coli* (105 isolates [34.5%]) and the predominant *Shigella* serotype was *Shigella sonnei* (88 isolates [56.1%]). A high rate of antibiotic resistance was observed among *E coli*, with 40 of 53 (75.5%) Shiga toxin-producing *E coli* isolates resistant to amoxicillin and tetracycline, and eight (5.2%) *E coli* isolates resistant to more than six antibiotics. Most *Shigella* isolates were resistant to tetracycline (95%) and trimethoprim-sulfamethoxazole (91.7%), with greatest antibiotic resistance observed among *S sonnei* (53 of 88 [60.2%] isolates). Antibiotic resistance is widespread in diarrheagenic *E coli* and *Shigella* in children with acute diarrhea in Tehran, Iran; hence, updated strategies for appropriate use of antimicrobial agents in Iran are needed.

Key Words: Antibiotic resistance; Diarrheagenic pathogens; *E coli*; Iran; *Shigella*

Diarrheal diseases caused by bacterial pathogens are a major problem worldwide, especially in developing countries (1,2). Five types of diarrheagenic *Escherichia coli*, including enterotoxigenic *E coli* (ETEC), enterohemorrhagic *E coli*, enteropathogenic *E coli* (EPEC), enteroinvasive *E coli* and enteroaggregative *E coli*, as well as *Shigella* species, are the most common causes of acute diarrhea in Iran (3,4). In Vietnam and Thailand, *E coli* is among the most common isolates from patients with diarrhea and has a high prevalence of resistance to antibiotics (5,6). *Shigella* is also a major cause of gastroenteritis, leading to annual deaths of three to five million children younger than five years of age in developing countries (7).

Antibiotic treatment of common bacterial infections plays a crucial role in reducing morbidity and mortality of diseases; however, overuse and misuse of antibiotics in the treatment of diarrhea could lead to increased antibiotic resistance (8). The progressive increase in antibiotic resistance among enteric pathogens in developing countries (9,10) is a research priority

La prévalence et la résistance antimicrobienne de l'*Escherichia coli* diarrhéogène et des espèces de *Shigella* liés à la diarrhée aiguë à Téhéran, en Iran

On a effectué une étude pour déterminer la prévalence et la résistance antimicrobienne des espèces de *Shigella* et des isolats d'*Escherichia coli* diarrhéogènes cultivés à partir de prélèvements de patients atteints de diarrhée aiguë à Téhéran, en Iran. Entre mai 2003 et mai 2005, on a prélevé et titré 1 120 échantillons de diarrhée pour dépister des entéropathogènes bactériens au moyen des méthodes classiques et moléculaires. On a isolé des agents étiologiques auprès de 564 échantillons (50,3 %), ce qui incluait 305 cas d'*E coli* (54 %), 157 espèces de *Shigella* (27,8 %) et 102 cas d'autres genres de bactéries (18 %). Le principal *E coli* était un *E coli* producteur de toxines Shiga (105 isolats [34,5 %]) et le principal sérotype de *Shigella*, le *Shigella sonnei* (88 isolats [56,1 %]). On a observé un fort taux d'antibiorésistance dans les cas d'*E coli*, 40 des 53 cas d'isolats d'*E coli* producteurs de toxines Shiga (75,5 %) étant résistants à l'amoxicilline et à la tétracycline et huit isolats d'*E coli* (5,2 %) étant résistants à plus de six antibiotiques. La plupart des isolats de *Shigella* étaient résistants à la tétracycline (95 %) et au triméthoprim-sulfaméthoxazole (91,7 %), la plus grande antibiorésistance s'observant dans les cas de *S sonnei* (53 des 88 isolats [60,2 %]). L'antibiorésistance est généralisée en cas d'*E coli* diarrhéogène et de *Shigella* chez les enfants atteints de diarrhée aiguë de Téhéran, en Iran. Il faut donc trouver de nouvelles stratégies afin d'utiliser les antimicrobiens convenablement en Iran.

of the diarrheal disease control program of the World Health Organization (11). Knowledge of antimicrobial resistance of diarrheagenic pathogens is important in selecting the appropriate therapy, because many patients with gastroenteritis are treated empirically with antibiotics and the antimicrobials may not be effective. This is especially relevant to the developing world, where the rate of diarrheal diseases is highest and the use of antimicrobial agents is often indiscriminate (12). The purpose of the present study was to determine the prevalence and antimicrobial susceptibility of diarrheagenic *E coli* and *Shigella* isolates from Iranian children with acute diarrhea in Tehran, from 2003 to 2005.

METHODS

Definitions

Diarrhea was defined as at least three loose stools in 24 h, any number of watery stools, or one or two loose stools in 24 h accompanied by at least one of the following symptoms:

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nausea, vomiting, abdominal cramps or fever of 38°C. Acute diarrhea was defined as diarrhea that lasted 14 days or less at the time of presentation. Isolates from children with persistent diarrhea were not included in this study. Persistent diarrhea was defined as diarrhea which lasted for more than 14 days at presentation. When diarrhea was present intermittently, it was considered persistent when diarrhea occurred during at least six days in a two-week period.

Sample collection

The present study included children who presented with acute diarrhea from May 2003 to May 2005 from four hospitals (two of four were children's hospitals) in Tehran. The children were categorized into six different groups according to their age – zero to two months, three to five months, six to 11 months, 12 to 23 months, 24 to 35 months and 36 to 60 months. They were part of the routine 25% to 30% surveillance sampling of all diarrhea children seen at the children's hospitals and clinical centres in Iran. The study was endorsed by the Ethic Committee of Research Center for Gastroenterology and Liver Diseases. Informed consent was obtained from the children's parents.

Fecal specimens

All specimens were added to Cary-Blair and PBS transport media and transported on ice packs to the laboratory of the Research Department of Foodborne and Diarrheal Diseases of Shaheed Beheshti University of Medical Sciences, M.C. in Tehran. Stool samples were collected on the day of presentation at the outpatient department, immediately transported to the laboratory, stored at 4°C and processed within 24 h.

Bacterial isolation

Samples (swabs from Cary-Blair transport media) were cultured for *E coli*, *Shigella* and *Salmonella* species by streaking directly onto xylose-lysine deoxycholate agar, MacConkey agar and Salmonella-Shigella agar (Merck, Germany), and incubating for 24 h at 37°C, and by enriching in Selenite F broth at 37°C overnight. Enrichment cultures were subcultured on each of the media described above by incubating at 37°C overnight. Swabs from Cary-Blair transport media of samples were also cultured for *Campylobacter* species, on enrichment media including Preston enrichment broth base (HIMEDIA, Mumbai, India, M899) supplemented with *Campylobacter* selective supplement IV (HIMEDIA, Mumbai, India, FD158) with an antibiotic (*Campylobacter* Supplement 2, Blaser Wang, HIMEDIA, Mumbai, India, FD 006) and 5% defibrinated sheep blood and incubated for 48 h at 42°C under microaerophilic conditions. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and tested for Gram stain, production of catalase and oxidase and hippurate hydrolysis. For *Yersinia* species, swabs from PBS transport media were cultured on selective agar, CIN agar, (Merck K GaA Darmstadt, Germany) and incubated at 22°C for 24 h to 72 h.

Molecular diagnosis of diarrheagenic *E coli* and *Shigella* species

E coli-like and other Gram-negative colonies growing on the primary culture medium were tested by polymerase chain reaction (PCR) assay for virulence genes. For DNA extraction, a loopful of bacterial growth obtained from the culture plate was

suspended in 0.75 mL of sterile distilled water and boiled for 20 min.

Extracts of genomic DNA from mixed cultures were assayed by PCR using seven different sets of primers targeting *stx1*, *stx2*, *eae*, pCVD432 plasmid, *lt*, *st* and *ipaH* (13-16). A 2 µL portion of this suspension was added to 22 µL of PCR mixture (50 mM potassium chloride, 10 mM Tris-hydrochloride [pH 8.3], 1.5 mM magnesium chloride, 0.2 mM each deoxynucleotide triphosphate and 0.6 U of Taq polymerase) and 1 µL of primer mix 1 or 2 containing 10 ng primer/µL. All samples were amplified in a programmable thermocycler (Eppendorf AG 2233, Germany) by cycling at 95°C for 2 min to denature DNA, then 30 cycles for 30 s at 94°C, 30 s at 60°C to anneal the primers (*stx1*, *stx2* and *eae* [58°C], *lt*, *st* and pCVD423 [57°C] and *ipaH* [52°C]), 20 s at 72°C, and a final prolonged extension at 72°C for 10 min.

The PCR product bands were separated by electrophoresis through 1.3% agarose gel in 1× TBE buffer. DNA fragments were visualized by ethidium bromide staining and photographed under ultraviolet light illumination.

Up to 15 to 30 suspected colonies for diarrheagenic *E coli*-positive cases were tested for the virulence genes. *E coli* isolates that were *eae*-positive and *stx*-negative were identified as EPEC. Isolates positive for pCVD432 were identified as EAEC and those *stx1*- or *stx2*-positive, or both, were Shiga toxin-producing *E coli* (STEC). Those *E coli* isolates that were *lt*- or *st*-positive, or both, were ETEC. Positive controls with genomic DNA from *E coli* ATCC 35401 (*lt*-positive, *st*-positive), *E coli* ATCC 43894 (*stx1*-positive, *stx2*-positive, *eae*-positive), *E coli* RH 4620 (Pcvd432-positive) and *Shigella* ATCC 9290 (*ipaH*-positive) were included in each PCR run along with a nontemplate-containing reaction to detect false-positive results. For isolation the *Shigella* strains, PCR was done for *ial* gene by the *ial* lower GGAGGCCAACAATTATTTCC and *ial* upper CTGGATGGTATGGTGAGG primers (320 base pairs). Which sample was positive for both *ial* and *ipaH* genes presented as *Shigella* strains (17).

Biochemical identification and serological assay

Colonies morphologically resembling *Shigella* species were further identified by biochemical reactions according to standard methods (8) and confirmed by slide agglutination test using commercially available antisera from Mast Group Ltd (18). *E coli* isolates that were positive for both *stx1* and *stx2* genes were tested by O157 and H7 antiseras (MAST House, Merseyside, United Kingdom).

Antibiotic susceptibility testing

E coli isolates were tested for susceptibility to tetracycline (30 µg), ampicillin (10 µg), ceftazidime (30 µg), nalidixic acid (30 µg), gentamicin (10 µg), amoxicillin-clavulanic acid (30 µg), sulfamethoxazole (30 µg), ceftriaxon (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg) and streptomycin (10 µg) by the Kirby-Bauer disk diffusion method (MAST House, Merseyside, United Kingdom) (19).

Shigella isolates were also assayed for the same 11 antibiotics in addition to erythromycin (10 µg), cefixime (5 µg) and ampicillin-sulbactam (20 µg). The resistance break points were those recommended by Clinical and Laboratory Standards Institute, (20) and was reported as sensitive or resistant based on break points. Bacteria used for controls included *Staphylococcus aureus* ATCC 25923 and *E coli* ATCC 25922.

TABLE 1
Prevalence of enteropathogenic bacteria isolated from children with acute diarrhea (n=564) in Tehran, Iran, from May 2003 to May 2005

Enteropathogens	Number of isolates
<i>Shigella</i> species	157
<i>Shigella sonnei</i>	88
<i>Shigella flexneri</i>	48
<i>Shigella dysenteriae</i>	13
<i>Shigella boydii</i>	8
STEC	105
<i>stx1</i>	30
<i>stx2</i>	65
<i>stx1/stx2</i>	10
EAEC	92
EPEC	70
ETEC	38
LT-producing	13
ST-producing	17
LT/ST-producing	8
<i>Campylobacter</i> species	60
<i>Salmonella</i> species	42
<i>Yersinia</i> species	0
Total	564

EAEC Enteraggregative *Escherichia coli*; EPEC Enteropathogenic *E coli*; ETEC Enterotoxigenic *E coli*; STEC Shiga toxin-producing *E coli*

Statistical analysis

All data were entered into SPSS/PC 11.0 software (SPSS Inc, USA) and comparative statistics were calculated using the two-tailed χ^2 test and Fisher's exact test. $P \leq 0.05$ was considered to be significant.

RESULTS

The etiological agents were isolated from 564 of 1120 stool specimens (50.3%) from children with acute diarrhea. The enteropathogens isolated included 305 (54%) *E coli* and 157 (27.8%) *Shigella* species, and 102 (18%) belonged to other genera of bacteria (60 [10.6%] *Campylobacter* species and 42 [7.5%] *Salmonella* species) (Table 1). The most frequently isolated *E coli* was STEC (34.5%), whereas the predominant serotype of *Shigella* species was *Shigella sonnei* (56.1%) (Table 1).

The highest acute diarrhea rate was from children 12 to 24 months of age (n=266) during the dry seasons (from June 20 to September 20) (68.6%) (Table 2). The 153 pure diarrheagenic *E coli* strains were isolated and selected for antibiotic sensitivity testing. The number of isolates compared by PCR-positive samples show that the molecular technique is more sensitive than conventional tests.

The *E coli* and *Shigella* species isolates had a high prevalence of resistance to commonly used antibiotics such as tetracycline, ampicillin and trimethoprim-sulfamethoxazole. Results of antibiotic susceptibility testing of STEC revealed that 40 of 53 isolates (75.5%) were resistant to amoxicillin-clavulanic and tetracycline and 36 (67.9%) were resistant to trimethoprim-sulfamethoxazole, whereas none was resistant to ciprofloxacin, gentamicin, ceftazidime and ceftriaxone (Table 3). Although antimicrobial resistances are reported for the STEC isolates, antibiotics are not used for treatment of STEC infections; rather, these results are reported for epidemiological tracking

TABLE 2
Basic information and clinical symptoms of the 1120 children with acute diarrhea analyzed

Characteristic	
Seasons, n (%)	
Spring	215 (19.2)
Summer	537 (48.0)
Fall	227 (20.2)
Winter	141 (12.6)
Age (months), n (%)	
0–2	89 (7.9)
3–5	171 (15.3)
6–11	219 (19.5)
12–23	266 (23.7)
24–35	121 (10.8)
36–60	254 (22.7)
Sex ratio (male:female)	1.21:1
Age, months (mean \pm SD) (male)	33.07 \pm 6.89
Age, months (mean \pm SD) (female)	32.93 \pm 7.27
Diarrhea, n (%)	
Frequency (mean \pm SD)*	7.1 \pm 2.4
Watery	469 (41.8)
Mucus	429 (38.3)
Bloody	112 (10)
Unknown	110 (9.8)
Vomiting, n (%)	631 (56.3)
Anorexia, n (%)	314 (28)
Fever, n (%)	322 (28.7)
Abdominal pain, n (%)	245 (21.9)
Antibiotic consumption, n (%)	84 (7.5)

*Number of depositions per day

purposes only. Of the EPEC isolates, 19 (54.3%) were resistant to trimethoprim-sulfamethoxazole; however, all were susceptible to nalidixic acid, ciprofloxacin, gentamicin, ceftazidime and ceftriaxone. Of 45 EAEC isolates, 32 (71.1%) and 27 (60%) were resistant to trimethoprim-sulfamethoxazole and ampicillin, respectively. The percentages of ETEC isolates resistant to ampicillin, amoxicillin-clavulanic acid, streptomycin and trimethoprim-sulfamethoxazole were 60% (n=12), 60% (n=12), 50% (n=10) and 60% (n=12), respectively, and 20% were resistant to nalidixic acid and ciprofloxacin (Table 3). *Shigella* species isolates had the highest rates of resistance to tetracycline (95%) and trimethoprim-sulfamethoxazole (91.7%), and more than 90% of *Shigella* species isolates were susceptible to cefixime, ceftriaxone, ceftazidime, nalidixic acid, gentamicin, ciprofloxacin and ampicillin-sulbactam ($P < 0.05$) (Table 3). Eighty-five of 88 *S sonnei* isolates (96.5%) were resistant to tetracycline and trimethoprim-sulfamethoxazole, and 66 (75%) to erythromycin (75.1%). All the *S sonnei* and *Shigella flexneri* isolates were susceptible to chloramphenicol, ceftazidime and gentamicin, and 94.7% and 98.8% of the isolates were susceptible to nalidixic acid and ampicillin-sulbactam, respectively (Table 4).

Resistance to more than four antibiotics was observed in 40.6% (n=62) of strains of the diarrheagenic *E coli* isolates, and eight (5.2%) were resistant to more than six antibiotics (Table 5). The predominant multidrug resistance pattern in these isolates (13.7%) was ampicillin, amoxicillin-clavulanic, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole

TABLE 3
Antimicrobial resistance of *Escherichia coli* isolates from children with acute diarrhea in Tehran, Iran

<i>E coli</i>	Resistance, n (%)													
	AMP	AMC	CHL	CRO	CAZ	GEN	CIP	TET	NAL	STR	SXT	CHL	CIP	SAM
EPEC	16 (45.7)	13 (37.1)	7 (20)	0	0	0	0	13 (37.1)	0	8 (22.8)	19 (54.3)	8 (9)	0	1 (1.2)
STEC	35 (66)	40 (75.5)	32 (60.4)	0	0	0	0	40 (75.5)	1 (1.9)	33 (62.3)	36 (67.9)	23 (47.9)	0	0
EAEC	27 (60)	23 (51.1)	16 (35.5)	0	1 (2.2)	2 (4.5)	1 (2.2)	26 (57.8)	3 (6.7)	23 (51.1)	32 (71.1)	1 (7.6)	0	0
ETEC	12 (60)	12 (60)	1 (5)	0	4 (20)	6 (30)	4 (20)	9 (45)	4 (20)	10 (50)	12 (60)	0	0	0

AMC Amoxicillin-clavulanic acid; AMP Ampicillin; CAZ Ceftazidime; CHL Chloramphenicol; CIP Ciprofloxacin; CRO Ceftriaxone; EAEC Enterococcal *Escherichia coli*; EPEC Enteropathogenic *E coli*; ETEC Enterotoxigenic *E coli*; GEN Gentamicin; NAL Nalidixic acid; SAM Ampicillin-sulbactam; STEC Shiga toxin-producing *E coli*; STR Streptomycin; SXT Trimethoprim-sulfamethoxazole; TET Tetracycline

TABLE 4
Antimicrobial resistance of *Shigella* species isolates from children with acute diarrhea in Tehran, Iran

<i>Shigella</i> serotype	Resistance, n (%)													
	ERY	CEF	CFM	CRO	TET	AMP	CAZ	SXT	NAL	AMC	GEN	CHL	CIP	SAM
<i>S sonnei</i>	66 (75)	26 (29.5)	1 (1.1)	0	85 (96.5)	50 (56.8)	0	85 (96.5)	2 (2.3)	27 (30.6)	0	8 (9)	0	1 (1.2)
<i>S flexneri</i>	29 (60)	4 (8.3)	0	0	46 (95.8)	23 (47.9)	0	42 (87.5)	0	26 (54.1)	2 (4.0)	23 (47.9)	0	0
<i>S boydii</i>	11 (84)	6 (38.4)	0	0	11 (84.6)	5 (38.4)	0	10 (76.9)	2 (15.4)	8 (61.5)	0	1 (7.6)	0	0
<i>S dysenteriae</i>	6 (75)	1 (12.5)	0	0	8 (100)	3 (37.5)	0	7 (87.5)	0	1 (12.5)	0	0	0	0

AMC Amoxicillin-clavulanic acid; AMP Ampicillin; CAZ Ceftazidime; CEF Cephalothin; CFM Cefixime; CHL Chloramphenicol; CIP Ciprofloxacin; CRO Ceftriaxone; ERY Erythromycin; GEN Gentamicin; NAL Nalidixic acid; SAM Ampicillin-sulbactam; SXT Trimethoprim-sulfamethoxazole; TET Tetracycline

TABLE 5
Distribution of resistance patterns among *Escherichia coli* strains isolated from children with acute diarrhea in Tehran, Iran, from May 2003 to May 2005

Antibiotics	STEC	EPEC	EAEC	ETEC	Total	Percentage of <i>E coli</i> isolates
AMP, TET, SXT, STR	0	1	1	0	2	1.3
AMP, AMC, SXT, STR	3	0	1	1	5	3.3
AMP, AMC, CHL, SXT	1	0	0	0	1	0.7
AMP, AMC, TET, SXT	1	0	1	0	2	1.3
AMP, AMC, TET, STR	0	0	0	1	1	0.7
AMP, AMC, CHL, TET	5	0	1	0	6	3.9
CHL, TET, SXT, STR	1	0	0	0	1	0.7
AMP, CHL, TET, STR	1	0	0	0	1	0.7
AMP, AMC, CHL, TET, STR	2	0	0	0	2	1.3
AMP, CHL, TET, SXT, STR	0	0	2	0	2	1.3
AMP, AMC, CHL, SXT, STR	0	0	3	0	3	2.0
AMP, AMC, TET, SXT, STR	1	0	2	1	4	2.6
CHL, TET, SXT, AMC, STR	1	0	0	0	1	0.7
AMP, AMC, CHL, NAL, TET, SXT	1	0	0	0	1	0.7
AMP, CHL, NAL, TET, SXT, STR	0	0	1	0	1	0.7
AMP, AMC, CHL, TET, SXT, STR	15	1	5	0	21	13.7
AMP, AMC, CHL, GEN, TET, SXT, STR	0	0	0	1	1	0.7
AMP, AMC, GEN, NAL, TET, SXT, STR	0	0	1	1	2	1.3
AMP, AMC, CAZ, GEN, CIP, TET, SXT, STR	0	0	0	1	1	0.7
AMP, AMC, CAZ, GEN, NAL, CIP, TET, SXT, STR	0	0	1	3	4	2.6
Total	32	2	19	9	62	40.4

AMC Amoxicillin-clavulanic acid; AMP Ampicillin; CAZ Ceftazidime; CHL Chloramphenicol; CIP Ciprofloxacin; CRO Ceftriaxone; EAEC Enterococcal *Escherichia coli*; EPEC Enteropathogenic *E coli*; ETEC Enterotoxigenic *E coli*; GEN Gentamicin; NAL Nalidixic acid; SAM Ampicillin-sulbactam; STEC Shiga toxin-producing *E coli*; STR Streptomycin; SXT Trimethoprim-sulfamethoxazole; TET Tetracycline

and streptomycin ($P < 0.05$). Four *E coli* isolates were resistant to nine antibiotics (Table 5). According to the definition of multidrug resistance by Lockhart et al (21), five *E coli* isolates were multi-drug resistant. These isolates were resistant to at least eight antimicrobials from three classes. Of these isolated, four were ETEC and only one of them was among EAEC isolates (Table 6).

Ninety-one (57.9%) *Shigella* species isolates were resistant to more than four antibiotics, with 16 resistant to six antibiotics (Table 7). *S sonnei* had the greatest percentage of multidrug-resistant isolates, with 53 (58%) resistant to more than four antibiotics. Eight (16.6%) *S flexneri* isolates were resistant to six antibiotics, and one was resistant to seven (Table 7).

TABLE 6
Multidrug-resistant *Escherichia coli* strains isolated from children with acute diarrhea

Antibiotics	STEC	EPEC	EAEC	ETEC	Total
AMP, AMC, CAZ, GEN, CIP, TET, SXT, STR	0	0	0	1	1
AMP, AMC, CAZ, GEN, NAL, CIP, TET, SXT, STR	0	0	1	3	4
Total	0	0	1	4	5

AMP Ampicillin; AMC Amoxicillin-clavulanic acid; CAZ Ceftazidime; CIP Ciprofloxacin; EAEC Enterococcal *Escherichia coli*; EPEC Enteropathogenic *E coli*; ETEC Enterotoxigenic *E coli*; GEN Gentamicin; NAL Nalidixic acid; STEC Shiga toxin-producing *E coli*; STR Streptomycin; SXT Trimethoprim-sulfamethoxazole; TET Tetracycline

TABLE 7
Distribution of resistance patterns among *Shigella* species isolated from children with acute diarrhea in Tehran, Iran, from May 2003 to May 2005

Antibiotic	<i>S flexneri</i>	<i>S sonnei</i>	<i>S dysenteriae</i>	<i>S boydii</i>	Total	Percentage of <i>Shigella</i> isolates
ERY, TET, AM, SXT	2	16	1	0	19	12
ERY, TET, AM, AMC	1	0	1	1	3	1.9
CEF, TET, AMP, SXT	1	1	1	0	3	1.9
ERY, TET, AMC, CHL	1	0	0	0	1	0.6
ERY, TET, SXT, AMC	2	2	0	0	4	2.5
ERY, TET, SXT, CHL	3	0	0	0	3	1.9
TET, SXT, AMC, CHL	2	1	0	0	3	1.9
TET, AMP, SXT, AMC	1	2	0	0	3	1.9
ERY, CEF, TET, SXT	0	4	0	0	4	2.5
ERY, CEF, TET, AMP, AMC	0	1	0	1	2	1.3
ERY, CEF, TET, SXT, AMC	0	3	0	1	4	2.5
ERY, TET, AMP, SXT, AMC	0	6	0	1	7	4.5
TET, AMP, SXT, AMC, CHL	6	0	0	0	6	3.8
ERY, TET, AMP, SXT, CHL	1	3	0	0	4	2.5
CEF, TET, AMP, SXT, AMC	0	4	0	0	4	2.5
ERY, CEF, CFM, TET, SXT	0	1	0	0	1	0.6
ERY, CEF, TET, AMP, SXT	0	3	0	0	3	1.9
ERY, CEF, TET, AMP, SXT, AMC	1	3	0	1	5	3.1
ERY, TET, AMP, SXT, NAL, CHL	0	0	0	1	1	0.6
ERY, TET, AMP, SXT, AMC, CHL	7	1	0	0	8	5.1
ERY, CEF, TET, AMP, AMC, CHL	0	1	0	0	1	0.6
ERY, CEF, TET, AMP, SXT, CHL	0	1	0	0	1	0.6
ERY, CEF, TET, AMP, SXT, AMC, CHL	1	0	0	0	1	0.6
Total	29	53	3	6	91	57.5

AMC Amoxicillin-clavulanic acid; AMP Ampicillin; CAZ Ceftazidime; CEF Cephalothin; CFM Cefixime; CHL Chloramphenicol; CIP Ciprofloxacin; CRO Ceftriaxone; ERY Erythromycin; GEN Gentamicin; NAL Nalidixic acid; SAM Ampicillin-sulbactam; SXT Trimethoprim-sulfamethoxazole; TET Tetracycline

All the *Shigella boydii* isolates were susceptible to ceftazidime, gentamicin, ceftriaxone, ciprofloxacin and ampicillin-sulbactam; however, two isolates were resistant to six antibiotics. Eleven (84.6%) *S boydii* and all eight *Shigella dysenteriae* isolates were resistant to tetracycline (Table 4). In addition, more than 90% of *Shigella* isolates were susceptible to cefixime, ceftriaxone, ceftazidime, nalidixic acid, gentamicin, ciprofloxacin and ampicillin-sulbactam, and all *Shigella* species isolates were susceptible to ciprofloxacin. Among the diarrheagenic isolates, 58% of *Shigella* species and 40% of *E coli* were resistant to more than four antibiotics. No *Shigella* species isolated categorized as multidrug-resistance by this definition.

DISCUSSION

The present study provides results of prevalence and antibiotic resistance patterns of diarrheagenic *E coli* and *Shigella* species isolates from children younger than five years of age with acute diarrhea in Iran. *E coli* (305 isolates [54%]) and *Shigella* species (157 isolates [27.8%]) were the most prevalent bacterial enteric pathogens isolated. These results are in the agreement with previous studies in which diarrheagenic *E coli* and *Shigella* species were identified as the most common bacterial enteric pathogens in Iran (18,22). Other isolated bacteria (*Campylobacter* [10.6%] and *Salmonella* [7.45%]) were less commonly associated with acute diarrheal illness in Iran than the diarrheagenic *E coli* and *Shigella* species (18,23,24) ($P < 0.05$).

Determining the prevalence of diarrheal pathogens should save the way for better control of the disease in the country. Continued vigilance of the safety of food, health education of food handlers, and close attention to hygiene and sanitary

conditions can provide an effective barrier against the spread of shigellosis (25). The antimicrobial resistance may be as a result of inappropriate and wide use of different antibiotics to treat infection.

Resistance to currently used antimicrobial agents among enteric pathogens has increased dramatically worldwide during the past decade (24,26,27). In developing countries, trimethoprim-sulfamethoxazole, ampicillin and tetracycline are widely used to treat diarrhea because of their low cost and availability (8). The widespread use of these antibiotics has resulted in an increased prevalence of resistance to these antibiotics by diarrheagenic bacteria, thereby raising concern among general practitioners and pediatricians, especially in developing countries (28).

Studies in Vietnam revealed 86.4%, 77.2% and 19.1% of *E coli* isolates were resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, respectively (8), whereas in Egypt the occurrence of antibiotic resistance among *E coli* isolates from patients with acute diarrhea was 68.2%, 57.2% and 24.2% for ampicillin, trimethoprim-sulfamethoxazole and ampicillin-sulbactam, respectively (29).

According to the United States Centers for Disease Control and Prevention National Antimicrobial Resistance Monitoring System for Enteric Bacteria in 2004 (www.cdc.gov/narms), the percentage of *S sonnei* isolates resistant to trimethoprim-sulfamethoxazole and tetracycline increased from 38.5% and 22.1%, respectively, in 2003 to 53.1% and 36.1%, respectively, in 2004. Resistance of *S flexneri* isolates to trimethoprim-sulfamethoxazole also increased from 28.8% in 2002 to 45.9% in 2004. Overall, in all years from 1999 to 2004, more than 90%

of *Shigella* isolates tested was resistant to at least one Clinical and Laboratory Standards Institute subclass.

In addition, a report from Iran cited by the World Health Organization indicates that sulfamethoxazole-trimethoprim, tetracycline and chloramphenicol were the least effective antibiotics since 112 (80.0%), 90 (64.3%) and 78 (55.7%) of the diarrheagenic *E coli* isolates were resistant to these antibiotics, respectively (30). Approximately 95% of *Shigella* species isolates in our study was resistant to tetracycline and 91.7% to trimethoprim-sulfamethoxazole. Comparing our data with previous reports from Iran reveals the same resistance pattern to commonly used antimicrobials such as tetracycline, sulfamethoxazole-trimethoprim and ampicillin, except resistance to chloramphenicol. Results from our study indicate the sensitivity of the most recent isolates to chloramphenicol has increased as all diarrheagenic *E coli*, *S dysenteriae* and *S boydii* isolates were sensitive to chloramphenicol previously.

Our findings are in agreement with reports of others in Iran indicating diarrheagenic *E coli* and *Shigella* isolates are resistant to trimethoprim-sulfamethoxazole, tetracycline and erythromycin (9,18). However, our results indicate higher rates of resistance to commonly used antibiotics and the emergency of isolates resistant to more than six antibiotics compared with reports from Iran and other countries. These findings illustrate the need for an ongoing monitoring system for antimicrobial resistance in Iran. Many studies have determined that multidrug resistance is common among *Shigella* and *E coli* isolates, especially to ampicillin and trimethoprim-sulfamethoxazole which are frequently used to treat shigellosis (31-33). In these cases, ampicillin, trimethoprim-sulfamethoxazole and tetracycline should be excluded for the treatment of diarrhea and

quinolones, to which these isolates *E coli* and *Shigella* species were susceptible, can be used as an alternative treatment.

Our study revealed a high prevalence of multidrug antibiotic resistance among *Shigella* and *E coli* isolates to the antibiotics most frequently used to treat diarrheal illnesses in Iran ($P<0.05$). The emergence of multidrug resistance requires continuous monitoring of the resistance patterns and the development and implementation of best practices strategies for antibiotic usage to extend the efficacy of the few effective antibiotics that remain.

The pattern of antibiotic resistance was monitored for *Shigella* and diarrheagenic *E coli* isolates. According to the definition of multidrug resistance by Lockhart et al (21), five *E coli* isolates were multidrug resistant. These isolates were resistant to at least eight antibiotics from three classes (one extended-spectrum cephalosporin, one aminoglycoside and ciprofloxacin) (21). It is quite unique finding in that similar trend in antimicrobial resistance of diarrheagenic pathogens, and has not been observed in Iran. This may be due to inappropriate use of antimicrobials especially in blind cases. The changing patterns of resistance to common antimicrobial agents in Iran indicates that designing a surveillance system for antimicrobial resistance and the introduction of integrated guidelines for the appropriate use of antibiotics are urgently needed. The result of this study suggests antimicrobial resistance is widespread among diarrheagenic *E coli* strains.

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