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Antimicrobial Susceptibility of Bacteria Isolated from Diarrheal Diseases in Infants and Young Children in Iran

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Abstract
Background: The antibiotic resistance of enteric bacteria has profound clinical implications because it threats the life and causes many of serious diseases such as acute gastroenteritis. **Methods:** Stool samples were inoculated, isolated and identified using standard bacteriological methods. Specimens were cultured and disk diffusion susceptibility testing was performed according to National Committee of Clinical Laboratory Standard (NCCLS) guideline. A total of eight selected antibiotic disks were used in the test. **Results and Discussion:** A total of 7321 stool samples were collected from patients with acute diarrhea. Among these diarrheal patients, 433 of enteropathogenic bacteria were isolated. enteropathogenic Escheichia coli (EPEC) 146(%)Salmonella 83(%), Shigella 79(%) ,Candida albicans 119(%), and other pathogen 6(%) were isolated. . Among enteric pathogens, EPEC was the predominant bacterium. The antimicrobial profile of all isolated bacteria Shigella (36.57%) ,Salmonella (29.85%) ,EPEC (53%) and EPEC showed high resistance rate against the tested 7 antimicrobials. the highest antimicrobial resistance rates were found against Ampicillin (82%).the isolates showed maximum sensitivity to CP(98%) ,CRO (98%), CFM (96%), NA (88%) in Salmonella spp. In Shigella spp. the highest sensitivity were CFM (92%), CRO (91%) ,CP(89%). In EPEC isolates the highest sensitivity were related to C (86%). High level resistance to antimicrobials is due to unselected use of these drugs. Periodic monitoring of drug resistance in enteric pathogens in each geographical area helps in choosing the appropriate antimicrobial agent for empiric therapy.
Key word: EPEC, Antibiotic resistance. Diarrhea, Salmonella .Shigella

Introduction:

The antibiotic resistance of enteric bacteria has profound clinical implications because it threats the life and causes many of serious diseases such as acute gastroenteritis [1].Diarrhea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual. Though most episodes of childhood diarrhea are mild, acute cases can lead to significant fluid loss and dehydration, which may result in death [2].

Only Salmonella spp. and Shigella spp., are routinely investigated through routine culture in Gaza strip [3]. Other potential pathogens (such as Campylobacter spp., Yersinia spp., Aeromonas spp.), however, are not routinely diagnosed. This often causes misdiagnosis, and physician overlooks other pathogens, and thus epidemiological data are inaccurate. Data from the health laboratories all over Palestine showed that the detection rate of Shigella spp. is very low

(about 3 cases in2009) and even lower for Salmonella spp. (2 cases in 2009) **[**4].

Worldwide, the most common pathogens that cause acute gastroenteritis are: Salmonella spp., Shigella spp., Campylobacter spp., E. coli O157:H7, Listeriamonocytogenes, Vibrio cholerae, Yersinia enterocolitica, Rotavirus, Cryptosporidium spp., Entamoeba histolytica, and Giardia lamblia. These pathogens can cause potentially serious diseases, which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks **[**5].

Antimicrobial resistance is one of the world's most serious public health problems, many of the microbes (bacteria, viruses, protozoa) that cause infectious disease no longer respond to common antimicrobial drugs. The problem is so serious that unless concerted action is taken worldwide, we run the risk of returning to the pre-antibiotic era when many more children than now died of infectious diseases. The major infectious diseases kill over 11 million people per year. The prevalence rate of antimicrobial resistance all overall the world of diarrheal shigellosis is 10-90% for ampicillin and 5-95% for trimethoprim/sulfamethoxazole [6].

The major cause of death for children is affected by diarrhoeagenic bacteria E. coli spp.,Vibrio spp., Salmonella spp., Aeromonas spp.,Shigella, Yersinia enterocolitica, Rotavirus, Cryptosporidium spp., Entamoeba histolytica, and Giardia lamblia. These pathogens can cause potentially serious diseases, which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks [7].

Inappropriate prescription of antibiotics prompted resistance and increased infectious disease mortality not only in developing countries but also in developed countries. Aging populations, changes in behavior and a decline in the development of new antibiotics exacerbated a deteriorating situation [8].

This study performed microbiological investigation of some potential pathogens associated with diarrhea, to characterize the isolates, their antibiotic resistance and the epidemiological factors related to the diarrheal disease in patients from Iran The aim of the paper was to determine the frequency of isolation of these microorganisms, as well as their resistance to a panel of antibiotics, in outpatients and hospitalized patients with diarrhea, whose stool was examined at the Milad hospital of Tehran ,Iran.

Material and methods:

The study was performed in tehran. We tested the hypothesis that enteric phatogens, specifically, EPEC, Salmonella and Shigella, are associated with childhood (<10yr) diarrhea in Iran. All stool samples were transported to the laboratory in the sterile container, and transported in an icebox immediately after collection. Samples were completely labelled by the necessary data (date, time of collection, sample type, patient name).This study included infant and young children younger than five years with diarrhea, from January 2012 to December 2013. Information was also obtained from each patient regarding age, sex, onset of diarrhea, antibiotic intake, other relevant clinical information, and laboratory results.

Stool specimens were collected from the patients in clear, transparent, wide-mouthed bottles and transported to the microbiology department of the laboratory. The specimens were examined for consistency, color, and atypical components such as mucous, blood, and parasites, examined by light microscope for the presence of red blood cells, pus cells, parasitic ova, and protozoa, inoculated onto Blood, MacConkey, Salmonella

Shigella (SS) agar media, and Selenite F broth, and incubated at 37◦C for 24 h. The isolates were subjected to the following tests: Gram staining, citrate utilization, oxidase test, and subcultured on lysine iron agar (LIA), motility indole ornithine (MIO), and triple sugar iron agar (TSI).

Salmonella and Shigella

Each stool sample was directly cultured onto Xylose Lysine Deoxy colate agar (XLD) agar, Salmonella Shigella (SS) agar, Hektoen enteric (HE) agar. Approximately 1 g of each sample inoculated into 10 ml of Selenite F broth. The tubes and plates were incubated at 37.C for 18 to 24 hr. Selective Selenite F broth subcultured onto XLD agar, SS agar, and HE agar then the plates incubated at 37°C for 18 to 24 hr. The suspected colonies identified by colony morphology and biochemical characteristics. Salmonella spp. appears on SS, XLD and HE agar as colorless colonies with black centre owing to H2S production. Shigella spp. colonies identified on SS agar as colorless while on XLD and HE agar the colonies appeared as transparent red.

Antibiogrammes: The sensitivity to antimicrobial drugs was determined by the disk diffusion method and interpreted using the three scale system of Bauer-Kirby, as stated by NCCLS – Performance Standards for Antimicrobial Disk Susceptibility, using the following antibiotic discs: ampicillin, cefixime, Co-trimoxazole, Nalidixic acid, ciprofloxacin, ceftriaxone, and chloramphenicol.

Statistical Analysis

Statistical analysis was done with SPSS version 10.1 software using independent samples T-Test. P values <0.05 were considered significant.

Results and Discussion:

Using standard bacteriological culture methodsfour enteropathogenic bacteria were detected in twelve cases of the 7321 stool samples examined. Fig 1 shows the distribution of enteropathogenic bacteria EPEC. Salmonella, shigella, Candida albicance and other, were isolated Growth of enteric pathogens could be yielded from 433samples, in which EPEC isolates below 5 years of age were 146; salmonella constituted 83shigella spp 79, Candida albicance 119 other pathogen were 6.A total of 146 consecutive non repeat samples from children under 10 years old with diarrhea were included in our study. In the present study, the isolates belonging to EPEC serotypes were tested by diffusion disk, and were interpreted according to Clinical Laboratory Standards Institute guidelines (CLSI, 2010).

The highest number of EPEC isolated belonged to polyvalent serogroup was 3 serogroup(85%) , 4 : 3%,2: 5% and 1: 7% . 433 positive samples, 146 came out positive for EPEC .based on the results of antimicrobial testing resistance to : ampicillin, cefixime, Co-trimoxazole, Nalidixic acid , ciprofloxacin, ceftriaxone , and chloramphenicol were 82.19% ,79.45% ,64.38% ,59.5% ,36.98% ,34.93% ,13.6% respectively(fig : 1)

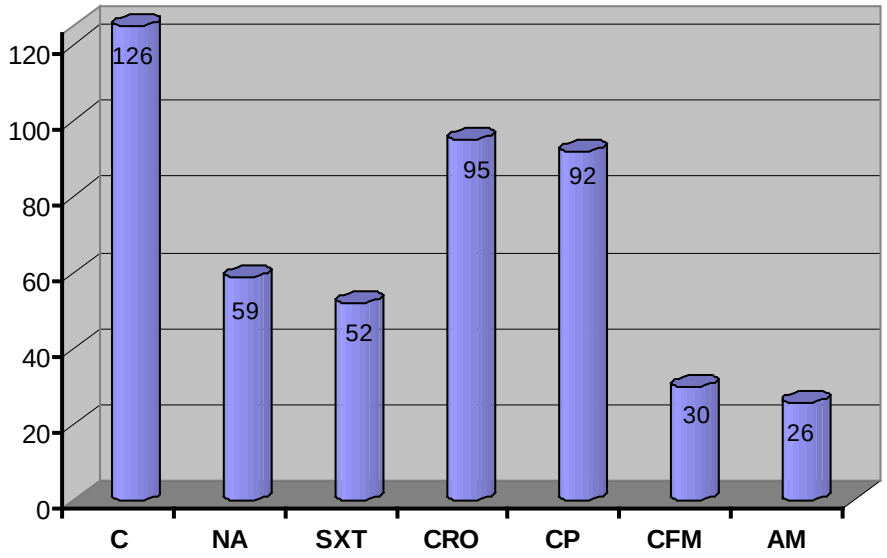

Fig 1: antibiotic sensitivity of 146 EPEC isolates to different antibiotics

The E coli positive cultures were set for serological_test, by slide agglutination test, using EPEC polyvalent O anti sera for EPEC, (I, II, III, and IV) separately. The highest number of EPEC isolated belonged to polyvalent serogroup was 3 serogroup(85%), 4: 3%, 2:5% and 1: 7%.however, present results were not agreement with Al-hilali and Al-charrakh in Iraq [9] All isolates showed 82.19% resistance to Ampicillin this study results were lower than Alaa Hani Al-Charrakh et al 2012.The rate of resistance to quinolons (Naldixic acid) and fluoroquinolones (Ciprofloxacin) were detected in 59.5% and 36.98% respectively (Figure 1). These Anti bacterial susceptibility pattern of bacterial agents isolates in different parts of the country provides essential information regarding the selection of antibiotics for all patients living in different areas. Tabatabaei in 2008 were studied on stool infections, the result of their study almost agreed with our study[10]. However, it might be possible that this high level of 3rd generation cephalosporin and Ampicillin in present study was most probably due to acquisition of β lactamase, possibly during therapy. Chloramphenicol is an antibiotic with a broud antibacterial spectrum and industrially manufactured by synthetic method. Two types of risks have been identified in relation to medicinal use of chloramphenicol. Firstly, aplastic anemia, a form of anemia when the bone marrow ceases to produce sufficient red and white blood cells, is the most dangerous effect produced by medicinally used Chloramphenicol [11]secondly, limited evidence exists for the genotoxic carcinogenicity of chloramphenicol in humans exposed to therapeutic doses [12].

Results of antibiotic susceptibility testing are summarized in Table 2. three enropathogenic isolates were resistant to Chloramphenicol

(Shigella spp: 18%, E. coli: 14% Salmonella 46%), Nalidixic acid (Shigella spp: 51%, E. coli: 60% Salmonella 12%),Co-trimoxazole (Shigella spp: 78%, E. coli: 64% Salmonella 86%),ceftriaxone(Shigella spp: 9%, E. coli: 35% Salmonella 2%),Ciprofloxacin(Shigella spp: 11%, E. coli: 37% Salmonella 2%),cefi xime (Shigella spp: 8%, E. coli: 79% Salmonella 4%), and ampicillin (Shigella spp: 81%, E. coli: 82% Salmonella 57%).

Serological identification of Shigella begins with the use of polyvalent antisera, which is used to identify the species (i.e., Susanne, S. flexneri S. dysenteriae).

Among Shigella, S. sonnei was the most common isolate (67.1%), followed by S. flexneri (25.3%), S. dysentriae (3.8%).

Unlike to our research the results of Tejashree et al (2013) imply to, 14(3.62%) Salmonella species were isolated, of which 5 (1.29%) were Salmonella typhi and 9 (2.33%) were Salmonella typhimurium. Of the 5 S typhi isolated, 1 each was resistant to furazolidone, nalidixic acid and doxycycline. Of the 9 Salmonella typhimurium isolated, 3 each were resistant to Nalidixic acid and doxycycline, 2 to norfloxacin, 1 to ampicillin. None was multidrug resistant. In Orissa, 0.7% Salmonella species were isolated and Salmonella spp. were resistant to Ampicillin and Furazolidone[13].

The survey results confirmed the most common sero groups of salmonella were in group D and the others were in A, B groups. To detect Salmonella spp. in feces or rectal swabs, approximately 1 g each of fecal material on swabs was added to each of 9 mL of selenite cystine broth and 9 mL of tetrathionate broth and incubated overnight at 42 °C and 37 °C,respectively. Subcultures were made onto xylose lysine deoxycholate (XLD) agar and brilliant green agar and incubated overnight at 37 °C. For detection of Shigella spp., approximately 1 g of feces or fecal material on swabs was enriched in 9 mL of gram-negative broth for 4–6 h at 37 °C and then plated on XLD agar and on MacConkey agar. Standard methods (16) were used for subsequent identification and serotyping of isolates. Shigella serotypes have been documented as important etiological agents for childhood diarrhea. Sh. sonnei, the predominant enteropathogen.

Resistance to antimicrobials is increasing throughout the world, but it is more extensive in developing countries. In the current study, nearly all of Shigella isolates were resistant to ampicillin (81%), Co-trimoxazole (78%), and Nalidixic acid.Isolated Salmonella were to lesser than the other enteropathogen bacteria, resistant tothese antibitics (table 2).all of three bacteria resistant to ampicillin and Co-trimoxazole (fig2). Multi-drug resistant non-typhoidal Salmonella species are widespread all over the World. Study done in north India by Taneja et al. revealed resistance to multiple drugs and an increased resistance to ciprofloxacin .Study done in Mexico by Zaidi M B et al. revealed a high prevalence of Salmonella in retail meat as well as in ill and asymptomatic children.

The investigation of Shigella drug susceptibility isolated in Crete, Greece revealed that 58.3% of isolates were resistant to ampicillin, while 30.5% of tested Shigella strains were resistant to trimethoprim/ sulfamethoxazole .The investigation of shigellosis in Spanish travelers that mostly visited India revealed that 12 of 200 tested isolates were resisted to quinolones[14].

Non typhoidal Salmonellae (NTS), responsible for sporadic cases and outbreaks of foodborne diarrhea, are zoonotic in etiology. Globally, NTS are estimated to cause93, 757,000 cases of gastroenteritis annually, resulting in 155,000 deaths.21 although most disease is self-limiting, invasive infections is a prominent feature of NTS infection in the

immunocompromised. The greatest impact of invasive NTS disease is seen in Africa, associated with a high incidence of human immunodeficiency virus in the region.22. The incidence of invasive salmonellosis appears to be much lower in Asia [15].

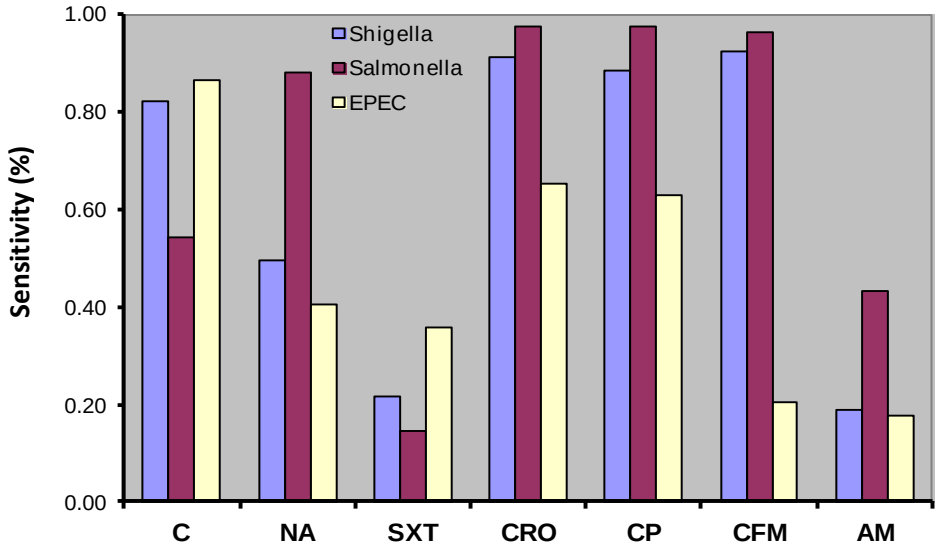

Fig 2: Antimicrobial susceptibility of isolated enteropathogenic bacteria

Table 1: Antimicrobial resistance properties inentropathogenic bacteria isolated from childhood diarrheic cases

Pathogens / **Antibiotics**	**SHIGELLA**		**SALMONELLA**		**EPEC**	
	s	r	s	r	s	r
C	65	14	45	38	126	20
NA	39	40	73	10	59	87
SXT	17	62	12	71	52	94
CRO	72	7	81	2	95	51
CP	70	9	81	2	92	54

CFM	73	6	80	3	30	116
AM	15	64	36	47	26	120

Table 2: Percentage resistance in bacterial isolates from childhood diarrheic cases

	C	NA	SXT	CRO	CP	CFM	AM
Shigella	0.18	0.51	0.78	0.09	0.11	0.08	0.81
Salmonella	0.46	0.12	0.86	0.02	0.02	0.04	0.57
EPES	0.14	0.60	0.64	0.35	0.37	0.79	0.82

Yousefi mashouf et al (2008) identified: the most common isolate was; Escherichia coli (EPEC) with 105 cases and the lowest isolate was Shigella with 18 cases. The most common serogroups of Salmonella were S.typhi and S.typhimorium and the lowest serogroup was S.para A.the most common serogroups of shigella was S.sonnei .results of this research seems to be consistent with our research [16].

S. sonnei infection is prevalent in developed countries. Historically, S. fl exneri has been the dominant etiologic agent of dysentery in the developing world, but S. sonnei is now replacing it and is emerging as a problem in developing areas undergoing public health development and improvements in water quality. Recently, drug resistant S. sonnei strains have been more frequently detected in developing areas such as Vietnam, Thailand, Bangladesh, and China.Reports from different regions of Iran such as Tehran and Shiraz alsoshow a tendency to increased incidence of S. sonnei strains,which are in concordance with our results[17].

In one study highest resistance rates to Ampicillin (95 %),Cotrimoxazole (84 %), and Amikacin (0.5 %) were seen which correlated with our study.But Ciprofloxacin resistance (18 %) was less when compared to our study. In yet anotherstudy , high resistance to Cotrimoxazole (88.6 %), Ampicillin (72.7%) was observed which correlated well with our study. But, low resistance reported in his study to Nalidixic acid (8 %) did not match our study.In the other study ,1.4 % of the Escherichia coli isolates showed resistance to all the antimicrobials tested. In our study, none of the isolates showed resistance to all the drugs tested [18].

The high antibiotic resistance also indicates a negative impact on therapy with these classes of antibiotics. The periodic monitoring of antibiotics to detect any changing patterns would be necessary for effective treatments. A further study to evaluate the extent of antibiotic resistance transmission and the impact of such transmission on the effectiveness of antibacterial use in human medicine is imperative.

Conclusion:

The present study showed that Escheichia coli (EPEC), Salmonella and Shigella species are predominant causes of bacterial diarrhea in children under 10 years old in this region. The results showed increase in profile for antibiotic resistance. Therefore it suggests that application of antibiogram test is necessary before antibiotic prescription for successful treatment and prevention of diarrhea caused by multi-drug resistance agents.

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