

Surveying the prevalence and pattern of antimicrobial resistance of *Yersinia enterocolitica* among diarrheal children attending health care centers in Qom

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ABSTRACT

Background: Diarrhea is the most common causes of mortality, accounting for 15-20% among children. It is caused by numerous microorganisms including, Shigella, Salmonella, enteropathogenic E. coli, and Yersinia enterocolitica. Yersinia enterocolitica is a gram-negative, facultatively anaerobic coccobacillus belonging to enterobacteriaceae. It causes numerous human diseases, mostly gastroenteritis.

Materials and methods: A total of 800 diarrheal children aged less than 10 years entered this study. Suspected stool samples were cultured on both conventional enteric and cold-enriched media. Conventional enteric media included MacConkey agar, Cefsulodin-Irgasan-Novobiocin (CIN) agar, and Salmonella-Shigella Deoxycholate (2%) agar, while for cold-enriched media PBS (phosphate-buffered saline) (PH=7.2-7.8) was used. Other enteric pathogens including Salmonella, Shigella, and enteropathogenic E. coli were also isolated.

Results: Of 800 suspected stool samples, 14 Yersinia enterocolitica were isolated (1.8%). Other enteric pathogens were as follow: 18 Shigella (2.3%), 32 enteropathogenic E. coli (4%), and 13 Salmonella (1.6%). Y. enterocolitica isolates were completely sensitive (100%) to gentamycin, kanamycin, ciprofloxacin, cefixim, cefataxim, and chloramphenicol, however, they were partially resistant to tetracycline (7.1%) and cotrimoxazole-nalidixic acid (14.3%). Yersinia enterocolitica isolates were completely resistant to ampicillin, penicillin, cephalotin, and erythromycin.

Conclusion: Suspected diarrheal children should be checked for Yersinia enterocolitica using cold-enriched environment, while antibiogram studies are strongly recommended for positive isolates.

Keywords: *Yersinia enterocolitica*, Diarrhea, Children, Iran.

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INTRODUCTION

Diarrhea is a common gastrointestinal disease occurring as acute or chronic forms especially in children. It could be associated with growth retardation or even death in children. Prior investigators have postulated numerous organisms

to cause diarrhea including viruses, bacteria, protozoa, and fungi (1).

Researchers have isolated Yersinia enterocolitica from patients in many countries worldwide. Most isolates are reported from Northern and Western Europe, United States, Canada, and Australia. Isolation of the bacterium in developing countries such as Iran is uncommon, partly due to inability to isolate the organism from

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stool specimens as a result of inexperience and lack of the use of proper techniques, however, it is also conceivable that geographical variation accounts for different isolation rates of this bacterium (2).

Y. enterocolitica is a widely distributed organism recovered from water, soil, contaminated food products such as vegetables, and also animals (3-6). *Yersinia* is a gram-negative coccobacillus that stained bipolarly during Gimsa or methylene blue staining. It was used to classify in Pasteurella family until 1944 when Vanloghon reclassified them as *Yersinia* genus in family of Enterobacteriaceae. Despite similarities with other enterobacteriaceae members, slight differences are exist such as slow growth in solid media, colonies of at most 1mm diameter, and better growth at cooler temperature (7,8).

Metchock et al reported yersiniosis most commonly in cooler months (9). In Maruyama et al study in Japan, *Y. enterocolitica* was isolated from less than 1% of stool samples (199 of a total of 26185 samples). Their isolates belonged to O:3, O:5, and O:9 serotypes, while O:3 was by far the most frequent one (97.5%). Similarly, most of their cases referred during cooler months (10).

Although yersiniosis has been described in all age groups, reports document enterocolitis most commonly in younger children. Regardless of sex, enteritis is the most important pathogenic feature among children. The usual presentation of acute yersiniosis is characterized by enteritis or enterocolitis with diarrhea that resolves spontaneously. Occasionally, terminal mesenteric ileitis resembles acute appendicitis, for which patients often undergo appendectomy (11).

PATIENTS and METHODS

Diarrheal children aged less than 10 years entered this study. Totally, 800 children including 435 boys and 365 girls were investigated. Suspected stool samples, i.e., mucoid, watery, or bloody stools, were cultured on both conventional

enteric and cold-enriched media in order to improve the chances of recovering pathogenic *Y. enterocolitica*.

Conventional enteric media included MacConkey agar, Cefsulodin-irgasan-novobiocin (CIN) agar, and Salmonella-Shigella Deoxycholate (2%) agar, while for cold-enriched media PBS (phosphate-buffered saline) (PH=7.2-7.8) was applied.

Stool samples were added to 4 ml of phosphate buffered saline (PBS) and kept refrigerated at 4°C for 10-20 days, then, transported to media and incubated at 37°C for 24-48 hours. Two separate samples were cultured for each plate, one to be incubated at 37°C and the other at room temperature. Finally, suspected colonies (tiny colorless colonies) were isolated and subcultured on TSI, Kligler's Iron agar (KIA), Simmon's Citrate agar, urea agar and Methyl Red-Voges Proskauer (MR-VP) broth. Nevertheless, further confirmation was achieved by ONPG, Ornithine decarboxylase medium, and mannitol and saccharose fermenting technique. Saccharose-free Kligler's Iron agar prevents misdiagnosis of yersinia (12,13).

Other enteric pathogens including Salmonella, Shigella, and enteropathogenic *E. coli* were also isolated.

RESULTS

Totally, 800 suspected stool samples were investigated among which 14 *Yersinia enterocolitica* were isolated (1.75%). Other enteric pathogens were as follow: 18 *Shigella* (2.3%), 32 enteropathogenic *E. coli* (4%), and 13 *Salmonella* (1.6%).

Table 1 presents age distribution of all children with either positive or negative stool cultures as well 14 yersiniosis cases. Most of the sufferers (28 subjects) aged 2-4 years, however, half of the *Y. enterocolitica* infected children aged less than 2 years.

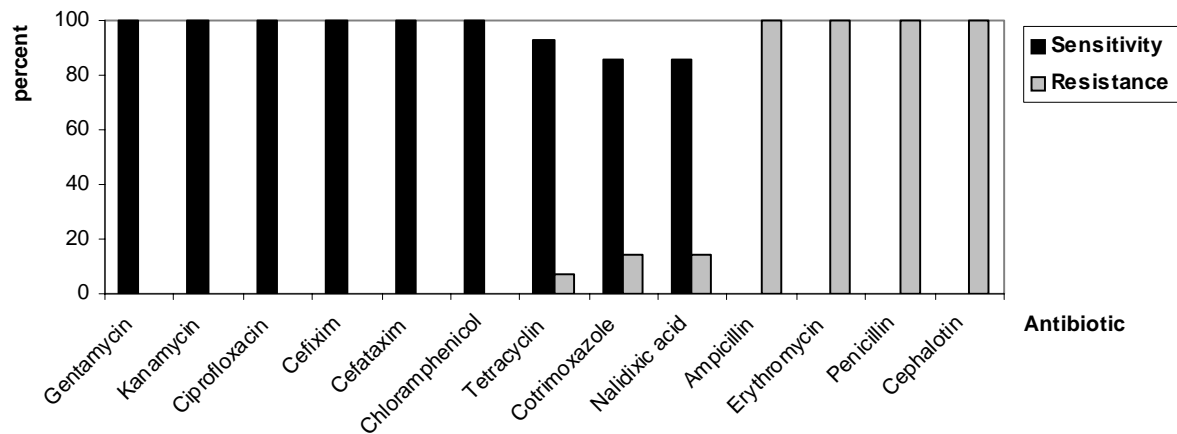
Table 1. Age distribution of children with positive or negative stool cultures and yersiniosis cases

		Age (year)					Total
		0-2	2-4	4-6	6-8	8-10	
Culture	Positive	24	28	13	7	5	77
	Negative	201	182	122	123	95	723
Total		225	210	135	130	100	800
Yersiniosis cases		7	5	2	0	0	14

Table 2. Seasonal distribution of enteric pathogens among less than 10-year old children

Season	No. of stool samples	Enteropathogenic E.coli	Shigella	Salmonella	Yersinia enterocolitica
Spring	220	4(12.5)*	3(16.7)	3(23.1)	5(35.7)
Summer	200	19(59.4)	9(50)	7(53.9)	3(21.4)
Fall	220	6(18.8)	5(27.8)	2(15.4)	2(14.3)
Winter	160	3(9.3)	1(5.5)	1(7.6)	4(28.6)
Total	800	32	18	13	14

* Numbers in parenthesis are percentage

**Figure 1.** Pattern of antimicrobial susceptibility of 14 *Yersinia enterocolitica* isolates

DISCUSSION

Prior investigators have conducted numerous studies on *Yersinia enterocolitica* since it is a worldwide human pathogen. Pathogenic *Y. enterocolitica* organisms are significant causes of human disease in many parts of the developed world, however, they are ubiquitous in the natural environment, and may be recovered from water, soil, animals, and food.

Determining the prevalence of *Y. enterocolitica* among diarrheal children is of utmost importance, however, it is rarely isolated partly due to the inexperience of laboratory staff and the need for especial media, i.e., cold-enriched environment.

We investigated 800 suspected stool samples among which 77(9.6%) were positive for 4 tested enteric pathogens. Of 14 *Y. enterocolitica* isolates,

9 (64.3%) were referred during winter and spring. This is in agreement with Nahed et al study (14). They had surveyed 10570 stool samples during a 7-year study, of which, 1120 (14%) samples were positive for enteric pathogens. They had isolated *Y. enterocolitica* in 142 (1.3%) stool samples (14).

Chatzipanagiotous et al studied the prevalence of specific antibodies against *Y. enterocolitica* plasmid encoded outer Hashimoto's protein (yops) in 71 patients with Hashimoto thyroiditis among whom 20 revealed to have positive ELISA tests confirmed with western blotting (15).

Omoigberale et al investigated 215 diarrheal patients in Nigeria of whom 47 (21.9%) had yersiniosis. Of 47 infected subjects, 15 (31.9%) aged 1-9 years and there was statistically insignificant difference according to sex. They had isolated *Y. enterocolitica* using Deoxycholate citrate agar (DCA) and MacConkey agar in both room temperature (37°C) and cold-enriched environment (16). The observed discrepancies between their studies and ours reflect a high contamination rate in Nigeria as compared with other societies.

Furtherore, Sherbini et al cultured 70 appendectomy and 80 ice-cream samples on special media under cold-enriched environment. Finally, they reported 17.1% of appendectomy and 26.3% of ice-cream samples to be infected with *Y. enterocolitica*. The susceptibility pattern of isolates showed complete sensitivity to chloramphenicol, gentamycin, tetracycline, and trimetoprim-cotrimoxazole and resistance to ampicillin. Their findings are more or less in consistency with ours (17).

In conclusion, *Yersinia enterocolitica* is a significant cause of gastroenteritis in children. Laboratory staff should be instructed to culture stool samples on special cold-enriched media in order to improve recovery rate.

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