Original Article

Frequency of viral, bacterial and parasitic enteropathogens among young children with acute diarrhoea in Saudi Arabia

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Abstract

Objective: The frequency of viral, bacterial and parasitic enteropathogens among 270 paediatric patients (<5 years of age) in various hospitals of Makkah and Jeddah cities of Saudi Arabia were investigated.

Methods: A total of 270 stool samples were collected from paediatric patients with signs and symptoms of gastroenteritis from different hospitals of Makkah and Jeddah cities of Saudi Arabia. Samples were investigated for bacterial, viral and parasitic enteropathogens using microscopic examination, immunological tests and bacterial culture techniques.

Results: Out of 270 stool samples tested in this study for various causative agents of gastroenteritis, total number of positive samples were 106 (39%). Of these, 90 (33%) had viral etiology, of which Rotavirus type A (serotype G) was found in the majority of cases 60 (22%), Adenovirus in 20 (7%) patients, and Astrovirus in the remaining 10 (4%) patients. Thirteen (5%) were of bacterial origins of which 9 (3%) were Salmonella species, 4 (2%) were Shigella species and only 3 (1%) of the samples were positive for Giardia lamblia.

Conclusion: This study indicated that most of the diarrhoeal diseases in young children in Saudi Arabia are due to viral etiology, where Rotavirus was predominant followed by Adenovirus and Astrovirus. While Salmonella and Shigella represent the bacterial etiology of paediatric acute diarrhoea and only Giardia lamblia was found as a parasitic cause of diarrhoea in young children in our study (JPMA 60:456; 2010).

Introduction

Gastroenteritis is defined as an inflammation of the stomach, large and small intestines.[1] It is one of the most common illnesses in humans worldwide.[2] Although it can affect individuals of any age, it presents a significant health risk to those at extremes of age, the very young and the very old.[3] It is the second most common cause of death among adults, and the leading cause of childhood death worldwide.[4] In children under 5 years, there are more than 700 million cases of gastroenteritis every year. The annual mortality associated with gastroenteritis has been estimated to be 3.5 to 5 million, with the majority of deaths occurring in developing countries.[5]

The major symptoms of gastroenteritis are nausea, vomiting, diarrhoea, loss of appetite, fever, weakness, abdominal cramps, and severe cases of the disease can lead to dehydration which in some cases is fatal.[3,6]

Infectious causes of paediatric gastroenteritis can be classified into three broad categories: bacterial, viral, and parasitic agents. Many species of protozoan parasites live in the gastrointestinal tract, infecting some 3.5 billion individuals worldwide. Three species are of particular importance: Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum.[7]

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programmes. The symptoms of acute bacterial intestinal infection are usually mild to moderate, and spontaneous remission occurs, but in some cases, the disease can cause rapid deterioration of a patient's condition.[8] Bacteria that cause gastroenteritis include, but are not limited to; Salmonella and Shigella (the two most common pathogens), Campylobacter jejuni, Escherichia coli, Clostridium difficile and Vibrio cholerae.[2]

Although at least 25 different bacteria and protozoa can cause an identical clinical syndrome of gastroenteritis, over 75% of gastroenteritis cases are caused by viruses.[9] Viral gastroenteritis is one of the most common infectious diseases worldwide, causing significant morbidity and mortality in children.[10] Four major viral pathogens are associated with gastroenteritis, three of them are RNA viruses (Rotavirus, Norovirus, and Astrovirus) and one DNA virus (enteric Adenovirus).[9]

The aim of this study was to determine the prevalence of bacterial, parasitic and viral aetiology of gastroenteritis in children less than 5 years of age presenting with diarrhoea in various hospitals of Makkah and Jeddah cities in Saudi Arabia. This information is important to

determine the contribution of these enteropathogens in childhood diarrhoeal diseases.

Patients and Methods

Ethical consideration included informed consent which was obtained from parents of each child before inclusion in the study. Every subject had been informed about the procedure of collecting stool samples before the procedure. Stool sample was collected from soiled diaper or directly from a potty-chair using the spoon that is attached to the cap of the containers, after that several spoonfuls of stool were placed into the collection container before being labeled with patient's name and sealed in the plastic bag provided.

A population-based prevalence study was done in randomly selected infants and young children suffering from signs and symptoms of gastroenteritis. A total of 270 stool samples were collected from patients from different hospitals in Makkah {Maternity and Children's Hospital (32 samples), Al-Noor Hospital (21 samples), Hera Hospital (15 samples)} and Jeddah {King Abdul Aziz University Hospital (98 samples), Children's Hospital in Azizzia (56 samples), Maternity and Children's Hospital in Musadia (48 samples)} cities of Saudi Arabia. The sample size of a minimum of 267 children was needed to meet the objectives of the study, with a 95% confidence interval, 6% error rate and around 350000 (under 5 years old children) population based in Makkah city of Saudi Arabia.

The study was carried out from March 2008 to August 2008. The age range of the patients was 1 day - 5 years, with a mean age of 2.4 ± 1.5 years, median age of 2 years and mode age of 4 years.

Macroscopic examination of stool samples was done for the presence of worms (Ascaris lumbricoides, Strongyloides stercoralis, Enterobius vermicularis, Trichuris trichiura and Taenia saginata), blood and physical characteristics such as color, appearance and odour .Microscopic examination of stool samples was done under 10x and 40x power after being stained with iodine for the presence of parasitic eggs and larvas. Bacterial culture of stool samples was done for the presence of a common enteric bacterial pathogens such as: Salmonella, Shigella, Campylobacter, Enteropathogenic E.coli, Enterotoxigenic E.coli and Vibrio cholera. These were performed by culturing the stool samples in different culture media including: MacConkey, XLD, DCA, campylobacter media, TCBS and selenite F broth.

MacConkey, XLD, DCA, TCBS and selenite F broth culture media were incubated at 37ºC for 24 hours and samples incubated in selenite F broth were re-cultured in XLD media for another 24 hours at 37ºC. Campylobacter media were cultured at 42ºC for 48 hours. Any bacterial growth was identified by specific biochemical tests (such as API20E) in addition to bacterial colonies characteristics.

Samples were also tested for the presence of gastroenteritis viruses (Rotavirus, Adenovirus type 40/41 and Astrovirus) using antigen detection ELISA kits [ACON laboratories, incorporated (Inc), USA] as described in manufacturers instruction.

In brief, washing buffer was diluted 1:10 with distilled water and samples were also diluted 1:11 with sample diluents. Hundred microliters of samples, positive and negative control were added to corresponding microtitre plate wells followed by the addition of 100µl conjugate. Plates were then incubated at room temperature for 60 minutes before being washed 5 times with 300µl diluted wash buffer using an ELISA washer (ETL testing laboratories Inc. USA). Hundred microliters of substrate were then added to all wells, before incubating the plates in dark for 15 minutes. Fifty microliters of stopping solution were then added before reading the results at an ELISA plate reader [E.ma, precision, microplate reader, USA] at 450 nm.

The results were statistically analysed by calculating the mean, median, mode, standard deviation, range and p value, and distributed according to age, nationality and gender differences using SPSS. P values less than 0.05 was considered significant.

Results

Out of 270 stool samples tested in this study for various causative agents of gastroenteritis, total number of positive samples were 106 (39%). Of these, 90 (33%) had viral aetiology, of which Rotavirus was found in the majority of cases 60 (22%), Adenovirus was detected in 20 (7%) patients, and Astrovirus in the remaining 10 (4%)

Table-1: Bacterial, parasitic, and viral etiology of diarrhea in 270 children.

Organism	Species	No. of Patients	Percent
Bacteria		13	5%
	Salmonella	9	3%
	Shigella	4	2%
Parasites		3	1%
	Giardia lamblia	3	1%
Viruses		90	33%
	Rotavirus	60	22%
	Adenovirus	20	7%
	Astrovirus	10	4%
Total	106		39%

Table-2: Distribution of positive stool samples according to age differences.

Age	Number of samples	Percent
≤ 1 year	57	21%
>1-2 years	88	33%
>2-3 years	39	14%
>3-4 years	52	19%
>4-5years	34	13%

patients, 13 (5%) were of bacterial origin of which 9 (3%) were Salmonella species, and 4 (2%) were Shigella species and only 3 (1%) of the samples were positive for Giardia lamblia (Table-1).

Of the total number of 106 positive samples; 61 (58%) were from males, and 45 (42%) were from females, however, this differences was not statistically significant (p = 0.3), with a 95% confidence interval (0.6 to 1.1).

Of the total of 106 samples, 88 samples (83%) were from Saudi nationals and 18 (17%) were of non-Saudi origin, but this difference was not statistically significant (p value = 0.1), (95% confidence interval 0.9 to 2.0).

Distribution of positive samples according to age differences is shown in Table-2. The majority of paediatric patients in this study 88 (33%) were under the age group 1-2 years. While the lowest number; 34 (13%) made-up the 4-5 years age group.

Discussion

Diseases causing diarrhoea are the most important cause of morbidity and mortality in developing countries. Five hundred million cases of acute diarrhoea occur annually in children aged less than five years throughout the world.[11,12]

In the present study, enteropathogens were detected in 106/270 (39%) paediatric patients under five years of age. In comparison, other parts of Saudi Arabia, such as the Central and Eastern regions, reported rates between 61% and 49% respectively in the age group under 5 years.[7,13]

The present study clearly shows that Rotavirus is the most common cause of acute gastroenteritis among children (22%). This is higher than that reported in Makkah, Saudi Arabia in 2005 by Ghazi et al, where the prevalence of Rotavirus in children <5 years was 10% (48/479).[14] However, our prevalence is slightly lower than that reported in other studies in Saudi Arabia. For instance, in a study in Jeddah, 34.6% of hospitalized patients were positive for Rotavirus, while 5.9% of out-patients were positive, all of them were under 5 years of age.[15] In Central Saudi Arabia, the prevalence was 44.3%, in the under 5 year age group,[7] 37.5% in Eastern Saudi Arabia and 42.2% in Western Saudi Arabia.[16] A study done in Al-Qassim reported very high prevalence rates for Rotavirus 66.2% in children less than 1 year and up to 4 years of age.[17]

In a study conducted in Karachi, Pakistan, Rotavirus was detected in 112/818 (13.7%) of stool samples from infants with diarrhea.[18] However, in another study carried out in India, Rotavirus was found in 22.6% of pediatric patients, who fell into the age group <1-3 years.[19] In the United Kingdom, Rotavirus infection prevalence was found to be 36% in children under 16 years of age,[20] while the prevalence rate of Rotavirus in different cities of South Africa varied from 13% to as high as 55% in under 5 year of age,[21] and in the USA the rate was 6.8% in children less than 6 years of age.[22]

In our study, Adenovirus was the second most common virus to be found with a prevalence rate of 7%. Other studies done in different parts of the world, showed prevalence rates ranging from as low as 0.8% in under 6 years old in the USA[22] to as high as 28% in children under 12 years of age in Ghana.[23] Our results were in accordance with the reported prevalence of Adenovirus.

The least common virus found in our study was Astrovirus (4%), this is in contrast to one study which found Astrovirus to be more common than Adenovirus.[22] When compared to other studies done worldwide it was reported that the prevalence of Astrovirus ranged from 0.4% in the under 5 year old age group in Tanzania[24] to 9.2% in <1 to 4 year old children in Al-Qassim, Saudi Arabia.[17]

Bacteria isolated in the current study were 5%, of which Salmonella made up 3%, and 2% was due to Shigella. In studies done elsewhere in Saudi Arabia, Salmonella was found in 3% of patients, while Shigella was isolated in 2.6% in 1-5 years of age.[15] In two other studies done world wide, Salmonella was found in 5.8% of patients in Australia in <1 up to 14 years of age,[19] and Shigella was found in 6% in Palestine in children under 5 years of age.[25]

Only 3 (1%) patients were positive in our study for Giardia lamblia. Another study in Jeddah, reported the same parasite in 3.1% patients between 1 year and 5 years of age.[15]

Conclusion

The result of this study concluded that most of the causative agents of diarrhoea in young children in Saudi Arabia are of viral origin, where Rotavirus ranks first followed by Adenovirus and Astrovirus. Salmonella and Shigella represented the bacterial etiology of paediatric acute diarrhoea and Giardia lamblia was the parasitic cause.

For future studies, we recommend further investigations such as: prevalence of various serotypes, electropherotypes and genomic analysis of enteropathogens in different parts of Saudi Arabia.

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Whenever I hear people talking about "liberal ideas," I am always astounded that men should love to fool themselves with empty sounds. An idea should never be liberal; it must be vigorous, positive, and without loose ends so that it may fulfill its divine mission and be productive. The proper place for liberality is in the realm of the emotions.

Johann Wolfgang von Goethe
German dramatist, novelist, poet, & scientist (1749 - 1832)