Occurrence of Rotavirus in Infants and Young Children with Acute Gastroenteritis in Gaza, Palestine

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الملخص

تواجد فيروس الروتا (الفيروس الدولابي) عند الأطفال المصابين بالتهابات الأمعاء الحادة في غزة- فلسطين

ينتمي فيروس الروتا (الفيروس الدولابي،Rotavirus) لعائلة تتميز بان مادتها الوراثية تكـون في صورة قطع من الـRNA ويعتبر هذا الفيروس من أهم أسباب الإسهال بين الأطفـال فـي العالم.

في غزة-فلسطين لا يزال الإسهال مشكلة صحية كبيرة فهي تأتي في المراتـب الأولـى بـين الأمراض الأكثر حدوثا بعد أمراض الجهاز التنفسي والإسهال هو أحد المسببات الرئيسية للوفاة بين الأطفال تحت سن خمس سنوات ومن هنا تأتي أهمية دراسة مسببات الإسهال في فلسطين.

وفي قطاع غزة لم يتم إجراء دراسة فيروس الروتا خلال السنوات العشر الأخيـرة ولا توجـد بيانات صحيحة حول مدى انتشاره بين الأطفال لهذا كان الاهتمام الأكبر في هذه الدراسة مركزا على مدى تواجد هذا الفيروس.

تم جمع 150 عينة من عينات براز أطفال يعانون من الإسهال ادخلوا إلى المستشفى المركـزي للأطفال في قطاع غزة (مستشفى النصر للأطفال) وكانت أعمارهم اقل من خمس سنوات جمعت جميع العينات في فترة الصيف لعام 2005م.

وجد أن فيروس الروتا هو المسبب الرئيسي للإسهال في الأطفال تحت سن خمس سنوات حيث شكل 28% من مجموع الحالات.

ويتميز الإسهال في هذه الحالة بأنه مائي 95.2%، وغالبا يكون مصحوبا بارتفاع فـي درجـة الحرارة 73.8% وقيء 92.9% وقد يؤدي في كثير من الحالات إلى الجفاف في حوالي 14.3%

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من الحالات ويعتبر الأخير من أخطر مضاعفات الإسهال، ولذا حين يصاب الطفل بالإسهال يجب ويتطلب العناية في المستشفى ومتابعة حالته لملاحظة أي بوادر للجفاف قد تظهر عليه.

ووجد أن المضادات الحيوية تستخدم بكثرة في علاج حالات الإسهال بالرغم من أن منظمة الصحة العالمية تنصح بعدم استخدامها لأنها غير مفيدة في مثل هذه الحالات ولها أضرار جانبية عديدة مثل اكتساب بعض أنواع البكتيريا المقاومة للمضادات الحيوية.

وجد أيضا أن الأطفال الذين أعمارهم اقل من سنتين هم أكثر عرضه للإصابة بالفيروس من الأطفال الأكبر عمرا وإن عزل الفيروس من حالات الإسهال التي تحتاج إلى الرعاية الصحية في المستشفى مهم جدا وذلك لأنه من أهم مسببات الإسهال المعدية داخل المستشفى (Nosocomial infection) حيث يجب تشخيص المرض في سرعة ودقة وذلك لعزل الطفل المصاب عن الأطفال الآخرين.

لذلك يجب توفر فحص سريع لتشخيص الفيروس في عينة البراز كما يجب استخدام لقاح ضد الفيروس للحد من انتشاره كما يجب تحسين الوضع الصحي عن طريق توفير المياه الصالحة للشرب، تحسين نظام الصرف الصحي، تحسين النظافة الشخصية والتثقيف الصحي، والحث على استمرار الرضاعة الطبيعية لمدة سنة على الأقل ويجب البحث عن المسببات الأخرى للإسهال وعدم إعطاء علاج قبل تشخيص الحالة وعدم استخدام المضادات الحيوية إلا بوصفات طبية.

ABSTRACT

Rotavirus belongs to the Reoviridae family, a group of segmented double stranded RNA viruses. The virus is a major cause of gastroenteritis and diarrhea in infants and young children worldwide .

Rotaviral gastroenteritis may result in mortality for populations at risk such as infants, the elderly and immunocompromised patients. Moreover, it is not possible to distinguish diarrhea caused by rotavirus clinically.

Timely diagnosis of rotavirus infection in patients with acute diarrhea helps to determine appropriate treatment, prevents the unnecessary use of antibiotics and minimizes the spread of the disease .

The most commonly used diagnostic tests for rotavirus infections are electron microscopy, enzyme linked immunosorbent assays, molecular tests, rapid immunochromatographic test and latex agglutination. Rapid antigen detection systems, such as immunochromatograpy have become the tests of choice in clinical settings.

In the present study, fecal samples from 150 children with ages ranging from 1 month to 5 years, living in Gaza, who presented with acute diarrhea episodes, were analyzed. The analysis was carried out using an immunochromatography-based diagnostic kit (The RotaStick One-Step test, Novamed Ltd, Jerusalem). The study was conducted during the peak diarrheal season (May-August) of the year 2005.

Rotavirus was detected in 28 % (42/150) of the fecal specimens examined, and the majority of patients 90% (38/42), who were positive for the virus were 1 to 24 months old, and the infection rate decreased with increasing age.

The highest rate of rotavirus antigen detection was observed among the 12 to 24 months age group 41.9%. Children infected with rotavirus were more likely to have watery stool (95.2%), fever (73.8%), vomiting (92.9%) and moderate dehydration (14.3%)

The findings of this study demonstrate that rotavirus is one of the most frequently detected, yet a routinely neglected pathogen during stool examinations in Gaza strip health laboratories.

To our knowledge, this is the first report on occurrence of rotavirus infection among children of Gaza since 1994. Therefore, based on this preliminary data further work is needed for better understanding of rotavirus diarrhea and its impact on infected children all over Gaza strip.

INTRODUCTION:

Acute gastroenteritis is one of the leading causes of illnesses and death in infancy and childhood throughout the world, especially in developing countries. In Asia, Africa and Latin America an estimated 1.3 billion diarrhea episodes and 4 to 10 million deaths occur each year in children less than 5 years of age (21,28,42).

Viral pathogens account for approximately 70% of episodes of acute infectious diarrhea in children, and rotavirus is the most commonly implicated agent (5,6,33).

Rotavirus is an icosahedral 65-70 nm, double shelled RNA virus of the family Reoviridae, a family of double-stranded segmented RNA genome viruses. The virus was first identified as a human acute diarrhea causing agent in 1973 (8,24).

World wide, group-A rotaviruses are responsible for 30–60% of all cases of severe watery diarrhea in young children (9,10). Although less often, rotavirus can also cause symptomatic or asymptomatic infections in older children and adults, especially in geriatric populations (40).

Each year, rotavirus causes approximately 111 million episodes of gastroenteritis requiring only home care, 25 million clinic visits, 2 million hospitalizations, and 352,000–592,000 deaths (median, 440,000 deaths) or approximately 2,000 children each day in children <5 years of age. This accounts for about one quarter of the deaths from diarrhea and about 5% of all deaths among children less than five years of age. Children in the poorest countries account for 82% of rotavirus deaths (29,42).

Rotavirus infection can be easily transmitted through the fecal-oral route, as the virus breeds in the gut and is secreted in large quantities with feces. Therefore, nosocomial infections with rotaviruses are dangerous, particularly in pediatric wards and neonatal nurseries, and their management is difficult (9,23).

Timely diagnosis of rotavirus infection in patients with acute diarrhea helps determine appropriate treatment, prevents the unnecessary use of antibiotics and minimizes the spread of the disease (13,16).

Several methods are available for detecting the rotavirus in stool specimens, the most commonly used ones include electron microscopy, latex agglutination (LA), enzyme-linked immunosorbent assays (ELISA), Polyacrylamide gel electrophoresis (PAGE), immunochromatographic methods and molecular tests. Immunochromatography-based methods are

reliable and the fastest and easiest to perform, and have found a wide use in detection of the rotavirus(11,20,27).

In Palestine, diarrhea is one of the major causes of many outpatient visits, and hospitalizations. The identification and diagnosis of diarrhea in Palestinian health laboratories is done only for Salmonella and Shigella species, through culture, biochemical and serological assays, while the parasites, e.g., Entamoeba and Giardia are diagnosed by direct microscopic examination. The rotavirus however, is not diagnosed. Moreover, there has been no reported studies regarding this virus since 1994 (3,31,36,39,41).

In Palestine, although bacterial and parasitic causes of gastroenteritis have been relatively studied, information concerning viral etiologies are not available. Moreover, this leaves many of the gastroenteritis causing agents unidentified due to limited diagnostic methods of detection of viral and other agents .

This study is an attempt to determine the occurrence of rotavirus infection in ≤5 years old children among acute diarrhea cases in Gaza.

MATERIALS AND METHODS:

Study Population:

During the peak of diarrheal season (May-August of 2005), 150 of the children up to 5 years of age who were admitted with acute diarrheal diseases to ElNasser Pediatric Hospital Gaza (the central pediatric hospital in Gaza strip), were enrolled in the study. Diar¬rhea was defined as the passage of three or more loose or watery stools in the preceeding 24 hours .

A questionnaire was completed for each patient containing the fol¬lowing information: age, gender, residence, clinical data, fever, vomiting, dehydration status and previous hospitalization.

Vomiting was defined as the forceful expulsion of gastric contents occurring at least once in a 24 hours period. Fever was defined as an under ¬arm measured temperature of >37.2°C, and dehydration level was assessed following the recommendations of WHO Program for Control of Diarrheal Diseases and was done by the pediatricians (29,34).

After the informed consent was obtained from the parents of the subjects, a pediatrician filled out the information relevant to clinical symptoms and illness onset on a standardized questionnaire, the rest of qustionnaire was filled by the parents of the children.

Sample Collection:

Fecal samples (one per each subject), from children with diarrhea were collected as soon as the children were admitted to the hospital by the help of their parents .

Each stool specimen was collected in a special container, kept at 4°C, and processed within 3 hours of collection.

Blood samples collected (2 ml) in heparinized tube or syringe were transported immediately on ice to the laboratory for pH measurement .

Stool Samples:

Fresh diarrheal fecal specimens were obtained from patients seeking medical care for gastroenteritis (hospitalized) at the ElNasser Pediatric Hospital-Gaza and transferred to the Medical technology laboratory of The Islamic university of Gaza, for laboratory testing. Specimen collection and transportation procedure was done according to Rotavirus test kit instructions. Rotavirus antigen detection was carried out by RotaStick one step test kit according to manufacturer's instruction.

Ethical Considerations:

An authorization to carry out the study was obtained from the Helsinki committee (Declaration of Helsinki the most widely accepted guideline on medical research involving human subjects) using an agreement letter prepared from The Islamic university of Gaza. Parents gave their consent for participation in the study and all the information that were obtained about the subjects as well as their parents were kept confidential.

LABORATORY INVESTIGATIONS:

Rotavirus Detection:

Stool samples were analyzed for group A rotavirus using RotaStick one step test kit for determination of rotavirus in human feces (Novamed ltd., Jerusalem) following the manufacturer's instructions.

Principle of the procedure

The principle of the kit depends on a rapid immunochromatographic test for the qualitative screening of human fecal samples for detecting the presence of rotavirus antigen .

In brief, about 0.1 g of stool specimen was added to 0.6 ml of buffer solution in a test tube. The content of the test tube was then mixed vigorously by vortex to suspend the specimen. After sedimentation of large particles to the bottom of the tube (2-5 min), the dipstick test strip was placed vertically into the sample tube and removed after 10 seconds or when the fluid had reached the middle of the test area of the dipstick. The test strip contains a mobile monoclonal (rabbit origin) anti-rotavirus antibody conjugated to colloidal gold particles. The strip contains another polyclonal (rabbit origin) anti-rotavirus capture antibody that is immobilized in the test area of the strip. If the stool sample extract contains rotavirus antigens, these form antigen antibody complexes with the gold particles. These complexes migrate along the dipstick to the immobilized capture antibody, and a positive test band becomes visible. An immobilized anti-rabbit antibody captures the remaining conjugate, forming a control band, which indicates proper performance of the test procedure. The test is judged positive when, in addition to the control band, a clearly distinguishable purple band becomes visible in the test window. Figure 1 below illustrates the interpretation of results.

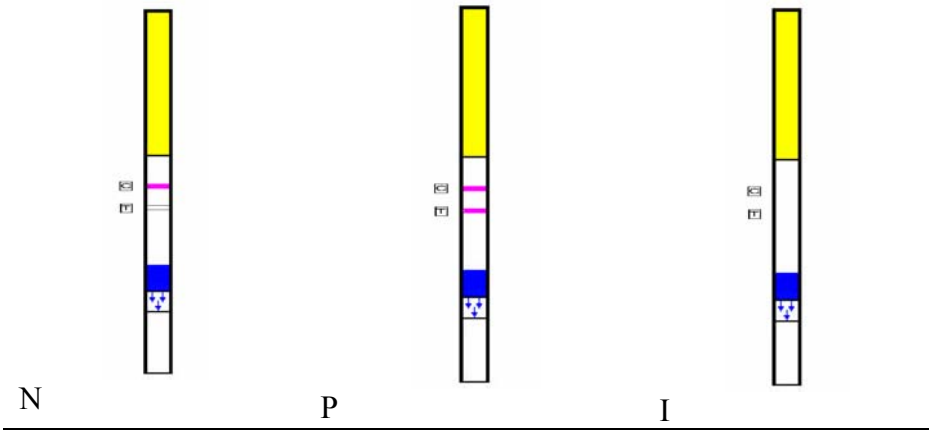

Figure 1. Interpretation of rotavirus results

N: Negative: only one pink/purple band appears in the Control window. No band is visible in the Test window, P: Positive: in addition to the Control band a clearly distinguishable pink/purple band also appears in the Test window, and I: Inconclusive: If no control band is visible (with or without a

visible band in the test window) the test is inconclusive. The test should be repeated using a new kit strip. C: Control window, T: Test window.

Blood pH Determination

Blood pH was determined by the Blood Gas Analyzers-Radiometer ABL 5 (Diamond Diagnostics USA), the sample was processed according to the manufacture's instructions.

Data Analysis:

The data was entered, sorted and analyzed by a personal computer using SPSS 8.0 statistical package, differences in proportions were assessed by a chi-square test, P values <0.05 were considered statistically significant.

RESULTS:

The study focused on detection of rotavirus antigen in 150 children less than 5 years of age in Gaza, Palestine. The results of the study can be summarized as follows:

The children enrolled in the study were divided into five age groups as illustrated in Table 1.

A clear higher incidence of diarrhea in the 0 to 2 years old subjects was observed. Infants below 12 months of age were particularly affected, accounting for 95 cases (63.3%), followed by 31 cases (20.7%) in the age group 13-24 months, Table 1.

Rotavirus was detected in 28 % (42/150) of all the fecal specimens examined (Figure 2), and the majority of patients who were positive for the virus, 90% (38/42), were 0 to 24 months old, and the infection rate decreased with increasing age.

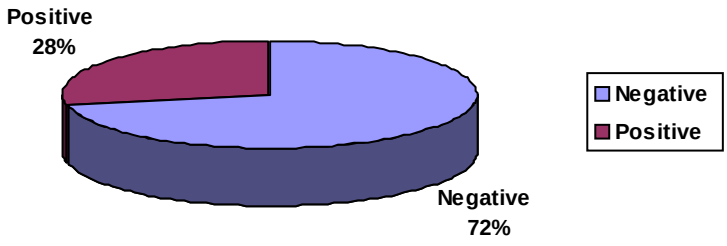

Figure 2. positive cases of rotavirus in the fecal samples.

Most of rotavirus-positive cases 25/42 (59.5%) were in the age group 0-12, followed by 13/42 (31.0%) in the age group 13-24 (Table 1). Moreover the highest rate of rotavirus antigen detection was observed among the 13 to 24 months age group since 13 out of the 31 diarrhea cases (i.e., 41.9%) examined in this age group were positive for rotavirus, followed by the age group 0-12 month (26.3%) and it decreased over age (Figure 3).

Table1. Distribution of rotavirus infection detected in 0 to 60 months old children with acute diarrhea.

Age	Rotavirus Antigen				
	No of patient examined	Positive		Negative	
		No	%	No	%
0-12 months	95	25	26.3	70	73.7
13-24 months	31	13	41.9	18	58.1
25-36 months	8	2	25.0	6	75.0
37-48 months	7	1	14.3	6	85.7
49-60 months	9	1	11.1	8	88.9
Total	150	42	28	108	72.0

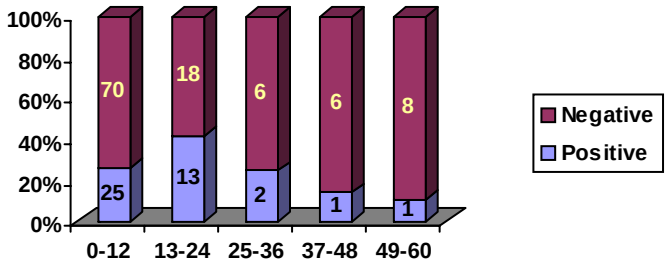

Figure 3. Rotavirus-positive versus negative cases detected in the various age groups.

Clinically; children infected with rotavirus were more likely to have watery stool (95.2%) with statistical significance (P value < 0.05), fever (73.8%), vomiting (92.9%) and moderate dehydration (14.3%), Table 2.

Table 2. Clinical presentation of children with and without rotavirus among the cases.

Clinical presentation	Number and percentage of samples				
	Rotavirus positive (n=42)	%	Rotavirus negative (n=108)	%	P-value
Vomiting	39	92.9%	92	85.2%	0.205
Fever	31	73.8%	87	80.6%	0.365
Dehydration	6	14.3%	11	10.2%	0.477
Watery Stool	40	95.2	89	82.4	**0.042**

Metabolic acidosis was significantly more frequent in rotavirus-positive cases (35.7%), while metabolic alkalosis was less common (2.4 %), Table 3.

Table 3. Blood pH and rotavirus

Condition	Number and percentage of samples				
	Rotavirus positive (n=42)	%	Rotavirus negative (n=108)	%	P-value
Metabolic acidosis	15	35.7%	15	13.9%	**0.002**
Metabolic Alkalosis	1	2.4	17	15.7	**0.002**

Slightly more males (89/150) were admitted to the hospital due to diarrhea caused by rotavirus than females(61/150). The ratio of rotavirus infection, however, was 1.2 higher in the female subjects, (19/61 of the females and 23/89 of the males) Table 4.

Table 4. Rotavirus detection from stool samples in relation to the gender

Variable	Gender		Total
	Female	Male	
Rotavirus Negative	42	66	108
Rotavirus Positive	19	23	42
Total	61	89	150

Most of the children 59.3% (89 of 150) who had diarrhea and were admitted to the hospital came from the Gaza region, 38.0 % (57 of 150) were from the Northern Gaza strip and the rest was from Mid zone and the Southern Gaza strip.

The highest rate of rotavirus antigen detection (36.0%) was observed among the Gaza region group Table 5.

Table 5. Rotavirus-positive and negative cases with respect to residence area.

Residence area	Number and percentage of samples				
	Rotavirus positive (n=42)	% of the total	Rotavirus negative (n=108)	% of the total	Total
North Gaza	9	6.0%	48	32.0%	57
Gaza region	32	21.3%	57	38.0%	89
South & mid zone Gaza	1	0.7%	3	2.0%	4
Total	42	28.0%	108	72%	150

Pearson Chi-Square = 0.030

DISCUSSION:

Diarrhea remains one of the most common illnesses of children and one of the major causes of infant and childhood mortality in developing countries. Considering the usually scanty resources available in the third world countries, a reduction in diarrhea-related mortality may be possible by identifying high risk subjects and targeting them for intensive intervention. In the present study, we focused on rotavirus as an important etiologic agent of childhood diarrhea.

Rotavirus was detected in 28 % (42/150) of the fecal specimens collected from children of < 5 years of age with acute diarrhea, using an immunochromatographic assay.

When compared to other studies the percentage of rotavirus-positive specimen of our study is lower than that reported by many investigators such as the 32.5% of Youssef et al. (2000) in Jordan, the 34.6% of El-Sheikh and El-Assouli (2001) in Saudi Arabia, and the 45.0% of Nguyen et al. (2005) in Vietnam .

On the other hand, our percentage is higher than that reported by many other authors; 6.8% by Sallon et al (1994) in Gaza, Palestine, 8.8% by Yoshida et al. (1998) in Japan, 11.9% Rohner et al., (1997) in Switzerland, 14% by Dagan et al. (1990) in Southern Israel, 15.3% by Modarres et al (1995) in Iran, 19.2% by Orlandi et al. (2001) in Brazil, and 19.6% by Ballal and Shivananda (2002) in India.

Meanwhile, our finding is nearly congruent with that Battikhi (2002) in Jordan and Ali et al. (2005) in Zliten, Libya, where they reported that 26.6% of their samples had rotavirus infection, 28.6% by Pazzaglia et al. (1993) in Alexandria, Egypt, and 27% by Buser, et al. (2001) in Switzerland .

The low or high rates of rotavirus infections presented by different investigators can be explained by several factors including, the study population, the incidence rate of the virus in different environments, the living conditions and standards of the study group, and the season on which the study was conducted.

Out of the 150 diarrheal patients enrolled in the study, 64.0% were less than 1 year of age, and 84.0% were less than 2 years of age. This shows a strong tendency of diarrhea to occur among children less than 2 years of age. This finding is consistent with other studies conducted in the middle east countries and other various developing countries, (17,28,44) where the major burden of diseases due to rotavirus occurs in the first and second year of life.

The children enrolled in this study were divided into five age groups, 90% of all the cases of rotavirus occurred in children ≤ 2 years of age, which is in agreement with many other studies done in most parts of the world and which showed why these age groups represent the highest morbidity and mortality from rotavirus diarrhea. The prevalence of rotavirus infection in this age group emphasizes the importance of rotavirus vaccines, which have been undergoing field trials for several years (5,19,40.(

Moreover, our study indicated that there was a trend of decreasing rates of rotavirus infection in the older children. This might partly be explained by the fact that older children acquired protective immunity during previous, probably subclinical, exposures to rotavirus and therefore become more resistant to infection with this agent (22,26).

A total of 89 (59.3%) male and 61 (40.7%) female cases of acute diarrhea were examined in this study Table 4. Slightly more males were admitted to the hospital due to diarrhea, Rotavirus prevalence was higher in female cases 19 of 61 (31.1%) than in males 23 of 89 (25.8%), no reasonable explanation has yet been given for this distribution but it is possible that the cultural or behavioral norms in our study area are contributing factors. For example it is a common practice in many families of preferentially seeking medical care for boys.

It is not possible to distinguish diarrhea caused by rotavirus clinically, because diarrhea, vomiting, fever, and dehydration are not absolutely associated with rotavirus infection, through, and as reported by the current

study, some clinical symptoms are more common in rotavirus infection such as vomiting, dehydration and metabolic acidosis (12,14,28,45).

Most of the patients (89/150 ; 59.3%) admitted with acute gastroenteritis were from the Gaza region, 38.0% (57/150) were from Northern Gaza Strip, and 2.7% (4/150) were from Mid zone and Southern Gaza Strip. It is important to note that there is no pediatric hospital in Northern Gaza Strip. The low number of cases admitted from the Mid zone and Southern Gaza Strip is due to the continuous closures and separation of Gaza from the southern area by the Israeli occupation, and the presence of some hospitals in that area.

The present study revealed that rotavirus was the microorganism associated most frequently (36.0%) with gastroenteritis in Gaza region, (15.8%) found in Northern Gaza Strip. Many authors have shown that rotavirus infection is more common in urban area as compared to rural areas. The reason of increased rotavirus prevalence in Gaza city can be due to nosocomial infections in neonatal nurseries, particularly more common in the urban area than the rural one, and the management of rotavirus infection is difficult (2,23,30).

The incidence of rotavirus disease was observed to be similar in both industrialized and developing countries, suggesting that adequate control may not be achieved by improvements in water supply, hygiene, and sanitation. Consequently, the development, trial, and widespread use of rotavirus vaccines is highly recommended in order to prevent severe and fatal rotavirus disease (42).

The current study was done during the late spring and summer seasons, rotavirus infections however, are known to be more common in the winter season, consequently the true prevalence of rotavirus may be well over than 28.0% if the study has been done during the cold season, and this further potentiates the importance of rotavirus in the etiology of diarrhea (18,26,37).

No specific treatment of viral infection is available nor it is really required. The severe symptoms and fatal outcome from rotavirus diarrhea are due to dehydration, the acute loss of fluid and electrolytes. This can be treated with rehydration therapy, ie. replenishing the fluids and electrolytes that have been lost. For children who are not severely dehydrated, oral rehydration solution is the treatment of choice whereas for children who are severely dehydrated, in shock, and are unable to drink, intravenous therapy can be lifesaving. Antibiotics are not required and are contraindicated, use of antimicrobials adds to the cost of treatment, risks adverse reactions and enhances the development of resistant bacteria(13,16).

Timely diagnosis of rotavirus infection in patients with acute diarrhea helps determine appropriate treatment, prevents the unnecessary use of antibiotics and minimizes the spread of the disease (13,16). It is worth noting here that the detection of rotavirus in stool specimen by the employed method requires only 30 minutes to perform .

CONCLUSION AND RECOMMENDATION:

The single most effective way to prevent the spread of rotavirus is by careful hand washing after using the toilet and before eating, as well as sanitary handling of food. Children with rotavirus diarrhea in whom stool cannot be contained by diapers or toilet use should be excluded from contact with other children, especially in childcare settings, until diarrhea ends. Surfaces should be washed with soap and water. Chlorine-based disinfectants will destroy rotavirus and help prevent the spread of the disease resulting from objects in the environment.

Improved hygiene, education, drinking clean water, breast feeding which helps in providing passive immunity in the newborn for at least for 6 months are highly recommended in order to lessen the infection rate not only of rotavirus but also of other many enteropathogen.
Based on the preliminary data presented by this study, further work is needed in order to provide a broader picture of the burden of rotavirus in children less than 5 years old all over Gaza strip.

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