Studying the causative agents of diarrhea among children under five years in Wasit Province

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Abstract

Bacterial infections are one of common cause of children diarrhea which causing mortality and morbidity worldwide. In this study One hundred – seventy stool samples were collected from children under five years old suffering from diarrhea. these samples were collected from AL-Karama Teaching Hospital, AL-Zahra’a Teaching Hospital in the Kut city, Al-Shahid Firoz General Hospital in Al-Hay City, and Al-Azizia General Hospital in Wasit Province, during period months from October, November, and December from 2014. Then these samples examined and cultured, and isolates were identified by conventional methods, and test their sensitivity for antibiotics by disc diffusion method.

The results showed that the bacteria are isolates from 91 samples from the total number of samples, while remaining are parasitic infections. The percentage of infections caused by E. coli (91 samples) is 53.529 %, While the percentage caused by Entamoeba histolytica (63 samples) is 37.058 %, and infection by Giardia lamblia (11 samples) is 6.47 %, and infection by Monilinia fungi (3 samples) is 1.764 %, and infection by Hymenolepis nana (2 samples) is 1.176 %.
The most common bacterial caused diarrhea in children is *E. coli*, and then Klebsiella, while the common parasite caused diarrhea is *E. histolytica*, and then *Giardia lamblia*, Monillia fungi, and *H. nana*.

Also the results showed that Ceftizoxime is good antibiotic high effect against *E. coli*, while Erythromycin is less effect antibiotic against it.

**Introduction**

Acute diarrhea can be defined as the new onset of passage of three or more unformed stools in a 24-hour time period as passage of an increase number of stools of decreased form compared with the normal state. In any case the duration is less than 14 days. Acute diarrhea is frequently associated with one or more enteric symptoms like nausea, vomiting, increase in abdominal gas, abdominal pain or cramps, tenesmus (intense urge with straining but minimal or no bowel movement), fecal urgency or passage of stools containing gross blood and mucus (1).

Diarrhea is an important disease, and its complications worldwide cause of morbidity and mortality in children, especially in developing countries (2). The world Health Organization estimates that over 2.2 million deaths due to diarrheal infections, especially among children under five years of age (3), and without aggressive efforts to control diarrheal disease, Millennium Development Goal 4 which is to reduce childhood mortality by 2015 will remain out of reach. More than half of these cases are in Africa and South Asia where bouts of diarrhea are more likely to result in death or other severe outcomes (4). Acute diarrhea as a gastrointestinal related symptom may have some different causes such as infection. Infectious diarrhea leads to approximately three million deaths worldwide and 516 deaths in Iranian children younger than 5 years per year (5, 6). The rate of entropathogen isolation in acute diarrhea varied in different studies depending on the sampling methods and microbiological techniques. Some of them, the most common bacterial pathogen is diarrheagenic *E. coli* (5, 7, 8).

When diarrhea lasts more than 14 days, it is considered to be persistent. The etiologic agents are likely to be different in these cases. The most important group of pathogens is the intestinal protozoa, including *Giardia lambelia*, *Cryptosporidium* and *E.histolytica* other well-known parasitic causes of persistent diarrhea are *Cyclospora*, *Isospora* and *Microsporidium*. The bacterial enteropathogens can be implicated in a subset of persistently ill patients (9).

Infants attending day care centers may be exposed to enteropathogens secondary to environmental contamination when a day care center child develops diarrhea The most common causes of diarrhea outbreaks in day care centers are the low-inoculum pathogens including *Shigella*, *Giardia*, *Cryptosporidium* and Rotavirus.
Immunity develops in high-risk day care centers by repeated exposure to prevalent enteric pathogens (10). Travelers’ diarrhea is defined as acute diarrhea acquired by persons during international trips, usually occurring in someone from an industrialized region during visits to develop tropical and semitropical countries. Travelers’ diarrhea is frequently caused by a bacterial pathogen. Poor sanitation of the host country is an important factor associated with enteric disease. The most important vehicle for transmission is food with water and ice being less important (11). Food is an important vehicle for enteropathogens in all regions of the world. It is estimated that 76 million persons suffer from foodborne diseases each year in the United States. The illness leads to 325,000 hospitalizations and 5000 deaths per year, while enterotoxigenic E. coli (ETEC) is the most important etiologic agent in travelers’ diarrhea, the organism occasionally causes foodborne outbreaks in the United States. Enterotoxigenic E. coli has caused extensive outbreaks of diarrhea secondary to contamination of recreational lakes or water parks where Cryptosporidium is also implicated in outbreak disease. Surveillance data from the CDC demonstrated that 50% of waterborne gastroenteritis outbreaks related to treated water were due to Cryptosporidium and 25% of waterborne outbreaks from freshwater were due to ETEC and 25% were due to noroviruses (12).

The aim of study

The aim of this study is to detect causative agents of diarrhea among children under five years in Wasit Province, and detect antibiogram of it.

Collection of samples

One hundred- seventy stool samples were collected from out patients (children) suffering diarrhea from AL-Karama Teaching Hospital, AL-Zahra’a Teaching Hospital in the Kut city, Al-Shahid Firoz General Hospital in Al-Hay City, and Al-Azizia General Hospital in Wassit Province.

Methods

All samples study was done on children with diarrhea in laboratories of AL-Karama Teaching Hospital, AL-Zahra’a Teaching Hospital in the Kut city, Al-Shahid Firoz General Hospital in Al-Hay City, and Al-Azizia General Hospital in Wasit Province, and some information such as age, sex, and symptoms from each patient. Fecal specimen was collected in a sterile container, and also a rectal swab. All samples were collected tested for detection of bacterial, parasitic, and fungal agents.

Identification of bacteria

The stool samples was given a general stool examination with direct plating on blood agar, EMB and Mac Conkey agar, and inoculation into enrichment media (peptone and tetrathionate) Identification of bacterial isolates were based on biochemical testes, using of the analytical profile index (Api 20 E) system(13) (Figure 1)
Also use Nutrient agar for sensitivity test to antibiotics discs which used (14). All samples were cultured and incubated at 37°C for 18 – 24 hours and incubated at 37 °C for 24 hr. Next day individual colonies were selected and identified on the bases of morphological, cultural and biochemical characteristics (15).

**Determination of antibiotic resistance profile**

Bacterial isolates from stool samples were subjected to antibiotic resistance screening by disc diffusion method. For this purpose bacterial isolates from stool samples isolates was made on Muller-Hinton agar with help of wire-loop then commercially available antibiotic discs were placed on lawn of culture and plates were incubated at 37 °C for 24 hr. Next day presence or absence of zone of inhibition around the antibiotic discs was observed (16), and detect sensitive or resistance of bacteria to antibiotics according to CLSI 2012 (17).

Figure (2) Antibiotics were used Ampicillin, Ciprofloxacin, Gentamycin, Ceftizoxime, Amoxicillin, and Erythromycin.

Figure (2): Antibiotics sensitivity test.
Results

The purpose of this research is to detect the causative agents of diarrhea in children, and the susceptibility or resistance profile of multi-drug resistant isolates from stool samples. One hundred-seventy isolates from different pathological laboratories of Wasit Province were isolated and identified by routine methods. Identification of the causative organism and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages of diarrhea (18). Percentage and sex of children with diarrhea diagnosis is depicted in (Table 1).

Table (1): Percentage and Gender of children with diarrhea diagnosis

<table>
<thead>
<tr>
<th>Total No. Of Samples</th>
<th>Male</th>
<th>Percentage</th>
<th>Female</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Diarrhea Diagnosis</td>
<td>102</td>
<td>60 %</td>
<td>68</td>
<td>40 %</td>
</tr>
</tbody>
</table>

The percentage of bacterial isolates was *E. coli* (53.529 %) from total samples. The percentage of parasitic infections includes, *E. histolytica* (37.058 %), followed by *Giardia* (6.47 %), *Monillia* (1.764 %), and *H.nana* (1.176 %). Table 2.

Table (2): percentage of causative agents of diarrhea of total samples

<table>
<thead>
<tr>
<th>Causative Agents Of Diarrhea</th>
<th>Number Of bacterial isolates Or parasite</th>
<th>Individual % Of Bacteria and Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>91</td>
<td>53.529</td>
</tr>
<tr>
<td><em>E. histolytica</em></td>
<td>63</td>
<td>37.058</td>
</tr>
<tr>
<td><em>Giardia</em></td>
<td>11</td>
<td>6.47</td>
</tr>
<tr>
<td><em>H. nana</em></td>
<td>3</td>
<td>1.764</td>
</tr>
<tr>
<td><em>Monillia</em></td>
<td>2</td>
<td>1.176</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>100 %</td>
</tr>
</tbody>
</table>

All isolates of bacteria were screened for antibiotics sensitivity profile by disc-Diffusion method with commercially available disc of Ampicillin, Ciprofloxacin, Gentamycin, Ceftizoxime, Amoxicillin, and Erythromycin.
Table 3 indicates the resistance level against commonly used antibiotics in bacterial stool isolates. Nearly, all the isolates were found to be susceptible against most of the antibiotics, whereas isolates show more resistance to Erythromycin as compare to other antibiotics used (19).

Table (3): Total percentage efficacy of different antibiotics among bacterial stool isolates (Total No. of E. coli isolates 91).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc code</th>
<th>No. of sensitive isolates from total E. coli</th>
<th>No. of Resistant isolates from total E. coli</th>
<th>% Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>Am</td>
<td>2</td>
<td>3</td>
<td>2.197</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Cip</td>
<td>27</td>
<td>3</td>
<td>29.670</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>G</td>
<td>4</td>
<td>6</td>
<td>4.395</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>CF</td>
<td>42</td>
<td>1</td>
<td>46.153</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>PB</td>
<td>1</td>
<td>2</td>
<td>1.098</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>E</td>
<td>0</td>
<td>91</td>
<td>0</td>
</tr>
</tbody>
</table>

Total No. of E. coli isolates (91)

Discussion

In this study, we found bacteria more than half of stool samples of children with diarrhea (60%). Our findings are in agreement with the results of other studies from the developing countries (5,8). In this study, the frequency of bacterial diarrhea in children was significantly higher than the others in agreement with the results of other studies (7, 19). This may be due to the kind of nutrition and high rate of breast milk feeding in infancy in our region. E. coli was the most common cause bacterial diarrhea similar to many other previous studies (7, 20).

So we found parasitic infections (40%) from total stool samples of children with diarrhea. Our findings are in agreement with the results of other studies as most important causative agent for diarrhea (21). In this study, the frequent of parasitic diarrhea in children was E. histolytica, then Giardia lamblia, Monillia, and H. nana in agreement with AL-Najar, 1999 in Baghdad (22). Ceftizoxime and Ciprofloxacin is more frequent in our study. It has been showed that there is a direct relation between the antibiotic used and the frequency and the kinds of antibiotic-resistant strains in human beings (23).
The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements/plasmids (24). In our studies, the most effective antibiotic for bacterial isolates from children with diarrhea is Ceftizoxime showing 46.153 %, then Ciprofloxacin which show 29.670 % efficacy, while Gentamycin 4.395 %, Ampicillin 2.197 %, then Amoxicillin 1.098 % efficacy, while Erythromycin didn’t appear any effects on bacteria (Table 3), due to the Ceftizoxime and Ciprofloxacin are recent antibiotics and the good uses for them, so less frequent use take susceptibility to these antibiotics (25) while Erythromycin is used since far time and high frequent use it in bad using depends on the amount of dosage and time of dosage lead to induced mutation in this bacteria (Its generation gap is 15 minutes), take resistant for this bacteria and difficulty to treat because of their high frequency of drug resistance (26), and due to antibiotic resistance and limited antimicrobial activity of antibiotics some strains of disease causing bacterial diarrhea (27, 28).

Conclusion
Hence present study shows that the majority causative agents of diarrhea in children less than five years two types:
1- bacterial agents’ especially E. coli.
2- parasitic agents especially E. histolytica, Giardia lamblia, H. nana, and Monillia.
3- Also showed their different susceptibility for the antibiotics usually used for the treatment of bacterial diarrhea.

References
