Microbial Etiology of Acute Diarrheoa in Children Under Ten Years of Age in Diyala, Iraq / Hospital-Based Study

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Abstract

This study was conducted in the Al Batool Teaching Hospital in Ba'aqubah in Diyala city, from August 2019 to December 2019. In children under 10 years patients suffering from diarrhea with different gastrointestinal complaints, Stool samples were collected from each patients use for microscopic examination and Culture and sensitivity test. Diarrheal diseases are major problem of developing countries. Though precise data on childhood mortality associated with diarrheal diseases in Iraq is not available, it has been estimated that approximately 25% of child death are associated with diarrheal disease, particularly acute diarrhea. The purpose of this study was to detect causative agent of acute diarrhea in children less than 10 years of age. With the study of sensitivity to certain antibiotics. Bacterial pathogen infected cases 30 (60%) male and 20(40%) female, among the total enrolled cases the Escherichia coli was 30(60%), Klebsiella species was 17(34%) and kluyvera ascorbate species was 3 (6%).also the study detected that bacterial infection was found to be of highest percentage, 26(52%) in the age group less than 1year, The least infection2 (4%) was found in group between7-8. There were no detectable cases of bacterial infection among the age group of 9-10 years in this study.Amikacin showed efficacy in 26(96.6%) isolates of E.coli species, also showed efficacy in 16(94.1%)isolate of Klebsiella spp.Amikacin,cefepime,meropenem,gantamicin showed efficacy in 3(100%)isolate of Kluyvera ascorbate.

Keywords: *Abdominal infection, Kluyvera ascorbate, Diarrhea in children, Klebsiella*

Introduction

Diarrhea is defined by World Health Organization (WHO) as having 3 or more loose or liquid stools per day or as having more stools than is normal for that person [(1)]. Acute diarrhea, defined as an increased frequency of defecation (three or more times per day or at least 200 g of stool per day lasting less than 14 days, may be accompanied by nausea, vomiting, abdominal cramping, clinically significant systemic symptoms, or malnutrition[(2)] .

Diarrhea is an important cause of morbidity and mortality in children from developing countries. Children less than ten years of age have 3.3 diarrheic episodes per year, and more than one-third of the deaths in this age group are associated with diarrhea. Therefore, every year there are approximately billion diarrheic episodes and 4 million deaths in children less than five years of age (most from 6 months to 12 years) caused by this disease [(3)].

Kluyvera is a gram negative bacillus a. This bacillus which could be localized in the respiratory tract, gastrointestinal system and urinary tract has been observed to cause to clinically severe disease in various anatomic localizations in the last 30 years. It was shown to be a new member of the Enterobacteriaceae family using molecular methods and deoxyribonucleic acid (DNA) hybridization technique [(4)]. There are four Kluyvera species which have been defined until the present time including K. ascorbata, K. cryocrescens, K. georgiana and K. cochleae. Kluyvera is a small, Peritrichous, mobile, oxidase negative, catalase positive gram negative bacillus and ferments glucose [(5)].

Kluyvera Ascorbata is a gram negative microorganism belonging to the family Enterobacteriaceae. Although

it causes infections infrequently, it is responsible for causing a wide range of infections including severe sepsis (6). Kluyvera is a relatively newly described member of the Enterobacteriaceae family that rarely causes infections in humans. In the pediatric population, it is described in association with clinically significant infections ranging from urinary tract infections to sepsis with multiorgan failure [7].

The factors related to higher prevalence of diarrhea are lack of education of mother, lack of exclusive breastfeeding, breastfeeding for less than 1 year, roundworm infestation, nutritional status, immunization status, female sex, literacy, personal hygiene, overcrowding, garbage disposal, source of water supply, and toilet facility [8]. In China, diarrhea is the most common reason for children to visit health-care clinics, and the antibiotic resistance has become a growing problem due to misuse of antibiotics [9].

In many hospitals in developing countries lacking clinical microbiology laboratories, the cause of diarrhea in children is unknown. The seasonality of specific enteropathogens such as rotavirus or some parasites has been reported [10].

The aim of this study was to determine the prevalence of enteropathogens, including bacteria, virus, and parasites, causing diarrhea among children less than five years old in during the dry and rainy seasons .The main etiology of the diarrhea is related to a wide range of bacteria (such as Campylobacter jejuni, Escherichia coli, Salmonella spp., Vibrio cholerae, Yersinia enterocolitica, and Aeromonas spp.), enter parasites (Giardia spp., Cryptosporidium spp., and Entamoeba histolytica), and viruses (adenovirus, Norwalk virus, and rotavirus) [11].

Microbial Identification

After many years of experience, Appeared Vitek 2 –compact (biomerieux) device as a reference in terms of identification of microorganisms. used to analyze the samples and obtains the results. Offering both manual and automated solutions, adapted to guarantees rapid, accurate and standardized results for all types of specimens, thus optimizing workflow of microbiology labs. Stool sample was collected in a sterile container. Rapid reliable identification of these organisms is essential for accurate diagnosis and prompt effective treatment. We evaluated the ability of the VITEK 2 system (biomerieux, Inc., Hazelwood, Mo.) to identify these organisms rapidly and accurately[12].

Each organism suspension was prepared from the growth of pure cultures of bacteria cultivated on plates containing Trypticase soy (TSA) agar with 5% sheep blood and incubated overnight at 35°C. Bacterial cells were suspended in 2.5 ml of a 0.45% sodium chloride solution. The suspension used in the VITEK 2 system was adjusted to a McFarland standard of 0.5 by using a Densicheck (bioMérieux). The Biomerieux DensiCHEK Plus instrument is for use with VITEK and VITEK 2 systems to measure the optical density of a microorganism suspension in McFarland units [13].

Antibiotic sensitivity test:

Emergence of Antimicrobial Resistance and the Rationale for Performing Susceptibility Testing

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. Empirical therapy continues to be effective for some bacterial pathogens because resistance mechanisms have not been observed. Susceptibility testing of individual isolates is important with species that may possess acquired resistance mechanisms (e.g. members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus species, and Streptococcus pneumoniae) [14].

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The most widely used testing methods include broth microdilution or rapid automated instrument methods that use commercially marketed materials and devices. Manual methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods. Each method has strengths and weaknesses, including organisms that may be accurately tested by the method. Some methods provide quantitative

results (e.g., minimum inhibitory concentration), and all provide qualitative assessments using the categories susceptible, intermediate, or resistant. In general, current testing methods provide accurate detection of common antimicrobial resistance mechanisms. However, newer or emerging mechanisms of resistance require constant vigilance regarding the ability of each test method to accurately detect resistance [15] .

Material and Methods

Patients and samples.

This study was a cross-sectional study conducted at AL-Batool Teaching Hospital, Department of Microbiology-Laboratory consent was obtained from the children's parents or guardian before enrollment. A total of 50 stool samples were collected from the children under 10 years of age visiting AL- Batool Children's Hospital, Ba'aqubah, Diyala Proven with acute diarrhea in the periods between Augast 2018 to December 2018. From each participating children, clinical data were obtained and stool sample was collected in a sterile container. Vitek 2 –compact (biomerieux) device used to analyzed the samples and obtain the results [16] .

Statistical Analysis

Data of current study analyzed by using Chi-square- x2 test to compare between percentages.A level of significance of a=0.05 was applied to the test. (Spss v.22) programs used analyzed current data.

Rusults

Bacterial pathogen infected were 50 (100%) totaled cases, 30 (60%) among of theme were male, while the remaining 20(30%) were female, as shown in table. 1

Table 1.gender distribution of diarrheal cases.

Gender	N(%)
Male	30(60%)
Female	20(30%)

Bacterial infection was found to be of highest, 26(52%) in the age group less than 1year. The least infection2 (4%) was found in group between 7-8. There were no detectable cases of bacterial infection among the age group of 9-10in this study. Statistical analysis showed that there were very significant different ($p<0.001$) in distribution of bacterial infection as shown in table 2

Age groups	No.	%
Less than one year	26	52%
1-2	11	22%
3-4	7	%14
5-6	4	%8
7-8	2	%4
9-10	0	%0

Table 2. Age distribution of diarrheal cases.

Among the total enrolled cases the Escherichia coli was 30(60%), Klebsiella species was 17(34%) and kluyvera intermedia species was 3 (6%) .NO Shigella or salmonella detected in this study. Statistical analysis showed that there were very high significant different ($p<0.001$) in distribution of bacterial infection as shown in table.3

Table3.distribution of bacterial pathogen.

Type of organism	NO. (%)
Escherichia Coli	30(60%)
Klebsiella SPP.	17(34%)
Kluyvera Ascorbata	3(6%)

Among the *Escherichia coli* Amikacin resistance showed the highest 26(96.6%) efficacy, followed by Meropenem which showed 23(76.6) efficacy, while the least efficacy showed by Ampicillin 3(10%) .Statistical analysis showed a very high significant different in antibiotic sensitivity ($p<0.001$)as in table.4

Table 4.antimicrobial sensitivity of E.coli spp (n=30)

Antibiotics	Sensitive No. (%)	Resistance No. (%)
Ampicillin	3(10%)	27(90%)
Amoxicillin	17(56.6%)	13(43.4%)
Cefuroxime	18(60%)	12(40%)
Cefoxitin	22(73.3%)	8(26.7%)
Cefixime	18(60%)	12(40%)
Ceftazidzme	9(30%)	21(70%)
Ceftriaxone	13(43.3%)	17(56.7%)
Amikacin	29(96.6%)	1(3.4%)
Meropenem	23(76.6%)	7(23.4%)
Gentamycin	20(66%)	10(33.4%)
Ciprofloxacin	18(60%)	12(40%)
Trimethoprime/sulfamethaxazol	7(23.3%)	23(76.6%)

In *Klebsiella spp*, Amikacin showed efficacy in 16 (94.6) of cases, followed by cefoxitin which was résistance only in 3(17.7) cases, the least efficacy showed by ampicillin 4(23.5%) and Trimethoprim\sulfamethaxazol which was sensitive in 6(35.2%). statistical analysis showed a significant different in antibiotic résistance ($p<0.05$) as in table.5

Table 5.antimicribial sensitivity pattern of Klebsiella spp (n=17).

Antibiotics	Sensitivity No. (%)	Resistance No. (%)
Ampicillin	4(23.5%)	13(76.5%)
Amoxicillin	8(47%)	9(53%)
Cefurxime	7(41.1%)	10(58.9%)
Cefoxitin	14(82.3%)	3(17.7%)
Cefixime	8(47%)	9(53%)
Ceftazidzme	7(41.1%)	10(58.9%)
Ceftriaxone	7(41.1%)	10(58.9%)
Amikacin	16(94.1%)	1(5.9%)
Meropenem	13(76.4%)	4(23.6%)
Gentamycin	12(70.5%)	5(29.5%)
Ciprofloxacin	12(70.5%)	5(29.5%)
Trimethoprime/sulfamethaxazol	6(35.2%)	11(64.8%)

Among *Kluyvera ascorbata* Cefepime, Meropenem, Gentamicin and Amikacin were sensitive in all cases 3(100%) ,Tetracycline showed no sensitivity in all case, table 6.

Table 6.antimicribial sensitivity pattern of Kluyvera intermediate (n=3)

Antibiotics	Sensitive No. (%)	Resistance No. (%)
Piperacillin/tazobzctam	2(66.6%)	1(33.4%)
Ceftazidzm	2(66.6%)	1(33.4%)
Cefixime	3(100%)	0(0%)
Meropenem	3(100%)	0(0%)
Gentamicin	3(100%)	0(0%)
Amikacin	3(100%)	0(0%)
Ciprofloxacin	2(66.6%)	1(33.4%)
Tetracycline	0(0%)	3(10%)
Trimethoprime/sulfamethoxazole	1(33.4%)	2(66.6%)
Piperacillin	2(66.6%)	1(33.4%)

Discussion

The e bacterial pathogens were found to be a significant cause of acute diarrhea. However, while diarrheal diseases are prevalent in all ages, they are often more severe among children due to their small body size, and rapid ability to become dehydrated [17].

In this study out of 50 cases enrolled 30were male and 20were female. Higher positivity rate among boys was in agreement with the numerous studies . This high prevalence associated with males may be related to the reality that male children more active than females. Moreover, they spend longer period outside the home and they were more in contact with environmental conditions than females. This makes them more liable to infection than females [18]. Children below 10 years of age were enrolled in this study. The maximum number of samples were from the age group of less than 2 years in which the age group of less than 1 years constitutes maximum number 26 (52%)followed by the age group of 1-2years 11(22%). Similar result was also found by Moyo et al.[21] . *Escherichia coli* were detected in 30 (60%) of cases with Similar results were also reported by several other investigators [19].

In our study *Klebsiella spp* detected in 17(34%) of cases. A comparable studies also found (23).*Kluyvera ascorbata* detected in 3(6%) of cases ,studies showed that, diarrheal infections caused by kluyvera spp have been reported in neutropenia and non-neutropenia patients ,in patients with underlying malignant neoplasm and in those without any known disease process ,with or without antimicrobial therapy. This suggests the presence of poorly understood host conditions that can predisposed a person to opportunistic infection with *kluyvera ascorbate* [20].

No *Vibrio* spp. was isolated in this study. The spread pattern of *Vibrio* spp. suggested water borne infection in rainy seasons, although the quantity of water is large in the rainy season most water are contaminated with excreted microorganisms from water surface runoff [21] .

While discussing about the antibiotic sensitivity profile, among *Escherichia coli* Among the *Escherichia coli*, Amikacin showed the highest 26(96.6%) efficacy, followed by Meropenem which showed 23(76.6), Cefoxitin 22(73.3%), ciprofloxacin 18(60%) efficacy, while the least efficacy showed by ampicillin 3(10%) of cases, . According to Kaminski et al.[28] . Ciprofloxacin was 100% effective against *E. coli* and 50% of isolates were resistant to Ampicillin and Al Gallas et al. [6]. showed that isolates 20.4% isolates resistant to Ampicillin. Among Klebsiella Amikacin showed efficacy in 16(94.6) of cases, followed by Cefoxitin which was résistance only in 3(17.7) cases, the least efficacy showed by ampicillin 4(23.5%) and Trimethoprime\ sulfamethaxazol which was sensitive in 6(35.2%),study by Chris Rowe Tatted et al. (2017) kany) . showed that Amikacin sensitive in 66% of isolate,ampcilin in 51% cases, while Trimethoprime\sulfamethaxazol 39% sensitive. In total identified cases of kluyvera spp Cefepime, Meropenem, Gentamicin and Amikacin were sensitive in all cases 3(100%), Tetracycline showed no sensitivity in all cases, Similar result showed by Eda Karadag Oncel et al. (2015) [9].

Conclusions

The study indicated that the frequency of diarrhea was higher in male children compared to female children. Amikacin, gentamycin, were the most effective antibiotics while Ampicillin

Were the least effective antibiotics in vitro against the bacterial isolates. *E.coli* species were common among the bacterial pathogen causing acute diarrhea in children less than 10 years of age. These children can become a source of outbreaks. So the awareness on prevention of the infectious diseases, improving hygiene should be implicated to reduce the burden of infectious diseases.

Ethical Clearance: The project of this study was taken from the ethical committee of College of Medicine / University of Diyala.

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Conflict of Interest: Nil

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