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The Prevalence of Acute Bacterial Gastroenteritis among Patients Intended to Kosti Teaching Hospital, Sudan

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Abstract

Background: *Acute bacterial gastroenteritis is a condition in which the fecal specimen reveals a significant growth. It's very common especially in children and can lead to serious complication even death.*

Study design: *This is across sectional study conducted in White Nile State (Sudan) at University of El_Emam El_mahdi (Kosti) from August 2017 to November 2017.*

Method: *A total of 70 patients were enrolled. All patients were clinically identified to have gastroenteritis. Fecal samples were collected from each patient into sterile containers. The fecal samples were examined microscopically and inoculated in Blood agar; Xylose Lysine Deoxycholate; MacConkey agar and Thiosulfate Citrate Bile Salt Sucrose agar, and after incubation period of overnight the result were reported and sensitivity tests were done.*

Result: *Out of 70 patients, 18 were revealed a positive for significant gastroenteritis, with prevalence rate 25.7%. the highest frequency was noticed in age group (15-30 year) 25%. Salmonella paratyphi A was the most frequent isolate (38.8%) followed by Shigella species, Salmonella typhi (16.6%) each, Salmonella typhimurum), Vibrio cholerea (11.1%) each and campylobacter species (5.5%). Most of isolated organism showed variable sensitivity to antibiotics, where the sensitivity to Cefotaxime was (55.6%), ciprofloxacin (38.9%),*

Tetracycline (16.7%), where all isolated were resistant to Doxycycline (100%)

Conclusion: *Acute bacterial gastroenteritis is common with prevalence rate 25.7%, the most effective drug was Cefotaxime, ciprofloxacin where all isolates were resistant to Doxycycline.*

Keywords: Acute Gastroenteritis, *Vibrio Cholerae*

INTRODUCTION:

Diarrhea, also spelled diarrhosea, is the condition of having at least three loose or liquid bowel movements each day. It often lasts for a few days and can result in dehydration due to fluid loss. Signs of dehydration often begin with loss of the normal stretchiness of the skin and irritable behavior. This can progress to decreased urination, loss of skin color, a fast heart rate, and a decrease in responsiveness as it becomes more severe. Loose but non-watery stools in babies who are breastfed, however, may be normal[1], The most common cause is an infection of the intestines due to either a virus, bacteria, or parasite; a condition known as gastroenteritis. These infections are often acquired from food or water that has been contaminated by stool, or directly from another person who is infected. It may be divided into three types: short duration watery diarrhea, short duration bloody diarrhea, and if it lasts for more than two weeks, persistent diarrhea. The short duration watery diarrhea may be due to an infection by cholera, although this is rare in the developed world. If blood is present it is also known as dysentery[1]

A number of non-infectious causes may also result in diarrhea, including lactose intolerance, irritable bowel syndrome, non-celiac gluten sensitivity, celiac disease, inflammatory bowel disease, hyperthyroidism, and a number of medications[2], In most cases, stool cultures are not required to confirm the exact cause[3], Diarrheal disease may have a negative impact on both physical fitness and mental development. "Early childhood malnutrition resulting from any cause reduces physical fitness and work productivity in adults,"[4]. Further, evidence suggests that diarrheal disease has significant impacts on mental development and health; it has been shown that, even when

controlling for helminth infection and early breastfeeding, children who had experienced severe diarrhea had significantly lower scores on a series of tests of intelligence[4][5].

Diarrhea can cause electrolyte imbalances, renal impairment, dehydration, and defective immune system responses. When oral drugs are administered, the efficiency of the drug is to produce a therapeutic effect and the lack of this effect may be due to the medication travelling too quickly through the digestive system, limiting the time that it can be absorbed. Clinicians try to treat the diarrheas by reducing the dosage of medication, changing the dosing schedule, discontinuation of the drug, and rehydration. The interventions to control the diarrhea are not often effective. Diarrhea can have a profound effect on the quality of life because fecal incontinence is one of the leading factors for placing older adults in long term care facilities (nursing homes)[6].

Prevention of infectious diarrhea is by improved sanitation, clean drinking water, and hand washing with soap. Breastfeeding for at least six months is also recommended as is vaccination against rotavirus. Oral rehydration solution (ORS), which is clean water with modest amounts of salts and sugar, is the treatment of choice. Zinc tablets are also recommended.[1] These treatments have been estimated to have saved 50 million children in the past 25 years.[7] When people have diarrhea it is recommended that they continue to eat healthy food and babies continue to be breastfed.[1] If commercial ORS are not available, homemade solutions may be used[8]. In those with severe dehydration, intravenous fluids may be required[2]. Most cases; however, can be managed well with fluids by mouth[9]. Antibiotics, while rarely used, may be recommended in a few cases such as those who have bloody diarrhea and a high fever, those with severe diarrhea following travelling, and those who grow specific bacteria or parasites in their stool[10]. Loperamide may help decrease the number of bowel movements but is not recommended in those with severe disease[10].

MATERIALS AND METHODS

Laboratory investigations:

Stool samples has been collected by a septical procedure from patients and inoculate in Xylose Lysine Deoxycholate (XLD), Macconkey agar, Thiosulfate citrate bile salt sucrose (TCBS) aerobically at 37℃, Blood

agar (microearophilic condition at 42℃), identification, biochemical tests and antibiotic susceptibility tests.

Sample collection:

Stool samples were collected from patients as following:

. clean, dry, and sterile containers had been prepared.

. the containers had been label in matching with questionnaire paper number.

. The patient was informed to collect the specimen properly.

. The specimens were received and transport to the lab with maximum delay avoidance.

Culture of specimen:

Each specimen had been inoculated in subsequence media plates in case of *vibrio cholerae specimens were inoculated into Thiosulphate Citrate Bile Salt Sucrose, all inoculates were incubated at37c for overnight.*

Gram stain: -

Used to differentiate between Gram positive bacteria and Gram negative bacteria·

Preparation of smear:

• One loopfull or more is taken up with the inoculating wire and spread thinly on the slide**.**

• After making smear the slides had been left in safe place to air-dry, protected from flies and dust.

• The slide is then held in the palm of the hand high over a bunzen flame and dried.

• The film is fixed by passing the dried slide with the film down wards, three times slowly through the flame.

Method of gram stain:

1. Fix the dried smear by using the heat.
2. Cover the fixed smear with crystal violet stain for 60 seconds.
3. Rapidly wash off the stain with clean water.
4 . cover the smear with Lugol's iodine for 60 seconds.
5.Wash off the iodine with clean water.

6.Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.
7. Cover the smear with neutral red stain for 2minutes.
8. Wash off the stain with clean water.
9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.
10. Examine the smear microscopically, first with the 100- objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells. [21]

Biochemical tests: -

The following were used for identification of isolated microorganism:

Catalase test	Oxidase test
Iodole test	Urease test
Citrate utilization test	Kligler iron agar

Catalase test:

This test is used to differentiate those bacteria that produce the enzyme Catalase, such as *Staphylococci*, from non-Catalase producing bacteria such as *Streptococci* .

Method:

1. 2 ml of the hydrogen peroxide solution was Poured into a test tube.
2. Using a sterile wooden stick or a glass rod several colonies of the test organism was removed and immersed in the hydrogen peroxide solution.
3. Looked for immediate bubbling.

Oxidase test (cytochrome oxidase):

This test is used to assist in the identification of *pseudomonas*, *neisseria*, *Vibrio and pasteurella species* all of which produce oxidase enzymes.

Method:

- a piece of filter paper was putted in clean petri dish and add 2or 3 drops of freshly prepared oxidase reagent.
- using a piece of stick or glass rod (not an oxidized wire loop), a colony of the test organism was removed, and smeared it in the filter paper.

- Looked for the development of a blue-purple colour within a few seconds.

Indole test:
Testing for indole production is important in the identification of enterobacteria.

Method:-
1. the test organisms were ioculated in test tube containing 3 ml of sterile tryptone water.
2. Incubated at 35–37 _C for up to 48 h.
3. indole was tested by adding 0.5 ml of Kovac's reagent. Shake gently. Examine for a red colour in the *surface layer* within 10 minute .

Urease test:-
Testing for urease enzyme activity is important in differentiating *enterobacteria*.

Method:
A colony of the test organism was inoculated over the entire slope surface and incubated at 37°C. The medium was examined after overnight incubation.

Citrate utilization test:-
This test is one of several techniques used to assist in the identification of *Enterobacteria* .

Method:-
1. Using a sterile straight wire loope, i the test organisms were inoculated.
2. Incubate at 35 _C for 48 hours. a bright blue colour in the medium had been looked for.

Kligler iron:
Is a differential slope medium used to assist in the identification of *Salmonella* and other *Enterobacteria***.**

Methods:

KIA medium was inoculated by using a straight wire. First the butt was stabbed and then the slope was cultured in a zig-zag pattern.The tube was Incubated aerobically at 37°C for 24 hours, then the results were interpreted.

Immobilization test:

This test is used to identify *Vibrio and campylobacter species* from other gram negative bacteria.

• **Method:**

- loopful of growth from a nutrient agar subculture had been mixed in a drop of sterile Distilled water on one end of a slide.
- On the other end of the slide; another loopful of growth had been mixed in a drop of peptone water
- each preparation had been covered with cover glass and examined microscopically

Using the 40x objective.

*All biochemical tests results were reported.

Antimicrobial sensitivity disk: -

Antibiotic susceptibility was performed by employing Kirby Bauer disk diffusion method in this technique, antibiotics discs are placed onto plates of Mueller Hinton agar in accordance with the guidelines of clinical and laboratory standards institute all gram positive and gram negative were tested against the following antibiotic disks: Doxycyclin(10ug), tetracycline(30ug), ciprofloxacin (5ug) and Cefotaxime(30ug), After incubation of plates at 37C for 24hours, diameters of zone of inhibition were measured. Bacteria classified as susceptible, intermediate and resistant strains according to the criteria of the clinical and laboratory standards institute [11].

Results:

This study was conducted in university of El-emam El-mahdi from august 2017 to November 2017.

A total of 70 patients who visited Kosti teaching hospital were recruited to this study; All patients or the patient's parents were agreed to participate in the study and sign a consent form. the prevalence of acute bacterial gastroenteritis was (25.7%). 7/70(10%) of patients were

children; 63/70(90%) were adult; 48/70 (76.2%) were male and 15/70 (23.8%) were female. the seventy (70) fecal sample were showed significant growth as showed in table (1). A fecal specimen was collected from each patient. Blood and mucus were uncommon in the feces 2.8%(2/70). Of the 70 patients, 10 % (7/70) were pediatric (≤14years), 45.7 % (32/70) were adult (15–30 years), and the other 28.6 % (20/70) were (31–45 years), 15.7 % (11/70) were elder (46-60), P-value= 0.001 as showed in table (2). Fever and vomiting were the main symptoms accompanying acute diarrhea. Up to 10 % (7/70) of patients stayed in hospital for 1–2 days. There was a significant difference in the duration of diarrhea across the four age groups in the study.

E.coli was the most frequent isolated organisms 58.6% (41/70) followed by *Paratyphi A and pseudomonas spps* 10% (7/70) for each, *citrobacter spps* 5.7% (5/70), *S.typhi and Shigella spps* 4.3% (3/70) for each, *S.typhimurum* and *V.cholerea spps* 2.9%(2/70) for each and campylobacter spps was the least frequent isolated 1.4%(1/70), as showed in table(3) .

Note that the isolated species of *E. coli, Pseudomonas, citrobacter* were enrolled as nonpathogenic in this study as is showed in table (4).

It has been noticed that *S. paratyphi A* was most frequency in age group (15-30 year) 5/7; while *V. cholerea spps* were exclusive on the eldest patient (46-60 year) 2/2 as it showed in table (5).

The antibiotic discs used were ciprofloxacin(5ug), Cefotaxime(30ug), Tetracycline(30ug), Doxycyclin (10ug).Among these, Cefotaxime was the most effective antibiotic(55.6%); followed by Ciprofloxacin (38.8%),Tetracycline (16.7%) and the least effective antibiotic was Doxycyclin (0%) which means all isolated organism were resistant to Doxycyclin (100%), as showed in table(6).

It has been noticed that the *S.typhi; S.paratyphi A* were 100% sensitive to Cefotaxime respectively; where *S.typhimurum* was 100% resistant to it .

Also *Shigella spps* were only sensitive to tetracycline 100% as it showed in table(10); on the other hand *V.cholerea* spps were only sensitive to ciprofloxacin 100% and intermediate to tetracycline.

Table (1): showed the positive and negative result of cultivation of the specimens:

Valid	Frequency	Percent
Growth	**70**	**100%**
No growth	**0**	**0%**
Total	**70**	**100%**

Table (2): showed the frequency of bacteria isolated in relation to the Age:

Age	Number of patients	Frequency of positive result	Percent
≤14	7	2	28.5%
15-30	32	8	25%
31-45	20	3	20%
46-60	11	5	45.4%
Total	70	18	25.7%

P value = 0.001

Table (3): showed the frequency of bacteria isolate:

Organism	Frequency	Percent
S.typhi	3	4.3%
S.paratyphi A	7	10.0%
S.typhimurum	2	2.9%
Shigella spps	3	4.3%
V.cholerae	2	2.9%
campylobacter spps	1	1.4%
E.coli	41	58.6%
pseudomonas spps	7	10.0%
citrobacter spps	4	5.7%
Total	70	100.0

Table (4): showed the pathogenic and nonpathogenic isolate:

Valid	Frequency	Percent
Pathogenic	18	25.7%
Non pathogenic	52	74.3%
Total	70	100%

Table (5): Showed the frequency of pathogenic bacteria in relation to the age:

Bacteria	Pediatric	15-30year	31-45year	46-60year	Total
S.typhi	1	2	0	0	3
S.paratyphi A	1	5	0	1	7
S.typhimurum	0	0	0	2	2
Shigella spps	0	1	2	0	3
v. cholera	0	0	0	2	2
Campylobacter spps	0	0	1	0	1
Total	2	8	3	5	18

Table (6): Showed the susceptibility testing result:

Antibiotic use		Frequency	Percent
Ciprofloxacin	Sensitive	7	38.9%
	Resistant	11	61.1%
	Intermediate	0	0%
Cefotaxime	Sensitive	10	55.6%
	Resistant	6	33.3%
	Intermediate	2	11.1%
Tetracycline	Sensitive	3	16.7%
	Resistant	13	72.2%
	Intermediate	2	11.1%
Doxycycline	Sensitive	0	0%
	Resistant	18	100%
	Intermediate	0	0%

DISCUSSION:

The present study of diarrheal diseases was carried out on patients who were suffering from Diarrhea at Kosti teaching Hospital. A total of 70 stool samples were collected and processed macroscopically and microbiologically. they were cultured in appropriate culture media, identification was made and antimicrobial susceptibility testing was carried out for appropriate selection of antibiotic.

In this study, out of 70 stool samples, 70 (100%) were culture positive and 0 (0%) were culture negative. All Culture were positive which may be because of commensal organisms were not avoided in the study.

In the study carried out by Okon et al[12]. in which out of 144 specimens, enteropathogens were found in 89 (61.8%) and 55 (38.2%) cases yielded negative results. Of the 89 enteropathogens detected 48 (53.9%) were bacterial pathogens which were different from the result

of current study. In the study carried out by Sawsan et al[13], five hundred samples of stool were collected from patients with diarrhea (infants and children under ten years of age) admitted to the pediatric and Maternity Hospital in Erbil City. No infectious agents were found in 75 (15%) of the samples which is different from the result of present study. In the study reported by Nair et al[14]. in Kolkata, India where 27.9% of the Diarrhea patients had no potential pathogen which is different from the result of present study.

In this study, among the 70 culture positive cases, 48 (68.6%) were specimens from males patients, 15 (21.4%) were from females and 7 (10%) were pediatric. The growth was found to be higher in specimens obtained from male patients than in female patients. This may be because sample number from male patients were higher in number than female patients The maximum number of culture positive samples (32) were observed in patients within the age group 15-30 years [P.value=0.001], out of which 22 (68.8%) were males and 10 (31.2%) were females. This may be because of this age-group has high possibility of contact with contaminated food and drinks, and soil due to their daily outdoor activities.

Similar study was carried out by Okon et al[12], over one year period from January to December, 2010, in which out of 144 samples, the sex distribution was 80 (55.5%) males and 64 (44.4%) female respectively. In the study carried out by Cajetan et al[15]. in Abuja, Nigeria in which 184 (45.5%) were male and 220 (54.5%) were female which is different from the result of the present study. The variation in different series might be due to the variation in place, time and season pattern of feeding and socio-economic status of the cases.

This study had found that among non pathogenic bacteria *E.coli* was the most abundant isolated organism (58.6%) followed by *pseudomonas spps* (10%) and least isolated organism was *citrobacter freundii* (5.7%).

E.coli was considered as a normal flora due to lack of the conclusive test Sorbitol- MacConkey agar (SMACC) to determine that if E.coli were O157 H7 or not .

Antimicrobial susceptibility testing is important for the correct prescription of antibiotics for the treatment of patients. Antibiotic sensitivity testing is an in vitro method for estimating the activity of drugs which will assist clinician in selecting an antimicrobial agent

effective in inhibiting the growth of an infecting microorganism in vivo. The aim of antimicrobial therapy is to choose a drug which is selectively active against the most likely pathogens and least likely to cause adverse effects or promote resistance. Hence it is necessary to determine the antibiotic susceptibility of organisms isolated from infected patients. In this study, the antibiotic discs used were Ciprofloxacin (5ug), Cefotaxime (30ug), Tetracycline (30ug), Doxycyclin (10ug), Among these, Cefotaxime (55.6 %) was the most effective antibiotic followed by Ciprofloxacin (39.8%), Tetracycline (16.7%), and the least effective antibiotic was Doxycyclin which is resistant (100%).

CONCLUSION:

This study revealed 25.7% prevalence of acute gastroenteritis in patients from all four age group ;the study was conducted in Kosti teaching hospital .

It has been noticed that the most pathogenic isolated organism was *S.paratyphi A* 7/18 (38.8%) and *S.typhi; Shigella spp* 3/18 (16.6%) for each; however Cefotaxime and ciprofloxacin are the most effective drugs against most of fecal isolate where the all organism were resistant to Doxycyclin (100%).

Limitation of the study:

The study was considered that the isolated *E.coli* as normal flora due to the lack of confirmatory test which is sorbitol macconkey agar to detected wiether *E.coli* is O157 H7 or not.

Also there was no serological test to detect the exact serotype of *V.cholerea*; *Shigella spp* and *campylobacter spp* .

Recommendation:

- We recommend that other studies should run in other part of the country.
- Routine stool culture should be done in order to determine the pathogenic organism and the suitable drug for it.
- Serological and conclusive test should be provided to determine wiether the isolated organism in this study were new strains or not especially the drug resistant ones.

- Antibiotic should prescribe carefully in order to avoid recurrencey and the risk of developing antibiotic resistance.

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