

Original article

The common aerobic bacteria causing diarrhea in children in Omdurman hospital-Sudan

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ABSTRACT

Background: Diarrheal disease is a major health problem throughout the world, and is responsible for high morbidity and mortality among children, especially in developing countries. **Objectives:** This study aimed to identify frequency of common aerobic bacteria causing diarrhea among children, by using stool culture and to determine the antibiotic susceptibility patterns of the isolated bacterial species. **Methods:** This was a descriptive cross-sectional study performed in Omdurman Pediatric hospital. It was carried out in the period from April- November 2022. It included children with diarrhea disease ranged between 2-5 years old attending Omdurman pediatric hospital. A total of 60 samples were collected from children aged 2 - 5 years old, the swabs from the stool were inoculated on Carry-Blair medium, and the swab was then cultured on Xylose Lysine Deoxycholate Agar (XLD) and deoxycholate citrate agar (DCA) media and incubated in 37 °C for 24 hours. **Results:** Result showed that, *Salmonella* species isolates were (3.3%), *Shigella* (16.7%), *Yersinia enterocolitica* (4.8%), and the other Coliform (45%). *E.coli* isolates representing (30.2%), were tested for sorbitol fermentation, for all isolates should negative findings. **Conclusions:** The study concluded that the pathogens frequently associated with diarrhea in children were *Shigella*, *Yersinia enterocolitica*, and *Salmonella* respectively.

Introduction

Diarrheal disease is a major problem throughout the world, responsible for high morbidity and mortality among children, especially in developing countries [1]. Diarrhea is the frequent passage of loose, watery, soft stool with or without abdominal bloating, pressure, and cramps commonly referred as gas. Diarrhea is also defined as an increased in stool mass, frequency or fluidity with or without vomiting. It is caused either by multiplication of bacteria in the intestine or from the effect of toxins.

Diarrhea and related complications can cause severe illness. The most significant cause of severe illness is loss of water and salts (electrolytes). In diarrhea, fluid passes out of the body before it can be absorbed in the intestines. When the ability to drink fluids fast enough to compensate for the water lost with diarrhea is impaired, dehydration can result. Most deaths from diarrhea occur in the young children, whose health may be put at risk from a moderate amount of dehydration [2].

Malnutrition modifies the risk of contracting diarrhea, these factors combine to facilitate the spread of enteropathogen. A part from well- described enteropathogen, such as *Shigella* spp, *Escherichia* spp, *Salmonella* spp, or enterotoxogenic *Escherichia coli* (ETEC), there are a number of other organisms from which diarrheal disease is controversial [3].

Most of the pathogenic organisms that cause diarrhoea and all the pathogens that are known to be major causes of diarrhoea are transmitted primarily or exclusively by the faecal–oral route [4].

Diarrhoea can be classified into five types: osmotic diarrhea, secretory diarrhea, inflammatory diarrhea, abnormal motility diarrhea and antibiotic-associated diarrhea [5].

The diagnosis of diarrhea includes the examination of faeces microscopically to visualise parasitic agents (*E.histolytica*, *Giardia lamblia*...etc.) and culturing of faeces, suspected food and vomitus on ordinary culture media or selective media, isolated organism is identified by biochemical tests.

There are three main types of therapy used in the case management of diarrhea: oral rehydration therapy, nutritional therapy, and drug therapy [6].

Prevention largely depends on sanitation (adequate disposal of sewage), clean food and safe water supply. Personal hygiene (washing hands after defecation) is a remarkably effective mean of preventing faecal-oral spread. The storage of food at room temperature must be avoided [7].

Diarrheal diseases kill two million children in developing countries each year. Diarrhea is very dangerous disease especially in children because it leads to dehydration and death after few days if untreated. Diarrhea is a leading cause of malnutrition in children under the age of five years.

As the rate of children's death by diarrheal diseases in our community is high, so identification of causative agents is needed.

The study aimed to determine the frequency of common aerobic bacteria causing diarrhea among children in Omdurman Pediatric Hospital from 2 to 5 years.

Material and methods

This was a descriptive cross-sectional study performed in Omdurman Pediatric hospital in the period from April 2022 to November 2022.

Study population

Children with diarrhea disease ranged between 2-5 years attending Omdurman pediatric hospital.

Sampling

Convenient non probability sampling was chosen to meet the objective of the study.

Inclusion criteria

Children with signs and symptoms of diarrhea were included in this study, it included only acute diarrhea with its clear definition in a time frame.

Exclusion criteria

Children with signs and symptoms of malaria, AIDS, measles, chronic diarrhea and malabsorption causes.were excluded from this study.

Sample size

A total of 60 stool samples were collected from children from 2 - 5 years old with diarrhea during the study period.

Collection of samples

Samples were collected randomly from children in clear, clean, dry, wide neck containers during the acute stage of diarrhea.

Transport media

The samples were transported in Carry-Blair medium.

Macroscopical examination

The appearance of the specimen was described regarding the color, consistency and its content of blood, pus, mucus or worms.

Microscopical examination

The wet preparation was observed for the presence of pus cell, RBCs, cyst, ova, trophozoite and bacterial cells.

Culture

Samples were inoculated on Xylose Lysine Deoxycholate agar (XLD) and Deoxycholate Citrate agar (DCA) media, and incubated aerobically at 37 °C for 24 hours.

Colonial morphology

Gram stain

The recovered colonies were stained with Gram stain. The smear was prepared by emulsifying small colonies with distilled water and left to air dry, fixed with heat, covered with crystal violet stain for 30-60 sec, after that, the stain was rapidly washed with tap water, covered with lugol's iodine for 30-60 sec, decolorized with acetone alcohol for few seconds, safranin as counter stain added for two minutes, washed with tap water, left to air dry. Microscopical assessment was done by using oil immersion

objective lens to observe bacterial cells' morphology, arrangement and Gram reaction.

Biochemical identification

Identification of Gram-positive bacteria

Catalase test

It has been used to identify staphylococci by using wooden stick, tested colony was immersed into test tube containing 2 ml of 3% hydrogen peroxide. Positive result was indicated by appearing of active bubbling in the test tube.

Coagulase test

It has been used to identify *S.aureus* by placing a drop of normal saline, emulsifying a colony of the tested organism in the drop, adding a drop of plasma to the suspension and mixing. Positive result indicated by clumping within 10 seconds, negative result indicated by no clumping within 10 seconds.

DNase test

It has been used to identify *S. aureus*, by using sterile loop, suspected colonies were inoculated under aseptic condition into DNA agar and incubated aerobically overnight at 37 °C. Positive test was indicated by clearance around the colonies within 5 minutes, negative result was indicated by no clearance around the colonies.

Bile esculin hydrolysis test

It was used to identify *enterococci*, based on ability of an organism to hydrolyze esculin. Using sterile straight loop, the organism was inoculated on sterile bile esculin agar and the inoculated media was incubated aerobically at 37°C for 24 hours. When the organism hydrolyzes the esculin it forms esculetin and dextrose, the esculetin reacts with the ferric citrate to form black color.

Identification of Gram-negative bacteria

Oxidase test

The test has been used to identify the bacteria which produce oxidase enzyme. A piece of filter paper was placed in a clean petri dish, then 2 drop of 1% freshly prepared oxidase reagent was added then by using a piece of wooden stick, the suspected colony was removed and smeared into the filter paper. A positive result was indicated by developing blue purple color within 10 sec, while negative result was indicated by no changing in color.

Kligler iron agar (KIA)

It is a differential medium and it is used to identify *enterobacteria*. Using a sterile straight loop, the tested organism was inoculated into the butt first, then by the same loop the slope was streaked in a zigzag pattern

and the inoculated medium was incubated aerobically at 37 °C for 24 hours. A yellow butt and red pink slope indicated the fermentation of glucose only, crack and bubbles in the media indicated gas production from fermentation, a yellow butt and yellow slope indicated fermentation of glucose and lactose, a red pink slope and butt indicated no fermentation of glucose nor lactose indicated hydrogen sulfide production.

Citrate utilization test

The test was used to identify *enterobacteria*. Sterile straight loop was used to inoculate the organism in 3 ml of sterile media and the inoculated media was incubated aerobically at 37 °C for 24 hours. The positive result was indicated by change of the indicator color from green to blue.

Urease test

The microorganism was inoculated on the slope surface of medium containing urea and it has been incubated aerobically at 37 °C for 4 -24 hours.

Indole test

It has been used to differentiate between the Gram-negative rod bacteria. The tested organism was inoculated in a tube containing 2 ml of sterile peptone water using sterile loop. The test tube was incubated aerobically at 37 °C for 24 hours, 0.5 ml of kovac's reagent was then added and the test tube was shaken gently and examined for a red ring on the surface within 20 minutes. A positive result was indicated by appearance of red ring on the surface of test tube.

Motility test

The organism was cultured in a semi-solid medium. The test was examined after overnight incubation at 37 °C, turbidity of medium and diffusing of organism on the surface of the agar around inoculum line indicated to a motile organism.

Sorbitol MacConkey Agar

It is a partially selective differential medium for the isolation of *E. coli* O157:H7 from stool samples.

Sensitivity test

By using disk diffusion method, a sterile loop has been used to isolate the microorganism and emulsify it in 3-4 ml of sterile physiological saline, the mix compared with the standard turbidity (0.5 McFarland), then inoculated to Muller-Hilton agar media, the antibiotic disc added, it was incubated for 24 hours at 37 °C and after that a clear zone around the colonies were read.

Data analysis

Collected data were analyzed by a computer system using statistical package for social science (SPSS)

program using the Chi square test and cross tabulation. Statistical significance was set at p-values < 0.05.

Ethical consideration

Participation in this study was completely voluntary to participate.

Results

A total of 60 samples were collected from children from 2 - 5 years old, the swabs from stool were inoculated in Carry-Blair medium, and the swabs were cultured on XLD and DCA media and incubated in 37 °C for 24 hours.

The identification steps showed that the *Salmonella* species isolates were (3.3%), *Shigella* (16.7%), *Yersinia enterocolitica* (4.8%), and the other *Coliform* (45%). The *E.coli* isolates (30.2%), were sorbitol fermentation negative.

Sensitivity test showed that *shigella* was positive to meropenem (60%), tetracycline (50%), ciproflucacin (50%), clindamycin (50%), ceftazidim (25%) and ampicillin (10%), meropenem (40%), tetracycline (50%), ciproflucacin (50%), clindamycin (30%), ceftazidim (50%) and ampicillin

(30%), meropenem (0%), tetracycline (0%), ciproflucacin (25%), clindamycin (20%), ceftazidim (25%) and ampicillin (60%).

Salmonella species were sensitive to meropenem (100%), tetracycline (100%), ciproflucacin (100%), clindamycin (0%), ceftazidim (50%) and ampicillin (0%), intermediate to meropenem (0%), tetracycline (0%), ciproflucacin (0%), clindamycin (0%), ceftazidim (50%) and ampicillin (0%) and resistant to meropenem (0%), tetracycline (0%), ciproflucacin (0%), clindamycin (100%), ceftazidim (0%) and ampicillin (100%).

Yersinia enterocolitica was sensitive to meropenem (100%), tetracycline (0%), ciproflucacin (100%), clindamycin (0%), ceftazidim (100%) and ampicillin (0%), intermediate to meropenem (0%), tetracycline (100%), ciproflucacin (0%), clindamycin (0%), ceftazidim (0%) and ampicillin (0%) and resistance to meropenem (0%), tetracycline (0%), ciproflucacin (0%), clindamycin (100%), ceftazidim (0%) and ampicillin (100%).

Figure 1. Age of patients

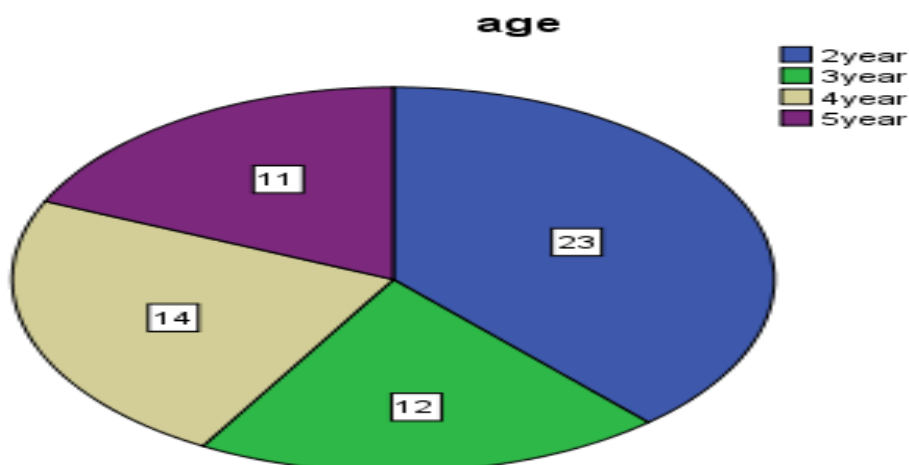


Figure 2. Gender of the children’s patients.

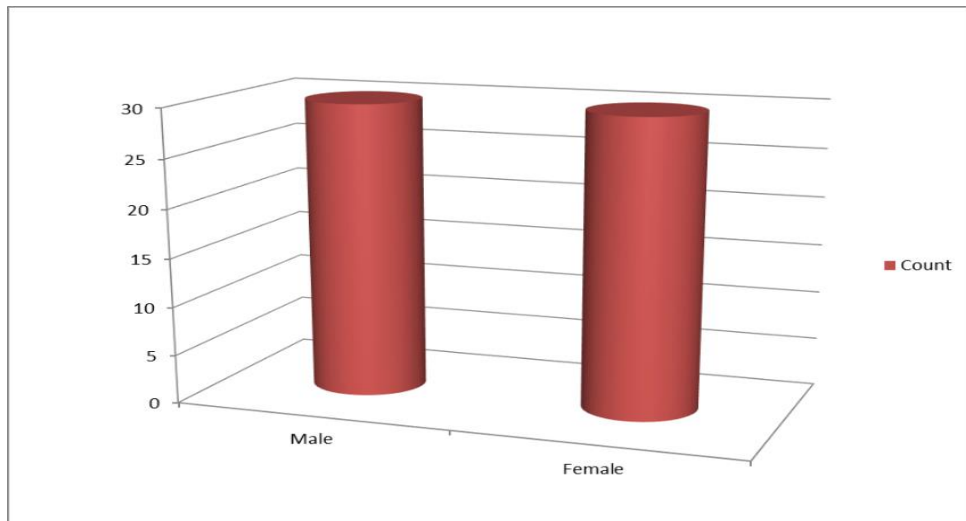


Figure 3. Macroscopical examination of the stool samples.

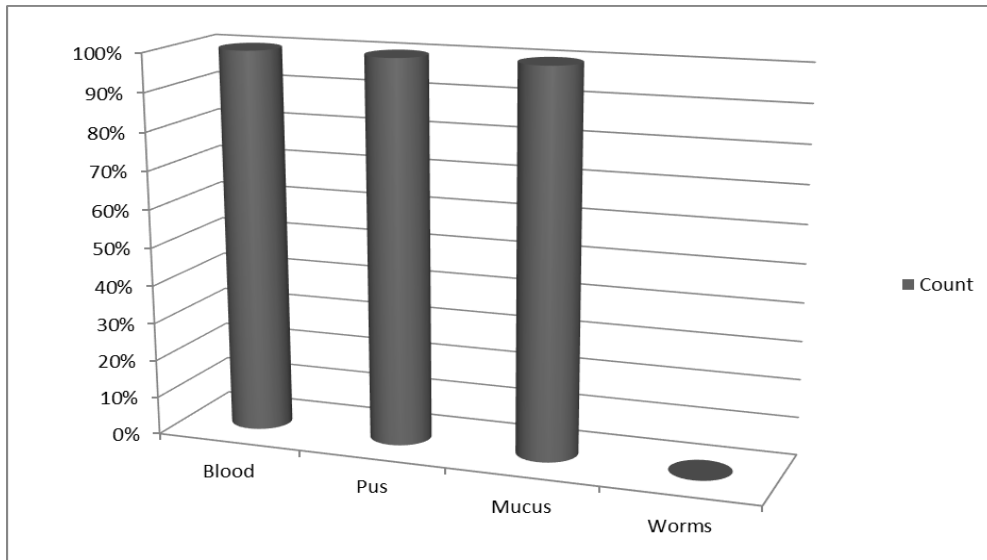
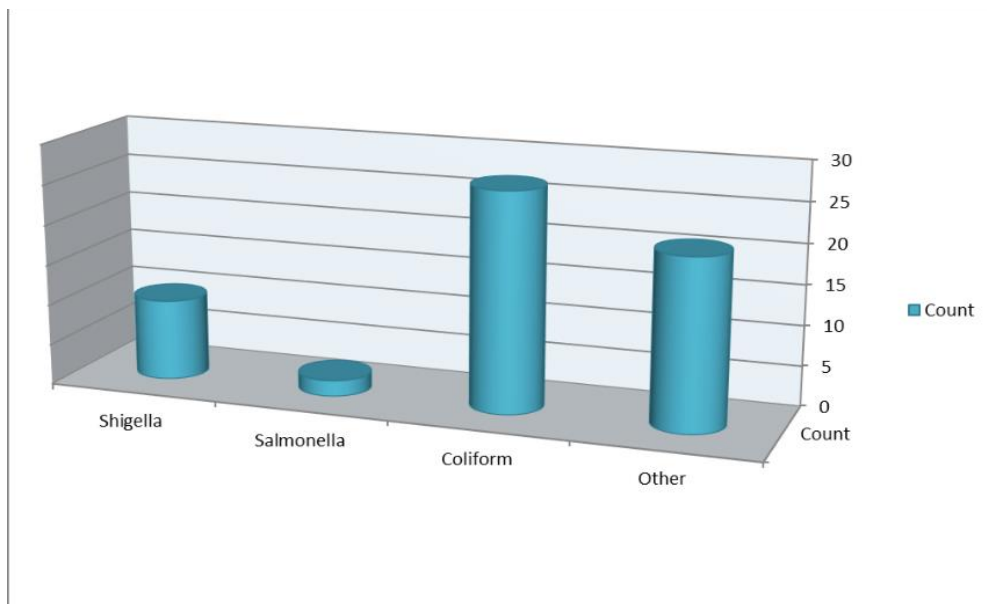


Figure 4. Common aerobic bacteria isolated from the patients



Discussion

In the early 1980s, diarrhea was the leading cause of child mortality, accounting for 4.6 million deaths annually worldwide, excluding China and Latin America [3].

A total of 60 samples were collected from children from 2 - 5 years old, the swabs from stool were inoculated in Carry-Blair medium, and the swab was cultured on XLD and DCA media and incubated in 37 °C for 24 hours.

The identification steps show that the *Salmonella* species isolates were (3.3%), *Shigella* (16.7%), *Yersinia enterocolitica* (4.8%), and the other *Coliform* (45%).

In the present study, *V. cholerae* strains were not found. The reason for this could be due to the low prevalence of this pathogen as shown in previous studies.

Existing interventions to prevent or treat diarrheal diseases have proven their efficacy in reducing mortality.

Use of antibiotics and intestinal antiseptics in the therapy of bacterial diarrhea. The use of antiemetics, antidiarrheics and spasmolytics is unnecessary and potentially risky, so that it is not recommended for children with AD [8].

Shigella species are the cause of approximately 10% of acute diarrhea in children aged 5 years or younger, but is also an important cause of diarrhea in older children and adults. It is the most common cause of dysentery (visible blood in the stool) in developing countries [9].

Salmonella infections usually resolve within 5–7 days and often do not require specific drug treatment. However, there are conditions where antimicrobial therapy using an appropriate agent is indicated, e.g. dysenteric presentation, infection in small infants, hosts with compromised immunity, etc. Globally, the strains are currently resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole [10].

Diarrheal diseases are one of the most common diseases in childhood. The most common cause is rotavirus. A key element in the approach of a child with diarrhea is determining their hydration status, which determines the fluid management [11].

Antibiotics should not be used to treat diarrhea in children unless there are specific indications for such use. Diarrheal episodes which last more than 14 days are associated with high mortality and severe malnutrition [12].

It mainly occurs in children until five years of age and particularly in neonates in the second half-year and children until the age of three years. Its primary causes are gastrointestinal infections, viral and bacterial, and more rarely alimentary intoxications and other factors [13].

Conclusion

The present study concluded that pathogens frequently associated from children with diarrhea in order of frequency were *Shigella* (16.7%), *Yersinia enterocolitica* (4.8%) and *Salmonella* (3.3%). The most active antibiotics against diarrhea in children were Meropenem, tetracycline, ciprofloxacin, ceftazidime and clindamycin. Ampicillin was not useful in treatment of diarrhea in children, because most organisms were resistant to it. The study recommended antibiotics and rehydration should be used in treatment of gastrointestinal tract infection.

Conflicts of interest :None.

Financial disclosure

None

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