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Frequency of Common Aerobic Bacterial Pathogens Causing Diarrhea Among Children Aged 2 to 5 Years in Sudan

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Abstract:

Diarrheal disease is a major problem throughout the world, and is responsible for high morbidity and mortality among children, especially in developing countries. This study aimed to determine the frequency of common aerobic bacterial pathogens associated with diarrhea in children aged 2 to 5 years in Sudan, using stool culture techniques, and to determine the antibiotic susceptibility patterns of the isolated bacteria species. This study was carried out in the period from February 2021 to April 2021. A total 100 Stool samples were collected from children with diarrheal symptoms and cultured to isolate aerobic bacterial pathogens. Antibiotic susceptibility testing was performed on all isolates. Ethical approval was obtained prior to data collection. Demographic and clinical information, including age, gender, clinical signs, symptoms, and microscopic findings, were recorded. Data analysis was conducted using SPSS software. The most frequently isolated pathogen was *Shigella species* (50%), followed by *Yersinia enterocolitica* (30%) and *Salmonella species* (20%). Although *Escherichia coli* was also isolated, all strains tested negative for sorbitol fermentation. Since no serological testing was performed, the isolated *E. coli* strains were presumed to be non-pathogenic. *Shigella* species were identified as the predominant bacterial cause of diarrhea among Sudanese children. The findings underscore the importance of regular microbial surveillance and antibiotic susceptibility testing to guide effective treatment strategies .

Keywords: WHO, AIDS, CAMP, XLD, DCA, TCBS, Diarrhea.

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Introduction:

Diarrheal disease is a major problem throughout the world, and is responsible for high morbidity and mortality among children, especially in developing countries.(1) Diarrhea is the frequent passage of loose, watery, soft stools with or without abdominal bloating, pressure, and cramps commonly referred as gas.(2) Diarrhea is also defined as an increased in stool mass, frequency or fluidity with or without vomiting. It causes either by multiplication of bacteria in the intestine or from the effect of performed toxins.(3) Diarrhea is commonly seen in tropical countries, although attacks are usually self –limiting and of short duration, the mortality can be high in the absence of good supportive treatment, especially in children and the malnourished.(4) Causes of diarrhea in endemic area include a wide variety of bacteria, viruses, protozoa and reaction of certain medication. Poor hygiene, water, and sanitation are common in communities with high level of diarrheal disease.(5) 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 organism from which diarrheal disease is controversial.(6) Most of the pathogenic organisms that cause diarrhea and all the pathogens that are known to be major causes of diarrhea are transmitted primarily or exclusively by the faeco–oral route.(7) Diarrhea can be classified into five types: osmotic diarrhea, secretary diarrhea, inflammatory diarrhea, abnormal motility diarrhea and antibiotic-associated diarrhea.(8) The diagnosis of diarrhea including the examination of faeces microscopically to see parasitic agents (E.histolytica, Giardia lamblia…etc) and culturing of faeces, suspected food and vomiting in ordinary culture media or a selective media, isolated organism is identified by biochemical tests.(9) Serological test such as latex agglutination and the demonstration of antibodies rising titre, neutralization test for detection of toxins and the molecular biology technique (PCR) can be used in the diagnosis the causes of diarrhea. There are three main types of therapy used in the case management of diarrhea: oral re-hydration therapy, nutritional therapy, and drug therapy.(10) Prevention largely depends on sanitation (adequate disposal of sewage), clean food and a safe water supply. Personal hygiene (washing hands after defecation) is a remarkably effective means of preventing faecal-oral spread. The storage of food at room temperature must be avoided.(11)

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Materials and methods:

Sampling:

Study design Prospective cross sectional study. Study area the study will be performed in Omdurman Pediatric hospital. Study period This study was carried out in the period from February 2021 to April 2021. Study population Children with diarrhea disease ranged between 2-5 years attending Omdurman pediatrics hospital. Inclusion criteria children with signs and symptoms of diarrhea were included in this study. Exclusion criteria children with signs and symptoms of malaria, AIDS, and measles were excluded in this study. Sample size all stool samples were collected from children with diarrhea during study period.

Methodology:

Collection of samples will be collected randomly from children in clear, clean, dry, wide neck containers during the acute stage of diarrhea**.** Culture samples will be inoculated in XLD and DCA media, and incubated aerobically at 37 °C for 24 hours. Sorbitol MacConkey Agar Is a partially selective differential medium for the isolation of *E. coli* O157:H7 from stool samples. Gram stain the recovered colonies will be stained with Gram stain. The smear will be 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 will rapidly be washed with tap water, covered with lugol‘s iodine for 30-60 sec, decolorized with acetone alcohol for few seconds, Safranin as counter stain will be added for two minutes, washed with tap water, left to air dry. Then microscopical will be done by using oil immersion objective to observe bacterial cells morphology, arrangement and Gram reaction.[12]

Biochemical identification:

Catalase test It will be used to identify staphylococci by using wooden stick, test colony will be immersed into test tube contain 2 ml of 3% hydrogen peroxide. Positive result will be indicated by appearing of active bubbling on the test tube. Coagulase test It will be used to identify S.aureus by placing drop of normal saline, emulsifing a colony of the test organism in the drop, adding drop of plasma to the suspension and mixing. Positive result will be indicated by Clumping within 10 seconds, negative result will be indicated by no clumping within 10 seconds. DNAse test It will be used to identify S. aureus, by using sterile loop, suspected colonies will be spot inoculated under a septic condition into DNA agar and after overnight aerobic incubation at 37 °C. Positive test will be indicated by the clearing around

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the colony within 5 minutes, negative result will be indicated by no clearing around the colony.[13] Oxidase test The test will be used to identify the bacteria which produce oxidase enzyme. A piece of filter paper will be placed in a clean Petri-dish, then 2 drop of 1% freshly prepared oxidase reagent will be added by using a piece of wooden stick, suspected colony will be removed and smeared into a filter paper. A positive result will be indicated by developing blue pure color within 10 sec, while negative result will be indicated by no changing in color. Kligler iron agar (KIA) It is a differentiation media and it will be used to identify of enterobacteria. Sterile straight lope will be used, a test organism will be stamped into the butt first, then the same lope will be used to streak the slope in zigzag pattern and the inoculated medium will be incubated aerobically at 37°C for 24 hours. Indole test It will be used to differentiate the Gram negative rod bacteria. The test organism will be inoculated in a tube containing 2 ml of sterile peptone water using sterile loop. the test tube will be incubated aerobically at 37°C for 24 hours, 0.5 ml of kovac's reagent will be added , then the test tube will be shaked gently and examined for a red surface layer within 20 minutes. A positive result will be indicated by appearing of red surface layer in the test tube. Motility test The organism will be cultured in semi-solid medium. The test will be examined after overnight incubation at 37°C, turbidity of medium and diffusing of organism on the surface of the agar around inoculums line will be indicated to a motile organism.**[14]**

Sensitivity test: By using Disk diffusion method, a sterile lope will be used to isolate the microorganism and emulsify it in 3-4 ml of sterile physiological saline, the mix will be comparing with the stander turbidity (0.5 McFarland), then it will be inoculated to Muller-Hilton agar media, the antibiotic disc will be added, it will be incubated for 24 hours at 37 °C and after that a clear zone around the colonies will be read.[15] Antibiotics selected Tetracycline, Clindamycin, Ceftaizidim, Ciprofluccacin, meropenem, Ampicillin.[15]

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 significant was set at p-values < 0.05.[16]

Result:

A total 100 samples were collected from children from 2 - 5 years old, the swabs from the up normal area in the stool were inoculated in Carry-Blair medium, and the swab culture in XLD and DCA media and incubated in 37°C for 24 hours. The most frequently isolated pathogen

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was *Shigella species* (50%), followed by *Yersinia enterocolitica* (30%) and *Salmonella species* (20%). Although *Escherichia coli* was also isolated, all strains tested negative for sorbitol fermentation. Since no serological testing was performed, the isolated *E. coli* strains were presumed to be non-pathogenic. *Shigella* species were identified as the predominant bacterial cause of diarrhea among Sudanese children aged 2 to 5 years. The findings underscore the importance of regular microbial surveillance and antibiotic susceptibility testing to guide effective treatment strategies. In sensitivity test *shigella* sensitive to Meropenem (60%), Tetracycline (50%), Ciprofluccacin (50%), Clindamycin (50%), Ceftaizidim (25%) and Ampicillin (10%), intermediate to Meropenem (40%), Tetracycline (50%), Ciprofluccacin (50%), Clindamycin (30%)· *Salmonella spices* sensitive to Meropenem (100%), Tetracycline (100%), Ciprofluccacin (100%), Clindamycin (0%), Ceftaizidim (50%) and Ampicillin (0%), intermediate to Meropenem (0%), Tetracycline (0%), Ciprofluccacin (0%), Clindamycin (0%), Ceftaizidim (50%) and Ampicillin (0%) and resistance to Meropenem (0%), Tetracycline (0%), Ciprofluccacin (0%), Clindamycin (100%), Ceftaizidim (0%) and Ampicillin (100%) . *Yersinia enterocolitica* sensitive to Meropenem (100%), Tetracycline (0%), Ciprofluccacin (100%), Clindamycin (0%), Ceftaizidim (100%) and Ampicillin (0%), intermediate to Meropenem (0%), Tetracycline (100%), Ciprofluccacin (0%), Clindamycin (0%), Ceftaizidim (0%) and Ampicillin (0%) and resistance to Meropenem (0%), Tetracycline (0%), Ciprofluccacin (0%), Clindamycin (100%), Ceftaizidim (0%) and Ampicillin (100%) .

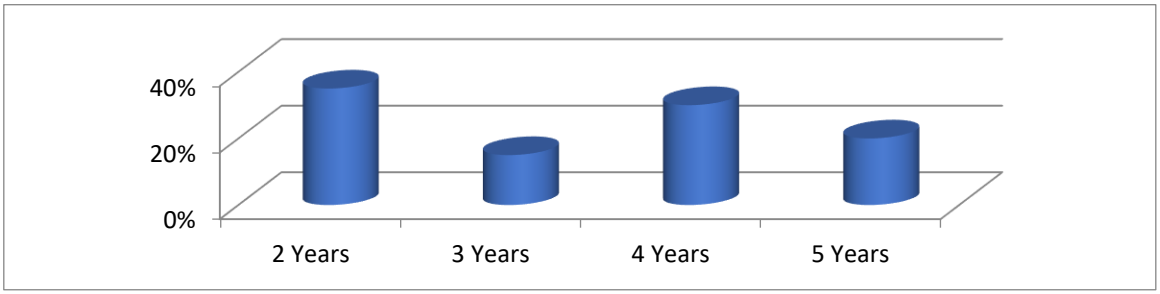

Figure-1: Age distribution of patient

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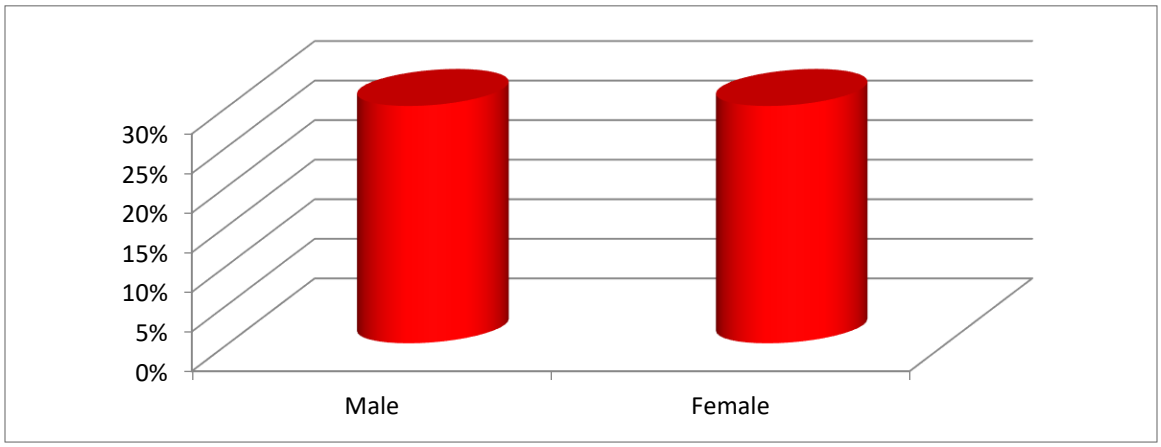

Figure-2: Gender of the children patients

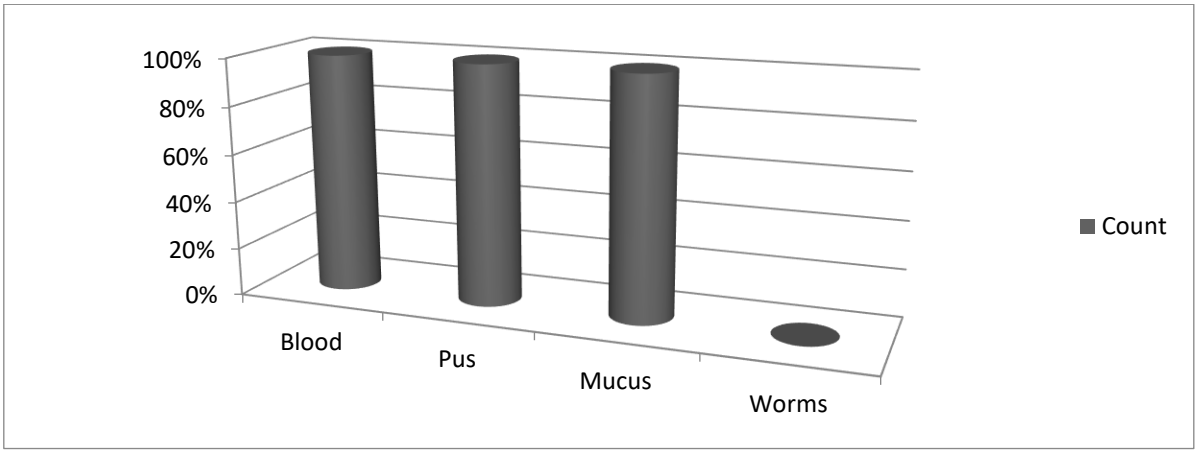

Figure-3: Macroscopical examination of the stool samples

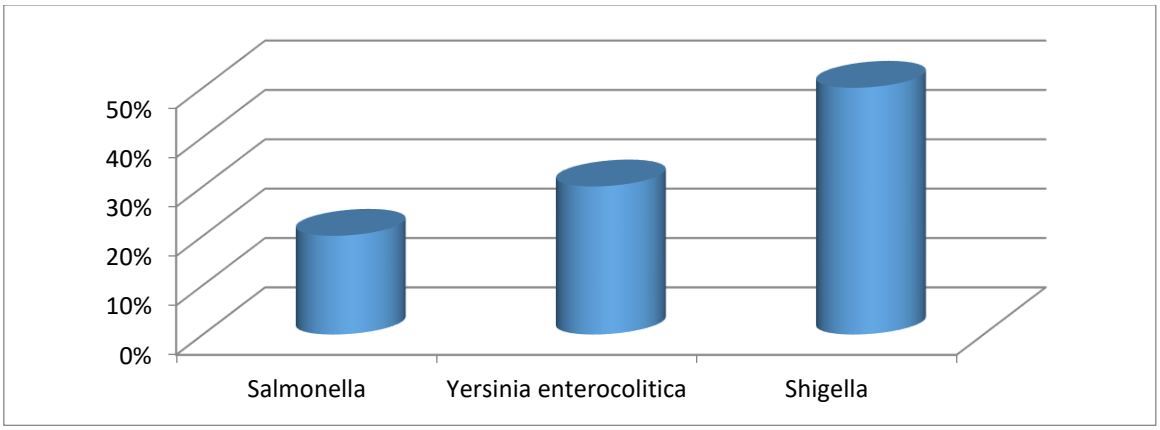

Figure-4: Common aerobic bacteria causative agent isolated of organism from children patients

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Discussion:

A total of 100 stool samples were collected from children from 2 - 5 years old, the swabs from the up normal area in the stool were inoculated in Carry-Blair medium, and the swab culture in XLD and DCA media and incubated in 37°C for 24 hours. The prevalence of isolated microorganism, Shigella species (50%), Yersinia enterocolitica (30%), and Salmonella species (20%). In the present study, V. cholerae strains were not found. The reason for this could be the low prevalence of this pathogen as shown in previous studies. In other study, Stewien, et al Shigella spp were the most prevalent pathogens isolated among children from 2-5 years old (53.1%).[17] This result was expected, because the frequency of shigellosis had increased with overcrowding, poor sanitation, and inadequate water supply. And this study agrees with our study. Tarig,et al in Omdurman Pediatric Hospital in 2010 and the result was showed Escherichia coli (37.5%), Staphylococcus aureus (25%), Enterobacter (12.5%), Citrobacter (9.4%), Shigella (6.6%), Salmonella typhimerium (3.1%), Providencia (3.1%), and Enterococcus faecalis (3.1%). And all E.coli strains, was isolated nonpathogenic strains. And this study agrees with our study. In the present study, the most active antibiotics against diarrhea in children were Meropenem, Tetracycline and Ciprofluccacin. Ampicillin was not useful in treating of diarrhea in children because resist to the most microorganisms.[18] In present study, the antimicrobials sensitivity was similar to what was found by Temu,et al who reported that all Shigella strains showed high resistance to Ampicillin.[19,20]

Conclusion:

A total 100 samples were collected from children from 2 - 5 years old, the swabs from the up normal area in the stool were inoculated in carry-Blair medium, and the swab culture in XLD and DCA media and incubated in 37°C for 24 hours. It can be concluded: The most frequently isolated pathogen was *Shigella species* (50%), followed by *Yersinia enterocolitica* (30%) and *Salmonella species* (20%). Although *Escherichia coli* was also isolated, all strains tested negative for sorbitol fermentation. Since no serological testing was performed, the isolated *E. coli* strains were presumed to be non-pathogenic. *Shigella* species were identified as the predominant bacterial cause of diarrhea among Sudanese children aged 2 to 5 years. The findings underscore the importance of regular microbial surveillance and antibiotic

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susceptibility testing to guide effective treatment strategies. The most active antibiotics against diarrhea in children were Meropenem, Tetracycline, Ciprofluccacin, Ceftaizidim and Clindamycin. Ampicillin was not useful in treating of diarrhea in children, because most organisms were resisting to it.

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