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In-Vitro Comparison of Antimicrobial Actions of Probiotics (*Lactobacilli* Species and *Saccharomyces boulardii*) with Standard Antibiotics for the Treatment of Diarrhea in Pediatric Population

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Abstract

Background and objectives: Irrational and repeated use of broad spectrum antibiotics for infectious diarrhea in children has resulted in their increased resistance along with several systemic toxic effects. Probiotics are also used in the management of infectious diarrhea as these are supposed to be favorable in promoting overall health benefits including stability of the intestinal flora. However, these agents are not used as an alternative to antibiotics as their exact bactericidal/bacteriostatic effects have not been evaluated on the basis of any clinical or *in-vitro* samples (Culture and Sensitivity test). Hence the aim of our study was to compare the culture and sensitivity patterns of standard antibiotics and two probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* used for the treatment of infectious diarrhea in children less than 5 years of age in a tertiary care hospital of Karachi, Pakistan. **Methodology:** This prospective quasi experimental study was conducted for a period of six months. After getting informed consent from parents/guardians, the stool samples were obtained from children of ages, 6 months to 5 years, presented with signs and symptoms of diarrhea in outpatient department (OPD) or being referred to microbiology department for stool C/S (culture and sensitivity). The sensitivity patterns of the cultured isolates were assessed for standard antibiotics according to the CLSI guidelines (2018), while the two probiotics (*Lactobacilli* and *Saccharomyces boulardii*) were evaluated by means of *Dried Modification method*. The data was

analyzed using statistical software SPSS version 19.0. **Results:** A total number of 325 stool samples were collected, out of which 152 samples were positive for pathogens *i.e. E. coli*, *Klebsiella* and *Salmonella typhi.* The sensitivity of combination of *Lactobacilli* for *E. coli*, *Klebsiella* and *Salmonella typhi* was 28.3%, 25% and 25% respectively. While, for *Saccharomyces boulardii* the sensitivity for *E. coli*, *Klebsiella* and *Salmonella typhi* was 37%, 32.1% and 25% respectively, which were slightly higher or equivalent to commonly prescribed antibiotics such as Amoxicillin/Clavulanic acid, Ceftazidime, Ampicillin, Cefotaxime, Cefuroxime, Ceftriaxone, Aztreonam, Trimethoprim/Sulfmethoxazole and Nalidixic acid. In comparison, the antibiotics which are not frequently used for infectious diarrhea showed higher sensitivities for all isolated organisms; as for *E. coli* the highest sensitivity was observed for Amikacin (96.7%), Gentamycin (95.7%) Imipenim (95.7%) and Piperacillin/Tazobactam (84.8%). Moreover, for *Klebsiella* the highest sensitivity was observed for Imipenim (98.2%), followed by Amikacin (94.6%), Piperacillin/Tazobactam (92.9%) and Gentamycin (89.3%). **Conclusion:** On *in-vitro* cultured samples, the two probiotics *Lactobacilli* and *Saccharomyces boulardii* have shown slightly higher or equivalent sensitivity in comparison to the most commonly prescribed antibiotics (Amoxicillin/Clavulanic acid, Ceftazidime, Ampicillin, Cefotaxime, Cefuroxime Ceftriaxone, Aztreonam, Trimethoprim/Sulfmethoxazole and Nalidixic acid). However, both probiotics displayed lower sensitivity in comparison to some broad spectrum but less commonly prescribed antibiotics (Amikacin, Gentamycin, Imipenim and Piperacillin/Tazobactam) in our clinical settings.

Keywords

Antibiotics, Probiotics, *Lactobacillus paracasei*/*Lactobacillus acidophilus*, *Saccharomyces boulardii*, *in Vitro*

1. Introduction

Diarrhea is one of the most common infectious diseases among humans globally [1]. It causes significant health risk particularly among pediatrics with most vulnerable age group affected is children less than 5 years of age, which is also, accounted for the high mortality rates in this age group [2]. ORS (oral rehydrating solution) has been a mainstay treatment in managing 90% of children with mild to moderate diarrhea [3]. Along with ORS zinc supplements are also found helpful in reducing the duration and volume of stools [4]. However, in pediatric infectious diarrhea antibiotics including Ampicillin, Cefexime, Ceftriaxone, Amikacin, Nalidixic acid and Ciprofloxacin are also required as their immunity is not sufficient to clear the infections [5]. Antibiotics on account of their toxicity, have limited use in pediatric age group [6] as well as their resistance is a rising threat to the human worldwide these days [7].

In Pakistan irrational and repeated use of antibiotics for infectious diarrhea in

children below 5 years of age has resulted in increased resistance and several toxicities of broad spectrum antibiotics, including alteration of the normal gut flora; which could lead to potential risk for future serious infections among children [8].

Evidence from the literature suggested the clinical use of probiotics for the treatment of bacterial gastroenteritis [9]. Probiotics are defined as the living microorganisms which when dispensed in the body at appropriate amounts prove to be favorable in promoting the health benefits, and at the same time it also increases the stability of the intestinal flora [10]. The probiotics used for the treatment of diarrhea in children are of bacterial or fungal origin. The bacterial strains of commonly used probiotics are *Bifidobacterium* and *Lactobacillus*, whereas, *Saccharomyces boulardii* is a yeast, which is a type of fungus [11]. The strains of *Lactobacilli* and *Saccharomyces boulardii* have proven its efficacy in reducing diarrhea [12].

Currently the probiotics are used along with antibiotics for infectious diarrhea but their direct bactericidal/bacteriostatic effects have not been tested or proved on the basis of any laboratory data or *in-vitro* culture and sensitivity tests. To the best of our knowledge, the current study is innovative across the globe. This study will help us to know the efficacy of probiotics *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* in comparison to the standard antibiotics against microbial organisms causing infectious diarrhea in children less than 5 years of age on the basis of stool culture and sensitivity.

2. Material and Methods

This prospective quasi experimental study was carried out in a pediatric unit and the microbiology laboratory of a tertiary care hospital, Dr. Ruth. K. M. Pfau Civil Hospital Karachi from December 2017 till May, 2018. The eligibility criteria for the recruitment in this clinical study were; children aged six months to five years, clinical diagnosis of acute diarrhea as per World Health Organization (WHO) criteria (*i.e.* having at least four liquid stools in the past 24 hours along with clinical signs and symptoms of dehydration on clinical examination). Children having systemic infection, malabsorption syndrome, severe acute malnutrition, blood in stool, have received antibiotics in last fourteen days or immediately require antibiotic for current infection were excluded.

The research was conducted following the ethical guidelines of Helenski declaration and Pakistan Medical and Research Council. Written informed consent was obtained from the parents or guardian prior to the recruitment of children with diagnosis of acute diarrhea. Moreover, the guardian or parents were comprehensively briefed about the research purpose and procedures involved. Importantly, the anonymity and confidentiality of the study participant's data was maintained throughout the research with no unauthorized person having access to the data. The research is approved by the institutional Ethical Review Com-

mittee (ERC), and the research was initiated after the approval was granted by the ERC.

Parents or guardian were informed and briefed to collect at least 5 gram of faeces of eligible study participants in a sterilized stool culture bottle. The data related to basic demographics was also collected for each eligible participants being recruited in this research.

Routine laboratory examination and stool culture were performed within four hours after collection of specimen. Initially, the stool samples were grossly examined for color and consistency. Later, the microscopic examinations of stool samples were performed to identify the presence of any cellular elements (*i.e.* red blood cells, white blood cells, pus cells), eggs, protozoa, cysts of parasites etc.

The stool culture was performed to identify the enteric pathogen causing acute diarrhea *i.e. E. coli* and *Klebsiella.* Standard procedures and steps (*i.e.* collecting in sterilized container and immediate processing within four hours) were followed for the stool culture process. At day 1 the stool sample was inoculated with *Salmonella Shigella* agar (SS agar), MacConkeys agar, and selenite enrichment broth being incubated aerobically at 37˚C overnight. Following day, subculture from selenite F broth on *Salmonella Shigella* agar was performed. At day 3, the stool cultures were re-examined for the presence of organisms *i.e. E. coli*, *Klebsiella and Salmonella.* The biochemical identification was later done for the confirmation of pathogenic strain by using; Simon citrate agar, SIM medium agar, Urea agar and TSI agar. The serological analysis was carried out by using *E.coli* and *Salmonella* and *Shigella* Antisera.

On each of the stool sample that showed positive growth of *E. coli*, *Klebsiella* and *Salmonella*, all standard antibiotics and two probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) *and Saccharomyces boulardii* were applied. The standard antibiotics applied were Amikacin, Amoxicillin, Gentamycin, Cefuroxime, Ceftazidime, Aztreonam, Ampicillin, Cefotaxime, Ciprofloxacin, Nalidixic Acid, Levofloxacin, Ceftriaxone, Imipenim, Trimethoprim-Sulfamethoxazole, Cefoperazone/Sulbactam and Piperacillin/Tazobactam, according to the Clinical Laboratory Science Institute (CLSI) 2018 guidelines [13]. The Zones of Inhibition (ZOI) of each antibiotic were measured. The calibrated vernier caliper was used to measure the diameters in millimeters (mm) of each antibiotic disc along with the clear surrounding clear area till the edges of the clear zone (showing no bacterial growth).

While the two probiotics, *Lactobacilli* and *Saccharomyces boulardii* were analyzed by dried modification method [14]. Standard procedure and protocol were followed where initially 2 µL of overnight culture was spotted on MRS agar. Later plates were dried at room temperature for half an hour and incubated an aerobically on 37˚C for 18 hours. Plates were overlaid with 10 ml of specific microorganism specific medium. Once the colonies were developed, the plates were again overlaid with 10 ml of soft microorganism specific medium and later laid for overnight culture of the target pathogenic strain. Following, 48 hours of in-

cubation the ZOI was measured and interpreted as (ZOI > 20 mm as sensitive and less than 10 mm as resistant).

3. Statistical Analysis

The data was analyzed using statistical software SPSS version 19.0. Initially, the data was validated twice for incorrect entries by checking with the study proforma. The categorical variables (*i.e.* gender, age categories and culture isolates) were presented as frequency/percentage while for their significance Chi square was applied.

4. Results

In the present research, stool sample from 325 children with confirmed diagnosis of acute diarrhea were collected and among those, 152 stool samples showed positive bacterial growth. Figure 1 gives details of the age categories (months) of 152 children with positive stool culture. Among all 152 positive culture isolates majority, 53 (34.9%) were found to be in the age group of 13 - 24 months, around eight percent in less than or equal to 12 months and 37 - 48 months of age categories. Figure 2 gives details of gender distribution. Majority, 57% were males while forty three 43% percent were females.

Table 1 shows the mean ZOI (mm) for individual organisms isolated from the stool samples of children with diarrhea for all antibiotics and the two probiotics *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii.*

Figures 3-5 show the sensitivity pattern of different antibiotics and Probiotics, *Lactobacilli* and *Saccharomyces boulardii* for different organisms isolated. For *E. coli* the highest sensitivity was observed for Amikacin (96.7%), Gentamycin

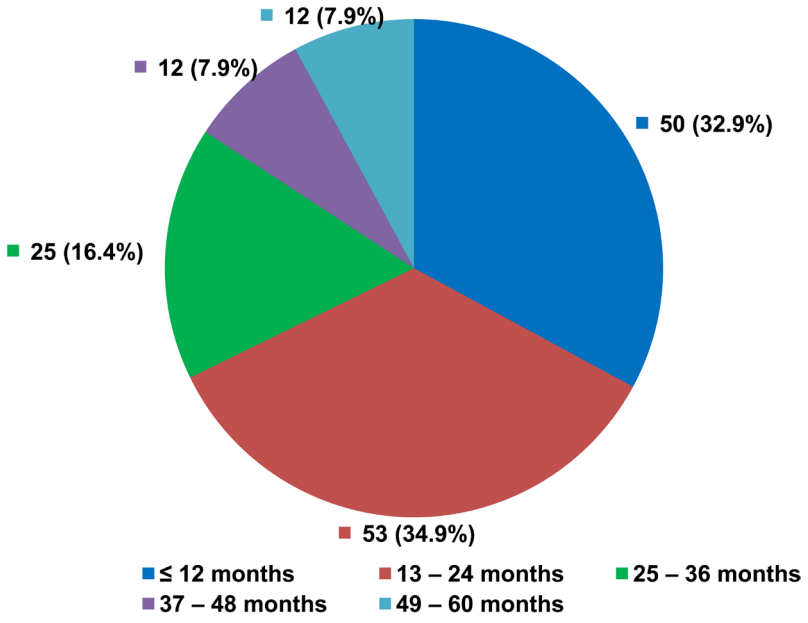


Figure 1. Age Distribution (n = 152).

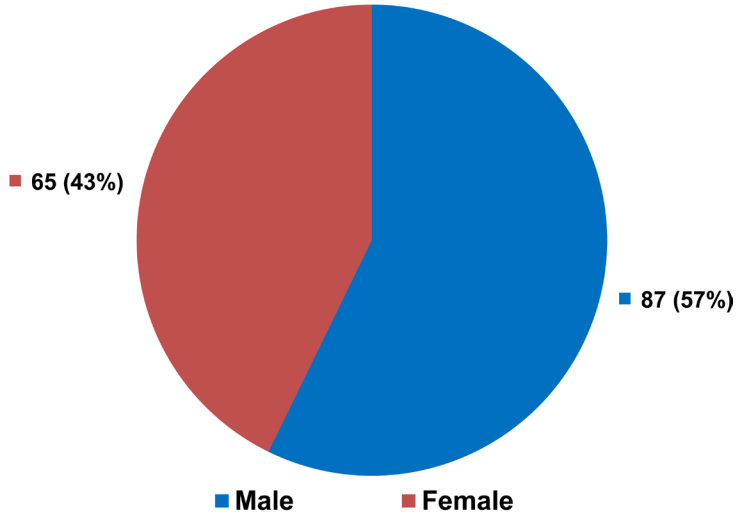
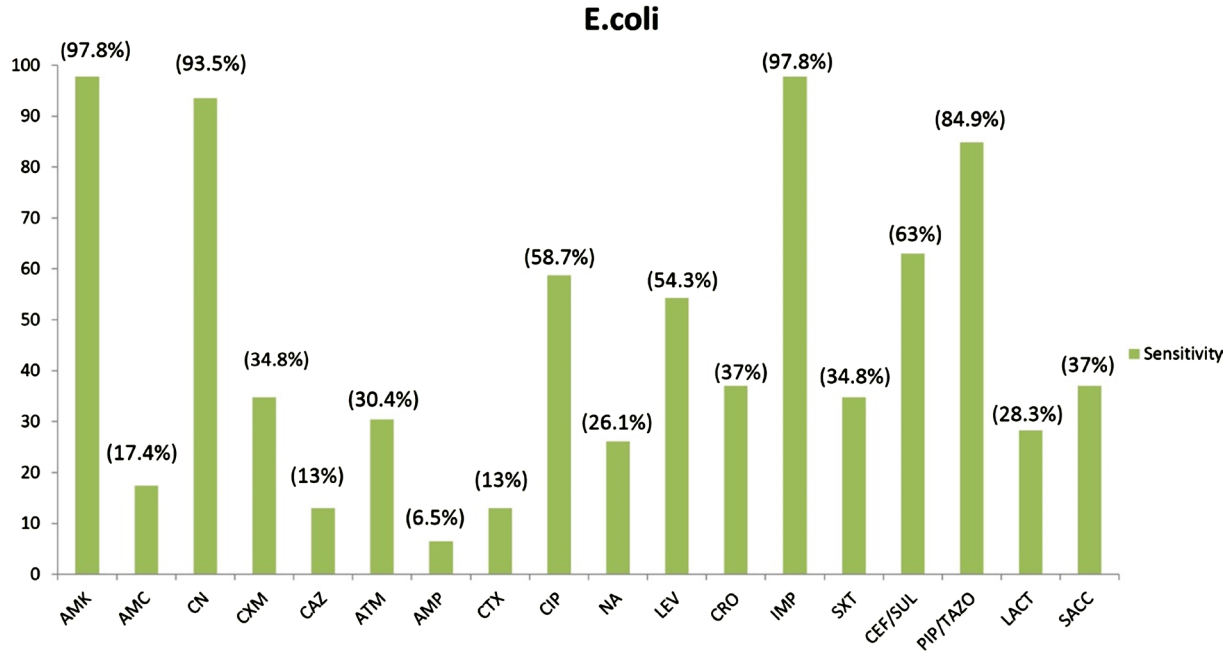


Figure 2. Gender Distribution.

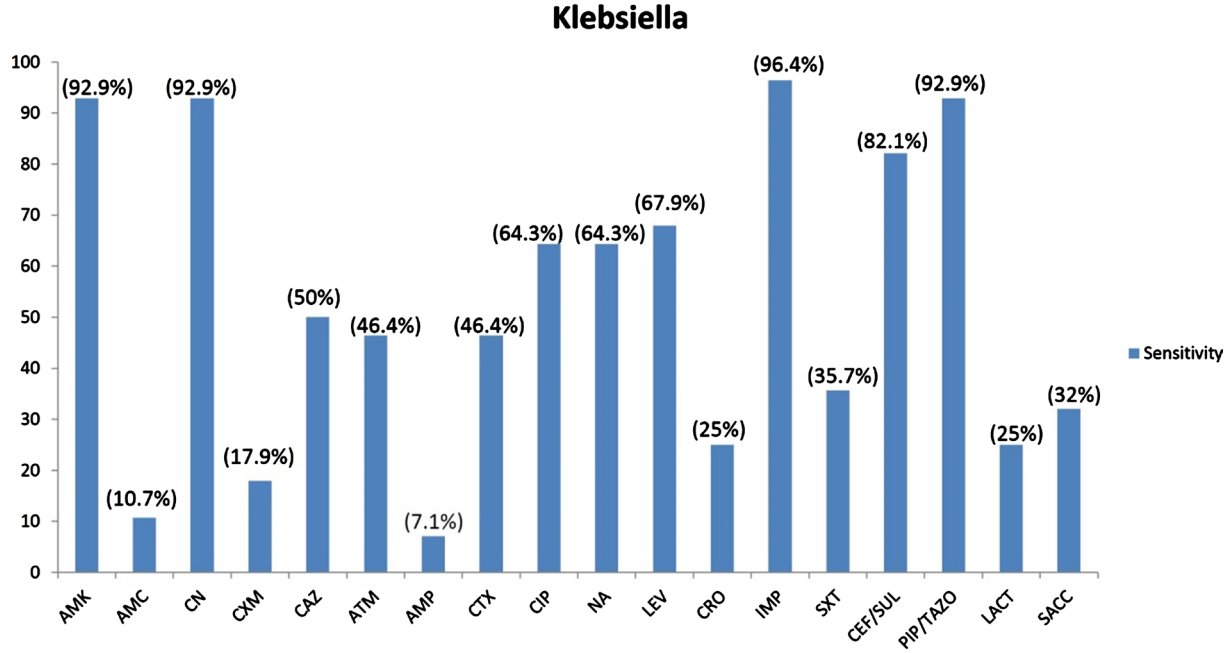
Table 1. Mean Zones of Inhibition (mm) of antibiotics and probiotics *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for all three Organisms isolated from the stool samples of children with diarrhea, total N = 152.

Antibiotics and Probiotics	*E. coli*	*Klebsiella*	*Salmonella typhi*
	Z.O.I (mm) Mean ± SD	Z.O.I (mm) Mean ± SD	Z.O.I (mm) Mean ± SD
Amikacin	18.78 ± 1.90	18.75 ± 1.73	18.25 ± 0.96
Amoxicillin	12.18 ± 3.59	11.64 ± 3.22	11.75 ± 0.96
Gentamycin	16.48 ± 1.76	16.50 ± 1.95	17.00 ± 1.83
Cefuroxime	14.64 ± 4.07	13.46 ± 3.81	13.50 ± 4.51
Ceftazidime	16.60 ± 3.49	18.00 ± 3.76	15.75 ± 1.50
Aztreonam	17.43 ± 3.91	18.25 ± 3.79	16.50 ± 3.42
Ampicillin	11.84 ± 1.82	12.27 ± 1.86	12.50 ± 0.58
Cefotaxime	21.18 ± 3.09	23.27 ± 4.03	19.50 ± 0.58
Ciprofloxacin	18.62 ± 4.79	19.14 ± 4.94	23.25 ± 0.50
Nalidixic Acid	14.55 ± 4.57	16.20 ± 4.58	13.25 ± 4.57
Levofloxacin	15.49 ± 3.90	15.86 ± 3.87	18.00 ± 1.16
Ceftriaxone	20.67 ± 4.26	20.13 ± 4.20	19.00 ± 3.37
Imipenim	23.57 ± 1.74	23.86 ± 1.59	23.25 ± 4.27
Trimethoprim-Sulfmethoxazole	11.16 ± 4.74	11.16 ± 5.27	10.25 ± 5.32
Cefoperazone/Sulbactam	19.03 ± 4.79	20.25 ± 4.38	19.25 ± 5.56
Piperacillin/Tazobactam	21.62 ± 2.90	21.71 ± 2.43	22.25 ± 2.06
Saccharomyces boulardii	11.58 ± 4.23	10.64 ± 3.65	9.25 ± 1.71
Lactobacilli (*Lactobacillus paracasei*/*Lactobacillus acidophilus*)	9.86 ± 3.24	9.24 ± 3.	9.25 ± 1.71



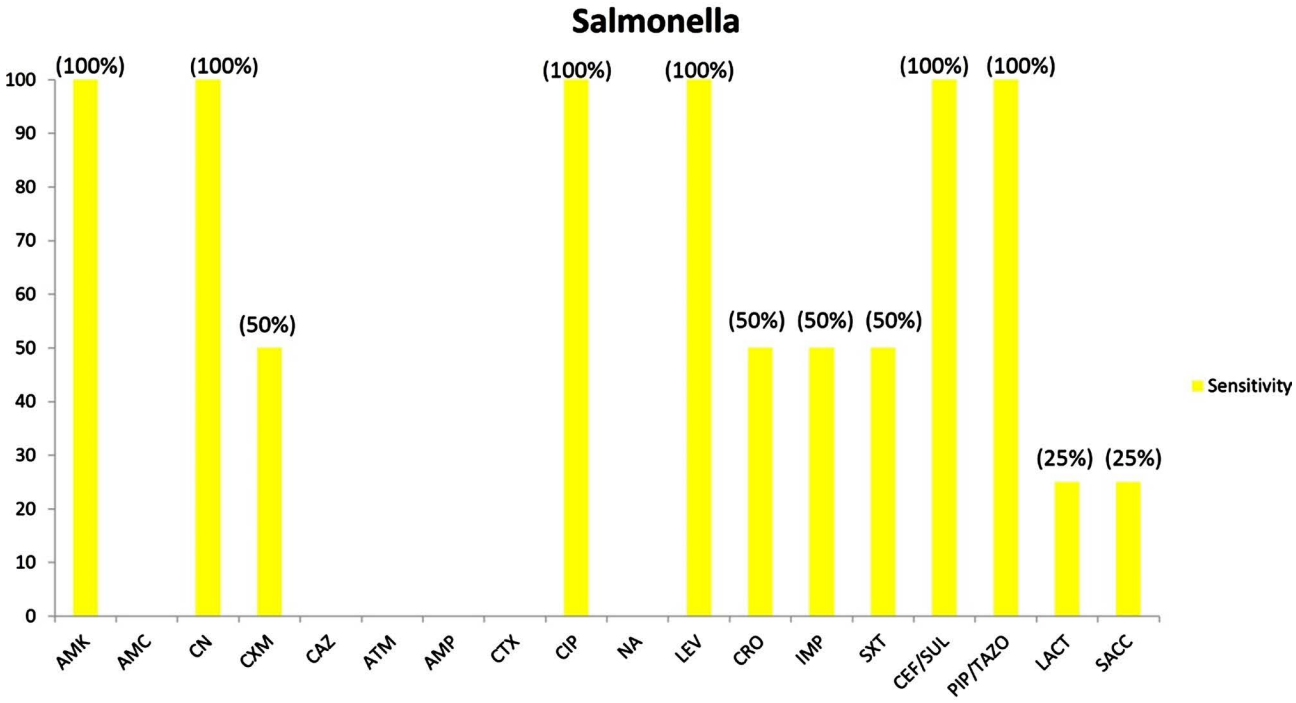
AMK; Amikacin, AMC; Amoxicillin-clavulanic acid, CN; Gentamycin, CXM; Cefuroxime, CAZ; Ceftazidime, ATM; Aztreonam, AMP; ampicillin, CTX; cefotaxime, CIP; Ciprofloxacin, NA; Nalidixic acid, LEV; Levofloxacin, CRO; Ceftriaxone, IMP; Imipenem, SXT; Trimethoprim-Sulfamethoxazole, CEF/SUL; Cefoperazone/Sulbactam, PIP/TAZO; Piperacillin/tazobactam, LACT; *Lactobacillus paracasei*/*Lactobacillus acidophilus*, SACC; *Saccharomyces boulardii.*

Figure 3. Sensitivity patterns of Antibiotics and Probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for *E. coli* (n = 92).



AMK; Amikacin, AMC; Amoxicillin-clavulanic acid, CN; Gentamycin, CXM; Cefuroxime, CAZ; Ceftazidime, ATM; Aztreonam, AMP; ampicillin, CTX; cefotaxime, CIP; Ciprofloxacin, NA; Nalidixic acid, LEV; Levofloxacin, CRO; Ceftriaxone, IMP; Imipenem, SXT; Trimethoprim-Sulfamethoxazole, CEF/SUL; Cefoperazone/Sulbactam, PIP/TAZO; Piperacillin/tazobactam, LACT; *Lactobacillus paracasei*/*Lactobacillus acidophilus*, SACC; *Saccharomyces boulardii.*

Figure 4. Sensitivity patterns of Antibiotics and Probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for *Klebsiella* (n = 56).



AMK; Amikacin, AMC; Amoxicillin-clavulanic acid, CN; Gentamycin, CXM; Cefuroxime, CAZ; Ceftazidime, ATM; Aztreonam, AMP; ampicillin, CTX; cefotaxime, CIP; Ciprofloxacin, NA; Nalidixic acid, LEV; Levofloxacin, CRO; Ceftriaxone, IMP; Imipenem, SXT; Trimethoprim-Sulfamethoxazole, CEF/SUL; Cefoperazone/Sulbactam, PIP/TAZO; Piperacillin/tazobactam, LACT; *Lactobacillus paracasei*/*Lactobacillus acidophilus*, SACC; *Saccharomyces boulardii.*

Figure 5. Sensitivity patterns of Antibiotics and Probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* for *Salmonella typhi* (n = 4)

(95.7%) Imipenim (95.7%) and Piperacillin/Tazobactam (84.8%). While, the other antibiotics which showed lower sensitivity are Amoxicillin/Clavulanic acid (17.4%), Ceftazidime (13%), Ampicillin (6.5%), Cefotaxime (13%) and Nalidixic acid (26.1%). Moreover, for *Klebsiella* the highest sensitivity was observed for Imipenim (98.2%), Amikacin (94.6%), Piperacillin/Tazobactam (92.9%) and Gentamycin (89.3%). Although, lower sensitivity is observed for Amoxicillin/Clavulanic acid (10.7%), Cefuroxime (17.9%), Ampicillin (7.1%) and Ceftriaxone (25%), for *Salmonella typhi* the highest sensitivity (*i.e.* 100%) was observed for Amikacin, Gentamycin, Ciprofloxacin, Levofloxacin, Cefoperazone/Sulbactam and Piperacillin/Tazobactam. Whereas, some of the antibiotics such as Cefuroxime, Ceftriaxone, Imipenim, Trimethoprim/Sulfmethoxazole revealed moderate sensitivity (*i.e.* 50%). Few of the antibiotics had shown no sensitivity. However, for *Lactobacilli* the sensitivity for *E. coli*, *Klebsiella* and *Salmonella typhi* were 28.3%, 25% and 25% respectively. While for the second probiotic, *Saccharomyces boulardii* the sensitivity for *E. coli*, *Klebsiella* and *Salmonella typhi* were 37%, 32.1% and 25% respectively.

5. Discussion

Though, different antibiotics have been used for the treatment of bacterial diarrhea, but considering growing resistance of antibiotics and harmful effects, [7]

researchers are more focused towards exploring the alternative means of treatment. Probiotics were first explored in 1907 by a Russian scientist Ellie Metchnikoff who linked the wellbeing and longevity of Bulgarian workers with the substantial ingestion of yogurt which contained large amounts of *Lactobacillus* species [15]. The other probiotic *Saccharomyces boulardii* was discovered in 1920 by a French microbiologist Henri Boulard, during a visit to Indochina, where he found that some people did not develop infectious diarrhea during the outbreak because they were already consuming a special type of tea made up of the outer skin of the tropical fruits lychee and mangosteens [9]. Generally, probiotics are preferred for the treatment of acute diarrhea by clinicians [8] and have been hypothesized in promoting the health benefits including the stability of the intestinal flora [16].

The possible mechanisms of action of *Saccharomyces boulardii* comprises, *in vivo* antimicrobial activity, immune system activation, antitoxin activity which helps to reduce the enterotoxin, enhances the enzymatic activity and favors the absorption and nutrition [17]. Locally, it has shown to secrete a heat-labile factor which decreases bacterial adherence [18]. One of the previous studies also revealed the immunomodulating effects on the intestine of the rats orally treated with *Saccharomyces boulardii*, attributed to increase the levels of secretory IgA and components of crypt cells of the small intestine [19]. Moreover, the probable mechanism of action of *Lactobacillus* species is to increase the mucin expression in human intestinal epithelial cells which blocks the invasion and adherence of pathogenic *E. coli* [20]. Additionally it is able to inhibit tumor necrosis factor (TNF) mediated inflammation and apoptosis in intestinal epithelial cells [21]. It has demonstrated mitogenic effects and has enhanced regeneration of mucosal lining [22]. However their direct antimicrobial effects are largely unknown due to lack of scientific evidence on the basis of *in-vitro* or culture and sensitivity tests. Hence their role as alternatives to antibiotics in bacterial gastroenteritis is not very much convincing as a single agent and used along with antibiotics.

The current study was aimed to evaluate the direct antimicrobial effects of probiotics on the basis of sensitivity testing of cultured stool samples of pediatric patients with diarrhea. This is evident from **Table 1** that probiotics, *Lactobacilli and Saccharomyces boulardii* had shown overall low zone of inhibitions for all organisms in comparison to majority of antibiotics. However, the zones of inhibition of *Saccharomyces boulardii for E. coli* were equivalent to trimethoprim/sulfamethoxazole and ampicillin, 11.16± 4.74 mm and 11.84 ± 1.82 mm respectively. No parallel or comparable data is available reporting the ZOIs for probiotics as our study is the first to assess these on isolated cultured samples of stool in pediatric patients with infectious diarrhea. The results of our study highlighted that the sensitivity of *Lactobacilli* for *E. coli*, was 28.3%, which is higher than majority of the commonly prescribed antibiotics including Amoxicillin/Clavulanic acid (17.4%), Ceftazidime (13%), Ampicillin (6.5%), Cefotaxime

(13%) and Nalidixic acid (26.1%). Similarly, for *Klebsiella* the sensitivity of *Lactobacilli* was 25% and was slightly better in comparison to commonly prescribed antibiotics including Amoxicillin/Clavulanic acid (10.7%), Cefuroxime (17.9%), Ampicillin (7.1%) and was equivalent in comparison to Ceftriaxone (25%).

Whereas, the sensitivity of *Saccharomyces boulardii* for *E. coli*, *was* 37%, slightly higher in contrast to most commonly prescribed antibiotics used for pediatric diarrhea including, Amoxicillin/Clavulanic acid (17.4%), Ceftazidime (13%), Ampicillin (6.5%), Cefotaxime (13%), Nalidixic acid (26.1%), and approximately equivalent to Cefuroxime (34.8%), Aztreonam (30.4%), Trimethoprim/Sulfmethoxazole (34.8%) and Ceftriaxone (37%). The sensitivity of *Saccharomyces boulardii* for *Klebsiella* was displayed to be 32.1%, better than the widely prescribed antibiotics, Amoxicillin/Clavulanic acid (10.7%), Cefuroxime (17.9%) and Ceftriaxone (25%). The lower sensitivity of commonly prescribed antibiotics for *E. coli* and *Klebsiella* in our study revealed that these antibiotics are prescribed irrationally and often misused because of their over the counter availability [23]. However the sensitivity of the two probiotics, *Lactobacilli* (25%) and *Saccharomyces boulardii* (25%) were lower as compared to all antibiotics in case of *Salmonella typhi.*

Furthermore our study also highlighted that two tested probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* had lower sensitivity against all of the three organisms isolated in our stool samples, when compared to more effective antibiotics which are not prescribed routinely for the treatment of infectious diarrhea in adults as well as in pediatrics. Overall for *E. coli*, the highest sensitivity was shown for Amikacin (96.7%), followed by Gentamycin (95.7%) Imipenim (95.7%) and Piperacillin/Tazobactam (84.8%). For *Klebsiella* the highest sensitivity was observed for Imipenim (98.2%), after that Amikacin (94.6%), Piperacillin/Tazobactam (92.9%) and Gentamycin (89.3). Nevertheless the use of majority of the above mentioned antibiotics is limited because of potential toxicities such as nephrotoxicity, ototoxicity, neurotoxicity and elevated levels of sodium, potassium and magnesium. Hence, these antibiotics should not be considered as first-line agents in the treatment of diarrhea in children and should be reserved as a final resort for serious and life-threatening infections. WHO has also warned that frequent use of these highly sensitive antibiotics may result in rise of the resistance of many of the gram negative organisms including *E. coli*, *Klebsiella and Salmonella typhi* [24] [25].

Although several studies have documented the beneficial effects of probiotics in diarrhea but their outcome was on the basis of clinical follow up of the patients with improvement or decrease in the duration of diarrhea. However the magnitude of therapeutic effects of probiotics was neither assessed nor compared with antibiotics, hence no related studies are available.

Besides, a couple of studies revealed that probiotics have become extensively popular and have rapidly achieved high level of use in Europe and Asia for the

cure of diarrhea [26] [27] [28]. The results of a meta-analysis, reported that *Saccharomyces boulardii* significantly reduced the duration of diarrhea (mean difference, −19.7 hours; 95% confidence interval, −26.05 to −13.34), stool frequency on day 2 (mean difference, −0.74; 95% confidence interval, −1.38 to −0.10) and day 3 (mean difference, −1.24; 95% confidence interval, −2.13 to −0.35), the risk for diarrhea on day 3 (risk ratio, 0.41; 95% confidence interval, 0.27 to 0.60) and day 4 (risk ratio, 0.38; 95% confidence interval, 0.24 to 0.59) after intervention compared with control [29].

The National Institute for Health and Clinical Excellence established in England also suggested the use of probiotics along with ORS for the treatment of acute diarrhea in children. The evidence-based clinical practice guidelines based on systematic reviews of randomized controlled trials displayed that *Lactobacilli* and *Saccharomyces boulardii*, were the efficacious agents in reducing the duration of diarrhea by one day [30] [31].

To the best of our knowledge the current study is innovative as for the first time, antimicrobial activity of the two most commonly prescribed probiotics, *Lactobacilli* and *Saccharomyces boulardii* were evaluated and compared with the standard antibiotics used against infectious diarrhea in children on the basis of stool culture and sensitivity. Furthermore the results of the current study also provided the scientific evidence of direct antimicrobial effects of probiotics which is an addition to their already described mechanisms of action. Even though in current study *in-vitro* testing of both probiotics clearly revealed that overall their sensitivities against organisms causing diarrhea were on lower side and not remarkably better in comparison to the majority of the standard antibiotics for infectious diarrhea in our pediatric clinical set up.

6. Conclusions

The results of the present study highlighted that both probiotics, *Lactobacilli* (*Lactobacillus paracasei*/*Lactobacillus acidophilus*) and *Saccharomyces boulardii* possess direct antimicrobial or bactericidal action but have overall low sensitivities against microbial organisms causing infectious diarrhea in our clinical set-up. Although both probiotics were found to have either equivalent or slightly higher sensitivities when compared to most commonly prescribed antibiotics (Amoxicillin/Clavulanic acid, Ceftazidime, Ampicillin, Cefotaxime, Cefuroxime, Ceftriaxone Nalidixic acid and Trimethoprim/Sulfmethoxazole). On the contrary, both of the probiotics had lower sensitivities in comparison to more effective and less commonly prescribed antibiotics (Amikacin, Gentamycin, Imipenim and Piperacillin/Tazobactam). Therefore, probiotics cannot be considered as sole agents or alternative treatments to antibiotics and hence antibiotics remain the mainstay of treatment for pediatric infectious diarrhea in our clinical set-ups.

However, the results of current study should be further validated by other multicenter studies conducted on diverse population.

7. Limitations of the Study

The study had certain limitations. Firstly, the study was conducted at only one hospital, where majority of patients are with low socio economic class and low education visits. Secondly, limited sample size and selection of only study site had restricted the generalization and external validity of the study findings. Thirdly, as the study was *in-vitro* where the laboratory analysis was undertaken to compare the sensitivity of antibiotics with probiotics with no follow-up involved.

Future Recommendations

Further similar experimental studies should be carried out on a large sample size in various clinical settings in order to validate the results of current study. Probiotics other than *Lactobacilli* and *Saccharomyces boulardii* should also be assessed and compared with antibiotics and with other probiotics used in the management of pediatric diarrhea.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Okitsu-Negishi, S., Nguyen, T.A., Phan, T.G. and Ushijima, H. (2004) Molecular Epidemiology of Viral Gastroenteritis in Asia. *Pediatrics International*, **46**, 245-252. https://doi.org/10.1046/j.1442-200x.2004.01896.x

[2] Jones, S. (2003) A Clinical Pathway for Pediatric Gastroenteritis. *Gastroenterology Nursing*, **26**, 7-18. https://doi.org/10.1097/00001610-200301000-00003

[3] Mattila, L., Peltola, H., Siitonen, A., Kyrönseppä, H., Simula, I. and Kataja, M. (1993) Short-Term Treatment of Traveler's Diarrhea with Norfloxacin: A Double-Blind, Placebo-Controlled Study during Two Seasons. *Clinical Infectious Diseases*, **17**, 779-782. https://doi.org/10.1093/clinids/17.4.779

[4] Cruchet, S., Furnes, R., Maruy, A., Hebel, E., Palacios, J., Medina, F. and Xóchihua, L. (2015) The Use of Probiotics in Pediatric Gastroenterology: A Review of the Literature and Recommendations by Latin-American Experts. *Pediatric Drugs*, **17**, 199-216. https://doi.org/10.1007/s40272-015-0124-6

[5] Choi, S.H., Kim, E.Y. and Kim, Y.J. (2013) Systemic Use of Fluoroquinolone in Children. *Korean Journal of Pediatrics*, **56**, 196-201. https://doi.org/10.3345/kjp.2013.56.5.196

[6] Hoffman, F.A., Heimbach, J.T., Sanders, M.E. and Hibberd, P.L. (2008) Executive Summary: Scientific and Regulatory Challenges of Development of Probiotics as Foods and Drugs. *Clinical Infectious Diseases*, **46**, S53-S57. https://doi.org/10.1086/523342

[7] Saeed, A., Abd, H. and Sandstrom, G. (2015) Microbial Aetiology of Acute Diarrhoea in Children under Five Years of Age in Khartoum, Sudan. *Journal of Medical Microbiology*, **64**, 432-437. https://doi.org/10.1099/jmm.0.000043

[8] Huff, B.A. (2004) Caveat Emptor. Probiotics Might Not Be What They Seem. *Canadian Family Physician*, **50**, 583-587.

[9] McFarland, L.V. (2009) Evidence-Based Review of Probiotics for Antibiotic-Associated Diarrhea and *Clostridium difficile* Infections. *Anaerobe*, **15**, 274-280. https://doi.org/10.1016/j.anaerobe.2009.09.002

[10] Lim, V.K. (2012) The Lack of Novel Antibiotic and Vaccine Development to Combat Resistance. APUA Corporate Sponsors, 20.

[11] Mandal, A. and Sahi, P.K. (2017) Probiotics for Diarrhea in Children. *Journal of Medical Research and Innovation*, **1**, AV5-AV12. https://doi.org/10.15419/jmri.66

[12] Guandalini, S. (2011) Probiotics for Prevention and Treatment of Diarrhea. *Journal of Clinical Gastroenterology*, **45**, S149-S153. https://doi.org/10.1097/MCG.0b013e3182257e98

[13] CLSI Guidelines (2018) Performance Standards for Antimicrobial Susceptibility Testing. 28th Edition (M100S).

[14] Touré, R., Kheadr, E., Lacroix, C., Moroni, O. and Fliss, I. (2003) Production of Antibacterial Substances by Bifidobacterial Isolates from Infant Stool Active against *Listeria monocytogenes*. *Journal of Applied Microbiology*, **95**, 1058-1069. https://doi.org/10.1046/j.1365-2672.2003.02085.x

[15] Metchnikoff, I.I. (2004) The Prolongation of Life: Optimistic Studies. Springer Publishing Company, Berlin.

[16] Markowiak, P. and Śliżewska, K. (2017) Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients*, **9**, 1021. https://doi.org/10.3390/nu9091021

[17] Łukaszewicz, M. (2012) *Saccharomyces cerevisiae* var. *boulardii*-Probiotic Yeast. InTech, London. https://doi.org/10.5772/50105

[18] Wu, X., Vallance, B.A., Boyer, L., Bergstrom, K.S., Walker, J., Madsen, K.L. and Jacobson, K. (2008) *Saccharomyces boulardii* Ameliorates *Citrobacter rodentium*-Induced Colitis through Actions on Bacterial Virulence Factors. *American Journal of Physiology Gastrointestinal and Liver Physiology*, **294**, G295-G306. https://doi.org/10.1152/ajpgi.00173.2007

[19] Czerucka, D., Piche, T. and Rampal, P. (2007) Yeast as Probiotics-*Saccharomyces boulardii*. *Alimentary Pharmacology & Therapeutics*, **26**, 767-778. https://doi.org/10.1111/j.1365-2036.2007.03442.x

[20] Mack, D.R., Ahrné, S., Hyde, L., Wei, S. and Hollingsworth, M.A. (2003) Extracellular MUC3 Mucin Secretion Follows Adherence of *Lactobacillus* Strains to Intestinal Epithelial Cells *in Vitro*. *Gut*, **52**, 827-833. https://doi.org/10.1136/gut.52.6.827

[21] Yan, F. and Polk, D.B. (2006) Probiotics as Functional Food in the Treatment of Diarrhea. *Current Opinion in Clinical Nutrition & Metabolic Care*, **9**, 717-721. https://doi.org/10.1097/01.mco.0000247477.02650.51

[22] Caballero-Franco, C., Keller, K., De Simone, C. and Chadee, K. (2007) The VSL # 3 Probiotic Formula Induces Mucin Gene Expression and Secretion in Colonic Epithelial Cells. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, **292**, G315-G322. https://doi.org/10.1152/ajpgi.00265.2006

[23] Hameed, A., Naveed, S., Qamar, F., Alam, T. and Abbas, S.S. (2016) Irrational Use of Antibiotics. Different Age Groups of Karachi: A Wakeup Call for Antibiotic Resistance and Future Infections. *Journal of Bioequivalence & Bioavailability*, **8**, 242-245.

[24] Fuchs, A., Bielicki, J., Mathur, S., Sharland, M. and Van Den Anker, J.N. (2016) Antibiotic Use for Sepsis in Neonates and Children: 2016 Evidence Update. WHO Reviews.

[25] World Health Organization (WHO) (2008) Second Meeting of the Sub-Committee

of the Expert Committee on the Selection and Use of Essential Medicines. Geneva. http://www.who.int/selection_medicines/committees/subcommittee/2/gentamicin_rev.pdf

[26] Saxelin, M. (2008) Probiotic Formulations and Applications, the Current Probiotics Market, and Changes in the Marketplace: A European Perspective. *Clinical Infectious Diseases*, **46**, S76-S79. https://doi.org/10.1086/523337

[27] Amagase, H. (2008) Current Marketplace for Probiotics: A Japanese Perspective. *Clinical Infectious Diseases*, **46**, S73-S75. https://doi.org/10.1086/523338

[28] Luong, M.L., Sareyyupoglu, B., Nguyen, M.H., Silveira, F.P., Shields, R.K., Potoski, B.A. and Toyoda, Y. (2010) Lactobacillus Probiotic Use in Cardiothoracic Transplant Recipients: A Link to Invasive Lactobacillus Infection? *Transplant Infectious Disease*, **12**, 561-564. https://doi.org/10.1111/j.1399-3062.2010.00580.x

[29] Feizizadeh, S., Salehi-Abargouei, A. and Akbari, V. (2014) Efficacy and Safety of *Saccharomyces boulardii* for Acute Diarrhea. *Pediatrics*, **134**, e176-e191. https://doi.org/10.1542/peds.2013-3950

[30] Gutiérrez, P.C., Polanco, I.A. and Salazar, E.L. (2010) An Evidence Based Iberic-Latin American Guideline for Acute Gastroenteritis Management in Infants and pre Scholars. *Anales de Pediatria*, **72**, 220-e1.

[31] National Collaborating Centre for Women's and Children's Health (2009) Diarrhoea and Vomiting Caused by Gastroenteritis: Diagnosis, Assessment and Management in Children Younger than 5 Years.