Egyptian Journal of Medical Microbiology Volume 28 / No.2 / April 2019 145-151 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Study of Some Enteropathogens Causing Acute Diarrhea in Infants and Children Less than 5 Years Old

[1]Amira E. Zaki*, [1]Wesam H. Amer, [2]Ahmed A. Abo Elezz, [1]Waseela M. Mohamed
[1]Medical Microbiology &Immunology Department, Faculty of Medicine, Tanta University
[2]Pediatrics Department, Faculty of Medicine , Tanta University

Key words:
Diarrhea, Enteropathogens, Rota virus, Antibiotic policy

Corresponding Author:
Amira Ezzat Zaki Eid , Demonstrator at Medical Microbiology &Immunology Department, Faculty of Medicine, Tanta University, Egypt.
Tel.: 01020195699
meroezzateid@gmail.com

ABSTRACT

Background: *Diarrheal disorders are a major health issue in pediatrics all over the world.* ***Objectives:*** *To isolate enteropathogens causing diarrhea and determine antibiotic sensitivity pattern.* ***Methodology:*** *120 stool samples underwent the following procedures; macroscopic and microscopic examination, preparation of wet smear, detection of fecal leucocytes ,carbol-fuchsin staining for detection of Campylobacter, Rota virus detection by immunochromatography, culture of samples on Mackongey agar, XLD, API for lactose non fermenting colonies, Identification of EPEC by polyvalent antisera , O157:H7 E.coli detection using Sorbitol Mackoncey and the isolates obtained were tested for antimicrobial sensitivity.* ***Results:*** *The detected enteropathogens were EPEC (46.6%), Rota Virus (20.8%), Salmonella (14.2%), Shigella (1.7%), K.oxytoca (8.3%)and Pseudomonas (4.2%).The antibiotic resistance pattern was ESBL; EPEC (53%), Salmonella (47%). ESBL and Ampc; EPEC (65%), Shigella (5%) and K.oxytoca (25%). Ampc resistance was EPEC while all Pseudomonas isolates were wild.* ***Conclusions:*** *Rehydration therapy, Rota virus vaccination and antibiotic policy are essential for management of diarrheal cases.*

INTRODUCTION

Diarrheal disorders are a major health issue in pediatrics worldwide, they are the second leading cause of death in infants and young children according to (WHO). In Egypt , diarrhea is the first cause of infant mortality. The most commonly recognized definition of diarrhea is based on (WHO) parameters that define diarrhea by an increase in frequency and /or fluidity of stool relative to the previous habit of the individual. [1]

There are several types of diarrhea, including acute diarrhea: typically lasting less than 14 days, persistent diarrhea; diarrheal episode that starts acutely, but lasting 14 days or more and chronic diarrhea; recurrent or long lasting diarrhea (for more than one month) due to major underlying cause such as secretory tumour, gastroenteritis: diarrhea due to intestinal infectious agent. [2]

Diarrhea may be either due to infectious or non infectious causes. For infectious diarrhea, infections are the major cause of severe morbidity and mortality among children is worldwide. Infectious diarrhea is due to different pathogens *as Rota virus, E.coli, Salmonella, Astrovirus, Calicivirus, Campylobacter, Shigella, Giardia, and Cryptosporidium species*. Signs and symptoms of diarrhea may include the following; dehydration, failure to thrive, malnutrition, electrolyte disturbance ,haemolytic uraemic syndrome .[3]

So diarrheal diseases need frequent and yearly estimation of its incidence and detection of the causative organisms to limit its complications.

METHODOLOGY

Study Population:

The study was carried out in Pediatrics and Microbiology and Immunology Departments, Faculty of Medicine, Tanta university . It included 120 children less than 5 years either from Outpatient Clinics or Inpatient Wards of Pediatric Department. Our execlusion criteria during this study were newborn and children older than 5years ,non infectious diarrhea, chronic and persistent diarrhea. Ethical approval for this study was provided by Ethics and Research committee, Faculty of Medicine, Tanta University.

Methods:

All patients were subjected to the followings; history taking including: age, sex, nutritional condition, developmental history ,present, past history of other illness and antibiotic course of treatment.

Clinical examinations included: general and systemic examination with special emphases on signs of infection such as fever >38, chills, rigors and signs of dehydration.

Microbiological Examination:

Sampling:

Stool samples were collected from different Inpatient and Outpatient cases in a clean wide mouthed

container and were transported as rapid as possible to Microbiology and Immunology Department laboratory.

Processing of Samples:

Macroscopy of stool samples was done to check for characteristic colour, presence of blood and mucous which its presence can act as a presumptive test for a suspected bacterial agent causing diarrhea.

Microscopic examination included: wet smear was done for exclusion of parasitic causes as protozoa, trophozoite, amoeba cyst, larvae, helminth eggs. Fecal leucocytes were detected using methylene blue stain. Carbol fuschin stain was used for the rapid detection of *Campylobacter* species in faecal samples.

Rota virus was detected using rapid detection immunochromatography (Rota virus Rapid test Device, Bio Tina GmbH**,**Germany).

Culture of samples on Mackoncey's agar, XLD, and incubation at 37 for 24 hours (Oxoid, UK).The isolates were identified by colony morphology, Gram stained film, biochemical reactions and motility testing.

Verification of results using API 20 *Enterobactericea* for lactose, xylose non fermenting colonies (BioMérieux,France).

Isolation and identification of *Enteropathogenic Escherichia coli* by serotyping using polyvalent antisera (O26,55,111,119,126) by mast assure pathogenic *E.coli* (O) antisera (Mast ,UK):liquid stable antisera for determination of O antigens for the serological identification of pathogenic *E.coli* .

Detection of O157:H7 *E.coli* using Sorbitol Mackoncey media(Liofilchem, Italy).

Antimicrobial susceptibility testing:

It was done on Mueller Hinton agar by Kirby Bauer-disk diffusion method using antibiotics as ofloxacin, gentamycin, sulfamethoxazole/trimethoprim, aztreonam ,ciproflxacin, ampicillin, ceftazidime, meropenam, cefotaxime, cefoperazone, cefoxitin, amikacin, and amoxycillin/clavulanic (Oxoid, UK) using CSLI guidelines[4]. ESBL detection was done by Modified double disc synergy test(MDDST) [5].

Statistical Analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.Qualitative data were described using number and percent. Quantitative data were described using Range (minimum and maximum), mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test, P values <0.05 were considered significant.

RESULTS

A total of 120 patients with acute diarrhea participated in the study. The age of studied cases was mainly between 1.5 and 2.5 years with a ratio 25.8 and 34.2 respectively. Seasonal distribution of cases was mainly during summer, more common in rural areas (64.2%). As regard type of feeding; bottle feeding was 30.8% and junk food 60.8%. Most of cases had previous history of antibiotic intake (37.5%). Bacterial enteropathogens were positive in 70.8% of cases with predominance of *EPEC* (46.7%) followed by *Salmonella* (14.2 %) then viral diarrhea by *Rota virus* (20.8%). The results are shown in table 1.

Table 1: Distribution of the studied cases according to epidemiological factor and causative enteropathogen (n=120):

Epidemiological factor	No.	%
Age of children (years)		
1.5 year	31	25.8
2 years	28	23.3
2.5 years	41	34.2
4 years	20	16.7
Min. – Max.	1.5 – 4.0	
Mean ± SD.	2.38 ± 0.83	
Median	2.50	
Sex		
Male	82	68.3
Female	38	31.7
Residence (geography)		
Urban	43	35.8
Rural	77	64.2
Type of feeding		
Bottle	37	30.8
Junk food	73	60.8
Season		
Winter	43	35.8
Summer	77	64.2
Previous antibiotic intake		
Positive	45	37.5
Negative	75	62.5
Enteropathogens		
Viral		
Rota virus	25	20.8
Bacterial	85	70.8
*EPEC**	56	46.7
Salmonella species	17	14.2
Shigella species	2	1.7
K.oxytoca	10	8.3
*Pseudomonas aeruginosa***	5	4.2
Parasitic	10	8.3
Entamoeba histolytica	6	5.0
Giardia lamblia	4	3.3

**Enteropathogenic E.coli, **co-pathogen with Enteropathogenic E.coli*

Viral diarrhea was mainly at age of 1.5 years (61.3%). Bacterial causes were; *E.coli* was the predominant particularly at 2 years old (82.1%) but *Salmonella* and *Shigella* species were more common at age of 4 years old (85%) and (10%) respectively. *Parasites* were present mainly at age of 2.5 years

(24.4%).Bottle feeding was significantly associated with all cases of viral diarrhea, however junk food was significantly associated with most of bacterial diarrhea cases. Viral diarrhea caused by *Rota virus* was mainly predominent in winter while bacterial diarrhea caused mainly by *E.coli* followed by *Salmonella* were mainly in summer as shown in table 2.

Table 2: Relation between age of children, type of feeding ,season and the causative enteropathogens (n=120)

Enteropathogen	*Rota virus* (n = 25)	*E. coil (EPEC)* (n = 56)*	*Salmonella* species (n = 17)	*Shigella* SPP (n = 2)	*Klebsiella-oxytoca* (n = 10)	*Pseudomonas* (n = 5)	*Parasite* (n = 10)	P value**
Age of children								
1.5 years(n=31)	19(61.3)	12(38.7%)	-	-	-	4(12.9%)	-	<0.001
2 years (n=28)	5(17.9%)	23(82.1%)	-	-	-	1(3.6%)	-	<0.001
2.5 years (n=41)	1(2.4%)	20(48.8%)	-	-	10(24.4%)	-	10(24.4%)	0.024
4 years (n=20)	-	1(5%)	17(85%)	2(10%)	-	-	-	0.039
Type of feeding			-	-	-		-	
Bottle feeding	25(100%)	12(21.4%)	17(100%)	2(100%)	-	5(100%)	10(100%)	<0.001
Junk food	-	44(78.6%)				-		0.006
Season								
Winter	25(100%)	8(14.3%)	-	-	4(40%)	5(100%)	6(60%)	<0.001
Summer	-	48(85.7%)	17(100%)	2(100%)	6(60%)	-	4(40%)	

**Enteropathogenic E.coli,* p value for comparing between the two categories
**: Statistically significant at $p \leq 0.05$

Vomiting, dehydration and fever represented a clinical triad in all viral cases. This triad was also detected among most of bacterial cases with variable degrees of severity of fever and dehydration. All viral stool samples were watery, yellowish and with fecal odour. However stool samples of bacterial diarrhea were semi formed or loose, greenish, offensive, contain mucous, undigested food particles and pus cells and that caused by parasites characterized mainly by a significant presence of its specific cyst ($p<0.001$). The results are shown in table 3.

Table 3: Relation between clinical and laboratory characters of the stool samples depending upon the various aetiological agents :

Clinical and laboratory characteristics	Enteropathogen							p*
	Rota virus (n = 25)	*E. coil (EPEC)* (n = 56)**	*Salmonella species* (n = 17)	*Shigella SPP* (n = 2)	*Klebsiella - oxytoca* (n = 10)	*Pseudomonas* (n = 5)***	*Parasite* (n = 10)	
Vomiting	25(100%)	50(89.3%)	10(58.8%)	1(50%)	6(60%)	0(0%)	0(0%)	<0.001*
Dehydration	25(100%)	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	10(100%)	-
Fever	25(100%)	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	10(100%)	-
Odour		-	-	-	-	-	-	<0.001*
Fecal	25(100%)	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	10(100%)	
Offensive	-							
Consistency								<0.001*
Watery	100%	-	-	-	-	-	-	
Semiformed	-	100%	100%	100%	10%	100%	100%	
Loose	-	-	-	-	90%	-	-	
Color								<0.001*
Yellow	100%	-	-	-	-	-	-	
Greenish	-	100%	100%	100%	100%	100%	-	
Brownish	-	-	-	-	-	-	100%	
mucous	0(0%)	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	10(100%)	<0.001*
Undigested food	0(0%)	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	10(100%)	<0.001*
Pus cell	0(0%)	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	0(0%)	<0.001*
Parasite cyst	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	10(100%)	<0.001*

: p value for comparing between the two categories *: Statistically significant at $p \leq 0.05$
***Enteropathogenic E.coli*
****co-pathogen with Enteropathogenic E.coli*

The antibiotic susceptibility pattern: all isolates; *E.coli, Shigella, Salmonella* species and *K.oxytoca* were sensitive to ofloxacin, carbapenems, sulfamethoxazole-trimethoprim and colistin. All *Pseudomonas* isolates were sensitive to carbapenems, colistin, ofloxacin, ciprofloxacin, gentamycin, amikacin, cefipime and ceftazidime. The result are shown in table 4.

Table 4: Antibiotic susceptibility of bacterial isolates from diarrheal stool samples:

Antibiotics		Enteropathogen					p*
	Disk content Mg	*E. coil (EPEC)*** (n = 56)	*Salmonella* species (n = 17)	*Shigella* SPP (n = 2)	*Klebsiella oxytoca* (n = 10)	*Pseudomonas* (n = 5)	
Ofloxacin	5	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	-
Ciprofloxacin	5	56(100%)	10(58.8%)	2(100%)	10(100%)	5(100%)	<0.001*
Gentamycin	10	56(100%)	-	-	10(100%)	5(100%)	<0.001*
Amikacin	30	56(100%)	-	-	10(100%)	5(100%)	<0.001*
Ceftazidime	30	9(16.1%)	10(58.8%)	1(50%)	5(50%)	5(100%)	<0.001*
Cefotaxime	30	15(26.8%)	10(58.8%)	1(50%)	5(50%)	-	0.029*
Cefazolin	30	15(26.8%)	10(58.8%)	1(50%)	5(50%)	-	0.029*
Cefoperazone	75	20(35.7%)	10(58.8%)	1(50%)	5(50%)	-	0.113
Cefuroxime	30	15(26.8%)	10(58.8%)	1(50%)	5(50%)	-	0.031*
Cefipime	30	20 (35.7%)	10(58.8%)	1(50%)	5(50%)	5(100%)	0.031*
Ceftriaxone	30	20(35.7%)	10(58.8%)	1(50%)	5(50%)	-	0.113
Cefoxitin	30	9(16.1%)	10(58.8%)	1(50%)	5(50%)	-	0.002*
Ampicillin	10	9(16.1%)	10(58.8%)	1(50%)	5(50%)	-	0.002*
Amoxycillin clavulanic	30	9(16.1%)	10(58.8%)	1(50%)	5(50%)	-	0.002*
Piperacillin tazobactam	110	9(16.1%)	10(58.8%)	1(50%)	10(100%)	3(60%)	<0.001*
Aztreonam	30	15(26.8%)	10 (58.8%)	1(50%)	5(50%)	3(60%)	0.056
Meropenem	10	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	-
Imipenem	10	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	-
Sulfamethoxazole trimethoprim	25	56(100%)	17(100%)	2(100%)	10(100%)	-	-
Colistin	10	56(100%)	17(100%)	2(100%)	10(100%)	5(100%)	-

p: p value for comparing between the different categories *: Statistically significant at $p \leq 0.05$
***Enteropathogenic E.coli*

ESBL was detected mainly in *E.coli* (53%; p<0.001) followed by *Salmonella* (47%; p<0.001). ESBL and Ampc was detected mainly in *E.coli* (70%; p<0.001) followed by *K. oxytoca* (25%; p=0.012). All *Pseudomonas* isolates were sensitive (p= 0.017). Thirty cases with negative history of antibiotic intake were wild, however 45 cases with positive history of antibiotic intake were ESBL, ESBL & Ampc and Ampc shown in table 5.

Table 5: Antibiotic resistance pattern of bacterial isolates from diarrheal stool samples and its relation to history of previous antibiotic intake :

Enteropathogen	Antibiotic resistance pattern				p*
	Wild (S) (n = 30)	Ampc (n = 25)	ESBL & Ampc (n = 20)	ESBL (n = 15)	
***E. coil* **(n=56)**	9(30%)	25 (100%)	14(70%)	8(53%)	<0.001*
***Salmonella* species(n=17)**	10(33%)	0	0	7(47%)	p <0.001*
***Shigella* SPP (n=2)**	1(3.3%)	0	1(5%)	0	p= 0.813
***Klebsiella oxytoca*(n=10)**	5(17%)	0	5(25%)	0	p= 0.012*
Pseudomonas (n=5)**	5(17%)	0	0	0	p= 0.017*
Antibiotic intake **Positive history** **Negative history**	 - 30(100%)	 20(80%) 5(20%)	 14(70%) 6(30%)	 11(73.3%) 4(26.7%)	<0.001*

p value for comparing between the two categories*: Statistically significant at $p \leq 0.05$
***Enteropathogenic E.coli*
****co-pathogen with Enteropathogenic E.coli*

DISCUSSION

A total of 120 stool samples from children under age of 5 years old were included in this study. Diarrheal cases were more common in children living in rural areas than those living in urban areas. This can be attributed to the poor quality of hygienic condition and sanitation practices in rural population similar results were reported by Rathaur et al.[6]

In our study, bacteria was the most common cause of diarrhea, that agrees with another study that showed a higher bacterial incidence (27.7%) than viral incidence (16.1%).[7] *EPEC* was the predominant bacterial enteropathogen detected among cases with a peak of incidence of cases in children aged 2 and 2.5 years old respectively. This is explained by the minimal role of breast feeding and start of weaning with use of bottle feeding and junk fookd These findings agree with a previous study demonstrated a significant association between *EPEC* and community acquired acute diarrhea[8] However, this pattern of distribution is in contrast in other areas of Africa and Malaysia, which had reported *E.coli* as the least common etiological agent causing diarrhea due to alteration in epidemiological features of the host as well environment that showed *Shigella* species as the commonest (54.3%) followed by *Salmonella* (38.4%) and *E.coli* (7.3%).[9]

Salmonella spp. was another important enteropathogen detected among 17 cases included in our study, their incidence was mainly at age of 4 years old ,this can be attributed to negligent attitude of parent's toward children at this age group and increased mobility of children due to their acquired walking ability. The main reservoir of infection in animals constitutes the principal source of diarrhea caused by *Salmonella spp*.[10]

Klebsiella oxytoca was another important enteropathogen detected in 10 cases. Its role was mainly among hospitalized cases receiving broad-spectrum antimicrobials, so it is considered antibiotic associated diarrhea. *K. oxytoca* has been considered a part of the normal gut flora, colonizing up to 9% of healthy subjects and the use of broad-spectrum antibiotics has been increased, this leads to the emergence of multidrug-resistant organisms in our locality. The previous use of antibiotics, including penicillins, cephalosporins, carbapenems, and fluoroquinolones, during hospitalization was significantly associated with the detection of *K.oxytoca*. This agrees with previous studies.[11-14]

Pseudomonas aeruginosa was detected during our study among 5 cases, it was isolated as a co-pathogen with *E.coli*. The role of *Pseudomonas* in acute diarrheal illness is controversial because the gastrointestinal tract is not the common infection site of *Pseudomonas*. The clinical features of *P. aeruginosa* enterocolitis were fever with mucoid diarrhea mimicking bacterial enterocolitis this agrees with a previous study [15] that described the relation between isolated *P.aeruginosa* and diarrheal illness in samples from children less than 5 years old during this study performed in Taiwan that classified *P. aeruginosa*-associated diarrheal diseases into 4 groups: Shanghai fever (5%), *P. aeruginosa* enterocolitis (15%), *P. aeruginosa*-related diarrhea (19%) and antibiotic-associated diarrhea (43%).Our study showed the mild form of co-infection with *E.coli*. This also agrees with another study done in Egypt.[16]

Rota virus was another important entero pathogen detected in our study. *Rota virus* cases were more detected at 1.5 years old (61.5%) this might be due to the fact that older children acquired protective immunity during previous exposures to *Rotavirus*, so they are more resistant to infection with this agent and we recommend the earlier application of rotavirus vaccine to children .[17]

As regards laboratory findings in bacterial stool samples these were mainly greenish semiformed or loose stool with offensive odour associated with pus cells, mucous and undigested food particles particularly with *Shigella* cases. This agrees with a previous study.[18] In viral cases, diarrhea was mainly watery yellowish and no pus cells. The fluid loss facilitates the occurrence of dehydration among cases that is supported by review studies on the pathogenesis of rotavirus infection and the mechanism by which rotavirus evokes intestinal secretion of fluid and electrolytes. [19]

As regards seasonal distribution of cases, the bacterial enteropathogens were detected mainly in the summer, that agrees with the findings in other studies which reported that *EPEC* infections show a marked seasonality and are associated with warm season peaks with effect of flies that facilitate transmission of diarrhegenic pathogens and school vacations allow children to increase their exposure to environmental pathogens and contaminated food. In winter, all cases of *Rota virus* were detected with its peak from September to December. This agrees with a study performed in Vietnam but differed from a Japanese study, in which *Rota virus* was rarely detected from September to December.[20,21]

As regards antibiotic susceptibility pattern of bacterial isolates *EPEC, Salmonella, Shigella, K.oxytoca and Pseudomonas* all were sensitive to carbapenems, colistin, sulfamethoxazole /trimethoprim this is in accordance with another study performed in Egypt. [16] Nearly all of bacterial isolates in our study were sensitive to SMX -TMP which is cheap and easy available drug .However unfortunately ESBL was detected among (53%) & (47%) of *EPEC and Salmonella* cases respectively. ESBL cases also carry plasmid resistant to sulfamethoxazole/trimethoprim, quinolones and

aminoglycosides so sulfamethoxazole/ trimethoprim was not effective in such cases .[22]

In this study, antibiotic resistance was high especially ESBL and Ampc mainly in *EPEC*. *E.coli* is a common agent of diarrhea, its strains are mostly exposed to different antibiotics injudiciously this was obvious in our study that among 85 bacterial cases, 45 cases (53%) with previous history of antibiotic intake showed different forms of antibiotic resistance, that is supported by other studies that showed antibiotic consumption is a well-known risk factor, and found a positive relationship between third-generation cephalosporin, other B-lactam or fluoroquinolone use and the acquisition of ESBL-producing organisms.[23]

ESBL was also detected in (41.2%) of *Salmonella* cases ,this is reported by other studies that showed emergence of ESBLs in gram negative bacteria that leads to third generation cephalosporines ineffectiveness which reduces therapeutic options. [24] That increasing incidence of decreased susceptibility to ciprofloxacin in zoonotic *Salmonellas* such as *S.enteritidis*, in England may be a consequence of the use of fluoroquinolone antibiotics in poultry and the primary method of spread to humans is assumed to be through the food chain. *Salmonella* resistance to ciprofloxacin was also detected in (41%) of cases this agrees with studies that reported resistance to ciprofloxacin among *non-typhoidal Salmonella* .[25,26]

CONCLUSIONS & RECOMMENDATIONS

Bacterial enteropathogens with predominant *EPEC* followed by *Rota virus* were the main enteropathogens detected during our study .Antibiotics abuse was significantly associated with emergence of drug resistance among the bacterial isolates. Bottle feeding was significantly associated with all cases of viral diarrhea however junk food was significantly associated with bacterial diarrhea. It was interesting to note that not only one but different co- pathogens were isolated from some stool samples. So we recommend proper diagnosis of aetiology, safe water supply , antibiotic policy in hospital and *Rota virus* vaccination as they are essential measures for minimizing the incidence of diarrhea and its complications.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

Acknowledgement

Collective and individual acknowledgments are owed to all professors and staff members of Medical Microbiology and Immunology and Paediatrics Departments, Faculty of Medicine, Tanta University.

REFERENCES

1. World Health Organization. Diarrheal disease Fact Sheet No330. http://who.int/mediacentre/factsheets/fs330/en/.2013. Retrived 18 June 2014.
2. Staff members of Tanta Pediatrics Department Gastrointestinal disorders-diarrheal diseases, staff members of Tanta Pediatric Department (editors) – Pediatrics Book (9th edition) 2012; 244-258.
3. Mehal JM, Esposito DH, Holman RC, Tate JE, Callinan LS, Parashar UD. Risk factors for diarrhea-associated infant mortality in the United States, 2005–2007. The Pediatric Infectious Disease Journal 2012 Jul 1; 31(7): 717-721.
4. Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 28st informational supplements. CLSI document M100-S28, Wayne PA. 2018.
5. Khan M, Thukral S, Gaind R. Evaluation of a modified double-disc synergy test for detection of extended spectrum β-lactamases in AMPC β-lactamase-producing proteus mirabilis. *Indian Journal of Medical Microbiology* 2008; 26(1): 58-61.
6. Rathaur VK, Pathania M, Jayara A, Yadav N. Clinical study of acute childhood diarrhoea caused by bacterial enteropathogens. Journal of clinical and diagnostic research: JCDR. 2014 May;8(5):PC01.
7. Varela G, Schelotto F. Síndrome urémico hemolítico en Uruguay. Aspectos microbiológicos y clínicos, aportes para su conocimiento regional. Revista Facultad de Ciencias de la Salud UDES. 2015 Jun 30;2(1):25-30.
8. Estrada-Garcia T, Lopez-Saucedo C, Thompson-Bonilla R, Abonce M, Lopez-Hernandez D, Santos JI et al. Association of diarrheagenic Escherichia coli pathotypes with infection and diarrhea among Mexican children and association of atypical enteropathogenic E. coli with acute diarrhea. Journal of clinical microbiology. 2009 Jan 1;47(1):93-8.
9. Sousa MÂ, Mendes EN, Collares GB, Péret-Filho LA, Penna FJ, Magalhães PP. Shigella in Brazilian children with acute diarrhoea: prevalence, antimicrobial resistance and virulence genes.

Memórias do Instituto Oswaldo Cruz. 2013 Feb;108(1):30-5.

10. Urio EM, Collison EK, Gashe BA, Sebunya TK, Mpuchane S. Shigella and Salmonella strains isolated from children under 5 years in Gaborone, Botswana, and their antibiotic susceptibility patterns. Tropical Medicine & International Health. 2001 Jan;6(1):55-9.
11. Beaugerie L, Metz M, Barbut F, Bellaiche G, Bouhnik Y, Raskine L et al. Infectious Colitis Study Group. Klebsiella oxytoca as an agent of antibiotic-associated hemorrhagic colitis. Clinical Gastroenterology and Hepatology. 2003 Sep 1;1(5):370-6.
12. Moreno A, Filho AF, Gomes TDT, Ramos ST, Montemor LP, Tavares VC et al. Etiology of childhood diarrhea in the northeast of Brazil: significant emergent diarrheal pathogens. Diagnostic Microbiology and Infectious Disease 2010; 66(1): 50-57.
13. Silva T, Nogueira PA, Magalhães GF, Grava AF, Silva LH, and Orlandi PP. Characterization of Shigella spp. by antimicrobial resistance and PCR detection of ipa genes in an infantile population from Porto Velho (Western Amazon region), Brazil. Memorias do Instituto Oswaldo Cruz 2008; 103(7): 731-733.
14. World Health Organization. Guidelines for the control of shigellosis, including epidemics due to Shigella dysenteriae 1. Geneva: World Health Organization; 2005; 64 p.
15. Chuang CH, Wang YH, Chang HJ, Chen HL, Huang YC, Lin TY et al. Shanghai fever: a distinct Pseudomonas aeruginosa enteric disease. Gut 2014; 63(5): 736-743.
16. Behiry IK, Abada EA, Ahmed EA, Labeeb RS. Enteropathogenic Escherichia coli associated with diarrhea in children in Cairo, Egypt. The Scientific World Journal 2011; 11: 2613-2619.
17. Jiang B, Gentsch JR, Glass RI. The role of serum antibodies in the protection against Rotavirus disease: an overview. Clinical Infectious Diseases 2002; 34(10): 1351-1361.
18. Poo MI, Lee WS. Admission to hospital with childhood acute gastroenteritis in Kuala Lumpur, Malaysia. Medical Journal of Malaysia 2007; 62(3): 189-193.
19. Yongsi HB. Pathogenic microorganisms associated with childhood diarrhea in Low-and-middle income countries: Case study of Yaoundé–Cameroon. International Journal of Environmental Research and Public Health 2008 Dec 2; 5(4): 213-229.
20. Nguyen TV, Le Van P, Le Huy C, Weintraub A. Diarrhea caused by Rotavirus in children less than 5 years of age in Hanoi, Vietnam. Journal of Clinical Microbiology 2004; 42(12): 5745-5750.
21. Fang ZY, Yang H, Zhang QJ, Sun J, Tang LW, Ma JY et al. Diversity of rotavirus strains among children with acute diarrhea in China: 1998-2000 surveillance study. Journal of Clinical Microbiology 2002; 40(5):1875-8.
22. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update *Clinical Microbiology Reviews* 2005; 18(4): 657-86.
23. Kaier K, Frank U, Hagist C, Conrad A, Meyer E. The impact of antimicrobial drug consumption and alcohol-based hand rub use on the emergence and spread of extended-spectrum β-lactamase-producing strains: a time-series analysis. *Journal of Antimicrobial Chemotherapy* 2009; 63(3): 609-614.
24. Vieira AR, Collignon P, Aarestrup FM, McEwen SA, Hendriksen RS, Hald T et al. Association between antimicrobial resistance in Escherichia coli isolates from food animals and blood stream isolates from humans in Europe: an ecological study. Foodborne Pathogens and Disease 2011; 8(12): 1295-1301.
25. Farzana R, Shamsuzzaman SM, Mamun KZ, Shears P. antimicrobial susceptibility pattern of extended spectrum Beta-lactamase producing gram-negative bacteria isolated from wound and urine in a tertiary care hospital, dhaka city, bangladesh. Southeast Asian Journal of Tropical Medicine & Public Health 2013; 44(1): 96-103.
26. Taneja N, Mohan B, Khurana S, Sharma M. Antimicrobial resistance in selected bacterial enteropathogens in north India. Indian Journal of Medical Research 2004; 120(5): 39-43.