Detection of diarrheagenic strains of E.*coli* from pediatric diarrheal infection in a tertiary hospital, Bangladesh

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Abstract

Background: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in Bangladesh and E.coli is an important agent of childhood diarrhea. Objectives: To determine the prevalence of diarrheagenic Escherichia coli (DEC) and its pathotypes in pediatric population with diarrheal infection using polymerase chain reaction (PCR). Methods: This was a descriptive type of cross sectional study conducted among the pediatric population with diarrhea admitted in Rajshahi Medical College hospital, Rajshahi, Bangladesh from July 2014 to June 2015. In this study, 268 children with diarrheal infection aged from 3 to 12 years were included. Stool samples were collected and identified as E. coli isolates by culture on MacConkeys agar media, microscopy and standard biochemical tests. Diarrheagenic strains of E.coli (DEC) were identified by multiplex PCR assays using six primer pairs by detecting the genes of enterotoxigenic E.coli (lt,st), Enteropathogenic E.coli (eae,bfp), Enteroaggregative E.coli (aat) and Enteroinvasive E.coli (iPah). Results: Among 268 stool samples,166 E. coli were isolated. Of the total 166 isolated E.Coli, 68(38%) were DEC identified by multiplex PCR. Among DEC, most frequently isolated pathotypes was EPEC (38, 44.7%), followed by ETEC (26, 30.5%), EAggEC (20, 23.5%) and EIEC (1, 1.1%). Conclusion: This study shows that DEC is an important pathogen causing diarrhea in pediatric group but yet there is no data available of strains responsible in the study area. Strain identification is essential for E. coli diarrhea and by using multiplex PCR assay, the simultaneous detection of strains in one PCR reaction can be done that makes a conclusive diagnosis of diarrhea.

Key words: diarrhea, DEC, ETEC, EPEC, EAggEC, EIEC, PCR.

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Introduction

Diarrhea is a major public health problem throughout the world and still continues to be one of the most important causes of morbidity and mortality among infants and children in developing countries .[1] Diarrheal disease is the second leading cause of death and causes 1.3 million deaths every year in children of under five years.[2] The causes of diarrhea include a wide range of bacteria, viruses and parasites .[3] In developing countries, 50-60% of cases are caused by bacteria and the peak incidence occur during the summer months. Bacterial causes include, *E.coli, Campylobacter jejuni, Campylobacter coli, Vibrio cholerae, Salmonella spp, Shigellaspp, Aeromonas, Plesiomonas, Bacterioidsfragilis*.In Bangladesh, *E. coli* was responsible for 68% cases followed by *Campylobacter* spp. (13%), *Pseudomonas* spp. (11%), *Klebsiella*spp. (5%), *Salmonella* spp. (2%) and *Shigella*spp. (2%).[4] In rural area, children suffer on an average of 4.6 episodes of diarrhea, of which about 2.3 million die every year.[5] Diarrheagenic strains of *E .coli* (DEC) is responsible for 41.33% cases of acute childhood diarrhea.[6]

The genome of *E. coli* consist of a single circular chromosome of about 4 to 5 million base pairs (bp) and multiple plasmids. Besides that phage genes and transposons are also present. These genes are encoded for various virulence factors of *E. coli*.[7] Diarrhagenic stains of *E. coli* are divided into six groups on the basis of their virulence properties, such as enterotoxigenic *E.coli* (ETEC) ,enteropathogenic *E.coli* (EPEC), enteroinvasive *E.coli* (EIEC), entero-haemorrhagic *E.coli* (EHEC), enteroaggregative *E.coli* (EAggEC) and diffusely adherent *E.coli* (DAEC).[8]

Identification of diarrheagenic strains of *E.coli* is limited in many developing countries because conventional microbiological method is unable to distinguish between normal flora and pathogenic strains of *E.coli*. Serotyping is the traditional method for detection of DEC. But it has limited sensitivity and specificity, it is tedious and expensive and is performed correctly only in few reference laboratories. So, it may therefore be insufficient and unreliable to define an isolate as truly pathogenic by using serotyping alone. Thus, detection of diarrheagenic strains of *E.coli* has focused increasingly on the identification and characterization of genome.[9]

Now a days PCR is considered as one of the molecular methods which is most reliable, rapid and sensitive technique for identification of diarrheagenic strains of *E. coli*.[10] The present study was designed to detect the diarrheagenic strains of *E. coli* using multiplex PCR. It was hoped that this study will be very helpful for the clinician for proper diagnosis and attract the researchers to do further study in this field.

Methods

A descriptive type of cross sectional study was carried out in the Departments of Microbiology of Rajshahi Medical College and of Dhaka Medical College and Department of Pediatrics, Rajshahi Medical College Hospital during the period from July 2014 to June 2015. A total of 268 stool samples were obtained from children of 3 to 12 years with diarrhea, who were not on antibiotic therapy and cultured on MacConkey agar media for isolation of *E.coli*. Suspected lactose fermenting colonies were further subcultured onto motility-indole-urease and Simmons citrate agar media and identified by colony morphology, Gram staining and standard biochemical tests.

Multiplex PCR

After confirmation, *E.coli* isolates were subcultured onto Muller Hinton agar media, then again inoculated into sterile ependorf tube containing tryptic soya broth. The tryptic soy broth containing bacterial growth was centrifuged at 4000 rpm for 10 minutes for formation of pellet. Then the pellets were re suspended with 300 microliter of sterile deionized water, boiled at 100^{0}C for 10 minutes and immediately kept on ice.It was again centrifuged at 14000 rpm for 10 minutes .The supernatant was used as DNA template for multiplex PCR. Six primer pairs were used listed as follows:[11,12,13]

Primers were diluted by mixing with different volume of Tris EDTA buffer according to manufactures instruction. Three different primers can be used at a time for multiplex PCR. A total of 25 µl of mixture was prepared with 8 µl of master mix, 3 µl of forward

Primer sequence for PCR:

Primer	Primer sequence	Amplicon size(bp)
Lt	5-TCTCTATGTGCATACGGAGC-3 5-CCATACTGATTGCCGCAAT-3	322
St	5-GCTAAACCAGTAGAGGTCTTCAAAA-3 5-CCCGGTACAGAGCAGGATTACAACA-3	147
Eae	5CCCGAATTCGGCACAAGCATAAGC 3 5CCCGGATCCGTCTCGCCAGTATTCG 3	881
Bfp	5-TTCTTGGTGCTTGCGTGTCTTTT-3 5-TTTTGTTTGTTGTATCTTTGTAA-3	367
Aat	5-CTGGCGAAAGACTGTATCAT-3 5-CAATGTATAGAAATCCGCTGTT-3	630
ipaH	GCTGGAAAAACTCAGTGCCT CCAGTCCGTAAATTCATTCT	424

primer (1 µl for each), 3 µl of reverse primer (1 µl for each), 3 µl of extracted DNA template and 8 µl of nuclease free water in a PCR tube. After a brief vortex, the tube was centrifuged in a microcentrifuge machine for few seconds. In this way 25 µl of mixture was prepared for amplification.

The PCR run for detection of ETEC, EPEC, EAggEC strains comprises of preheat at 94°C for 10 minutes, then 36 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 45 seconds, extension at 72°C for 2 minutes with final extension at 72°C for 10 minutes. For EIEC strains, each PCR run comprises of preheat at 94°C for 10 minutes, then 30 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 2 minutes, extension at 72°C for 1 minute with final extension at 72°C for 7 minutes.

The detection of ETEC, EPEC and EAggEC involved five primers (lt,st,bfp,eae,aat) and EIEC involved one primer (iPaH). The multiplex PCR was done to detect lt. st and aat genes in one PCR run, eae and bfp genes for second run and iPaH for third reaction. Then this amplified DNA was mixed with loading dye and gel electrophoresis was done. The gel was then removed from the tray and visualized by UV illuminator and photographed for documentation. The size of the amplified DNA was assessed comparing with the bands of supplied DNA ladder (Bio-Rad, USA).

Result

In this study, among 268 cases, 115(43%) were from 3-6 years of age group, 97(36%) were from 7-9 years of age group and rest 56(21%) were from 10-12 years. One hundred thirty (48.5%) cases belonged to the lower income group, 88 (32.8%) belonged to middle income group and 50(18.7%) belonged to higher income group. In our study, the prevalence of diarrhea is more in rural area.

Table 1 : Distribution of the children by age and sex.

Age (Years)	Male N (%)	Female N (%)	Total N (%)
3-6	64 (55.7)	51 (44.3)	115 (42.9)
7-9	50 (51.5)	47 (48.5)	97 (36.2)
10-12	33 (58.9)	23 (41.1)	56 (20.9)
Total, N (%)	147 (54.9)	121 (45.1)	268 (100.0)

Among 268 patients,211 (79%) cases of lactose fermenters, 54(20%) cases of non - lactose fermenters and 3(1%) of no growth of bacteria were found by stool culture. Within the lactose fermenters, *E.coli* was 166 (79%) and other than *E.coli* were 45(21%). Within

Table 2 : Socio-economic status and residence of the children

Residence	Socio-economic condition			
	Lower N(%)	Middle N (%)	Higher N (%)	Total N(%)
Urban	59 (22)	32 (12)	21 (08)	112 (42)
Rural	71 (26)	56 (21)	29 (11)	156 (58)
Total N (%)	130 (48)	88 (33)	50 (19)	268 (100)

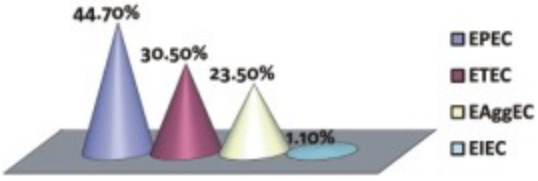

Figure 1:Distribution of detected strains of DEC

A total of 63 DEC, 26(41.2%) were in various combinations. lt +aat and eae+aat was the predominant combination and present in 4(6.34%) of isolates. 3(4.76%) in each were the lt+st and eae+bfp. 2(3.17%) in each were st+bfp, st+eae, st+lt+bfp, st+lt+eae, st+aat and lt+st+bfp+aat combinations. So, among 26 samples ETEC and EPEC each were 3(11.5%) ETEC and EPEC combination were 8(30.7%) followed by ETEC and EAggEC were 6(23%), EPEC and EAggEC were 4(15.3%) and ETEC, EPEC and EAggEC were 2(7.6%) (Table 3).

and 9(35%) were *lt+st* gene. Among EAggEC and EIEC, *aat*gene were 20 and *ipaH* gene was 1 in number. developing countries but also in developed countries. ETEC was listed as the highest priority for vaccine development because of their association with high morbidity and mortality rates.[14] In the present studies, 268 acute diarrhea patient were included. The patient were aged from 3 to 12 years , of them,115(43%) were from 3-6 years of age group, 97(36%) were from 7-9 years of age group and rest 57(21%) were from 9-12 years. Among them, 147 (55%) were male and 121(45%) were female. The ratio of male and female was 1.2:1. The age specific difference suggest that children having immature immune system may be exposed to contaminated formula milk, foods or environment or may have not been protected completely by breast feeding.[15] As the child grows, protective immunity develops and diarrhea is decreased. In Bangladesh, Qadri *et al.* reported in 2007 that the ratio of male to female among the diarrhea patients was 1:1.04[16]. Usually,the number of male and female depends on the availability of patients admitted in hospitals and has no influence on disease process. In Nigeria, Nweze reported in 2010 that sex had no effect on the distribution of diarrheagenic bacteria.[17]

Table 3: Distribution of genes in various combinations among DEC (N=68)

Gene combination	Number (%)	DEC	Number (%)
lt+st	3(4.76)	ETEC	3(11.5)
eae+bfp	3(4.76)	EPEC	3(11.5)
st+bfp	2(3.17)	ETEC and EPEC	8(30.7)
st+eae	2(3.17)		
st+lt+bfp	2(3.17)		
st+lt+eae	2(3.17)		
st+aat	2(3.17)	ETEC and EAggEC	6(23.0)
lt+aat	4(6.34)		
eae+aat	4(6.34)	EPEC and EAggEC	4(15.3)
lt+st+bfp+aat	2(3.17)	ETEC, EPEC and EAggEC	2(7.6)
Total	26(41.2)		26(100)

Discussion

E.coli is an important and unrecognized cause of diarrhea in infancy, not only in the isolated *E.coli,* 68 (38%) DEC were identified, of which 38 (44.7%) was EPEC, 26(30.5%) was ETEC, 20 (23.5%) was EAggEC and 1(1.1%) was EIEC on the basis of multiplex PCR. Among 38 EPEC, 21(55%) contained *eae* gene, 14(37%) contained *bfp*gene and 3(8%) contained *eae+bfp* gene. Among 26 identified ETEC, 6(23%) were *lt*gene, 11(42%) were *st* gene

About 48% pediatric diarrhea belonged to the lower income group and 33% from middle income group. In 2007, WHO reported 90% of all childhood diarrhea occurs in low and middle income groups.[18] Many studies reported among acute diarrheic children, 66% yearly visits to public clinic belongs to low income group and only 8% visit to private pediatrician office from high income group.[16] Diarrhea is more in rural than urban area as rural people are significantly less aware than urban people regarding safe food and drinks, using tube well water for drinking, using latrine. Their knowledge regarding ORS and its uses in diarrhea is very poor. These are may be the causes for more diarrheal patients from rural areas.

Among 268 cases of acute diarrhea, *E.coli* was 166 (79%) and 68(38%) isolates were identified as DEC by multiplex PCR. In previous studies in Bangladesh, 41.33% and 46.78% DEC were identified among diarrheic patients. Similarly in Iraq and Tanzania detection rate of DEC were 38% and 37.5%. However, the frequency of DEC varied in different countries, in Italy it was 6.3% and in Taiwan 5.74%. The prevalence and epidemiology of this pathogen as causative agent of diarrhea vary in the world from region to region and even between and within countries in the same geographical area.[19,20]

In this study, 38(44.7%) were identified as EPEC which was the most prevalent strain among DEC. EPEC was also the most prevalent strain among DEC in Iran, in a study conducted by Alikhani *et al.,* (2006) who reported 44.5% EPEC among diarrheic children.[21] Our study is dissimilar with the study of Arif and Salih (2010) in Iraq, Galane and Roux, (2001) in South Africa and Roy *et al.,* (2014) in Bangladesh.[22,23,4] This dissimilarity may be due to contaminated water, overcrowding and poor sanitation. Among 38(44%) EPEC, 21(55%) contained *eae* gene and 14(37%) contained *bfp* gene and these 92%(55%+37%) were considered as atypical EPEC(as contain only *eae* or *bfp* gene), 3(8%) were typical EPEC as they contained both *bfp* and *eae* genes.

Regarding ETEC, it was 26(30.5%) and the second frequent type of DEC. Similarly, Roy *et al*, in Bangladesh and Arif and Salih, in Iraq reported the isolation rate of ETEC was 35.29% and 26.3% respectively.[4,22] On the contrary,Yang *et al.* observed 66.7% ETEC were associated with diarrhea in Taiwan.[24] The results of a study in Vietnam by Nguyen *et al.*, in Mozambique by Mandomando *et al.,* and in Brazil by Bueris *et al.*, reported 2.2%, 6.8% and 3.7% of ETEC.[11,25,26] Among 26(35.5%) identified ETEC, 11(42%) contained *st* gene, 6(23%) contained lt gene and 9(34.6%) contained both *st*and *lt* genes. In Bangladesh, in a study by Nessa *et al., st* gene was 51.3%, *lt* gene was 25.6% and both *st+lt* genes was 23.1% and in another study by Roy *et al.,*st gene was 37.5%, *lt* gene was 29.17% and *st+lt* genes was 33.33%.[4,6] These findings are consistent with our study.

In the present study, EAggEC was 20(23.5%). Our study is similar with the study of Vilchez *et al.,* (2009) in Nicaragua and Roy *et al.,* (2014) in Bangladesh, they reported 27.8% and 26.4% of EAggEC among diarrheic children.[4,27] Now a days, EAggEC is emerging as an enteric pathogen and responsible for acute and most prominently persistent diarrhea (>14 days) and may cause malnutrition and growth retardation. These malnourished children are more prone to infection like diarrhea and the cycle continues. This strain have been associated with traveler's diarrhea in both developing and developed countries and have been isolated in immune compromised patients.[28]

In the present study, EIEC was 1 (1.1%) among DEC. A study carried out by ICDDR,B, 2002, in which no EIEC strain was detected.[29] Prats *et al.,* detected 0.2% EIEC among DEC positive cases in Spain in 2003.[30] Nguyen *et al.,* detected 2% cases of EIEC in 2005 in Vietnam[11]. The low infection rate of EIEC is nearly similar with our study.

EIEC is not frequently detected in developing countries of Africa and Asia and it is associated with occasional food borne or waterborne outbreaks.[31]

In the present study, among 63 DEC positive cases, 26(41.2%) contained more than one pathogenic genes in various combinations . Among co infections, ETEC +EPEC were 8(30.7%), ETEC+EAggEC were 6(23%), EPEC+EAggEC were 4(15.5%), ETEC+EPEC+EAggEC were 2(7.6%). In Bangladesh, Roy *et al.,* (2014) reported among the combination of DEC, EPEC + ETEC was 6 (27.27%), ETEC+EAggEC was 4(18.18%), EPEC+EAggEC and ETEC+EPEC+EAggEC were both in 3(13.6%) which were similar with our study.[4]

The findings of this study showed that DEC is a common cause of childhood diarrhea in Bangladesh. By using multiplex PCR assays, in one PCR reaction DEC can be diagnosed which can be a rapid and reliable diagnosis of diarrhea. If detection of DEC is done by multiplex PCR in tertiary hospitals or in clinical laboratory, it will be a great achievement to treat acute diarrheal infection in paediatric population.

References

1. WHO. Diarrhoeal diseases, 2010. Last accessed on June 28, 2016. Available at HO.int/vaccine_resarch/.../index.html.
2. Black RE, Cousens S, Johnson HL. For the child health epidemiology reference group of WHO and UNICEF. Global, regional and national causes of child mortality in 2008; a systemic analysis. Lancet 2010; 375: 1969-87.
3. Guerrant RL, Hugher JM, Lima NL, Crane J. Diarrhoea in developed and developing countries; magnitude, special settings and etiologies. Rev Infect Dis 1990; 12(1): 41-50.
4. Roy S, Shamsuzzaman SM, Mamun KZ. Molecular Detection of Diarrheagenic*Escherichia coli* from children with Acute Diarrhoeain Tertiary Care Hospitals of Dhaka, Bangladesh. *Asian J Med Sci* 2014; 5(2): 59-66.
5. Piechulek H, Al Sabbir A, Mendoza-Aldana. Diarrhoea and ARI in rural areas in Bangladesh. *J Trop Med* 2003; 34(2): 337-42.
6. Nessa K, Ahmed D, Islam J, Kabir FML, Hossain MA. Usefulness of multiplex PCR for detection of diarrhoeagenic*Escherichia coli* in a diagnostic microbiology laboratory setting Bangladesh. *J Med Microbiol* 2007; 01(02): 38-42.
7. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Esch. coli. Nat Rev Microbiol* 2004; 2: 123-140.
8. Donnenberg MS. Enterobacteriaceae. In: Mandell GL, Bennett JE, Dolin R. editors: Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: Elsevier Churchill Livingstone, USA. 2005: pp 2567-86.
9. Oscar G Duarte, Arzuza O, Urbina D, *et al*. Detection of *Escherichia coli*Enteropathogens by Multiplex Polymerase Chain Reaction from Children's Diarrheal Stools in Two Caribbean-Colombian Cities. *Foodborne Pathog Dis* 2010; 7(2): 199-206.
10. Nataro JP, Kaper JB. Diarrheagenic*Escherichia coli.ClinMicrobiol Rev* 1998; 11(1): 142-201.
11. Nguyen TV, Le Van P, Le Huy C, Gia KN, Weintraub. A Detection and characterization of diarrhoeagenic*E.coli*from young children in Hanoi, Vietnam. *J ClinMicrobiol* 2005; 43(2): 755-60.
12. Toma C, Lu Y, Higa N, *et al.* Multiplex PCR Assay for Identification of Human Diarrheageni c*Escherichia coli. J Clin Microbiol* 2003; 41(6): 2669-71.
13. Dutta S, Chatterjee A, Dutta P et al. Sensitivity and performance characteristics of a direct PCR with stool samples in acomparison to conventional techniques for diagnosis of *Shigella* and enteroinvasive*Escherichia coli* infection in children with acute diarrhea in

Calcutta, India. *J Med Microbiol* 2001; 50: 667-674.
14. LanataCF, Mendoza W, Black RE. Improving diarrheal estimates. World Health Organization 2002.
15. Akbar SA. A study of *Escherichia coli* Isolated from children with diarrhea in Kalar. (MS. Thesis) College of Eduation-Kalar, University of Sulaimani, Sulaimaniah, Iraq; 2008.
16. Qadri F, Saha A, Ahmed T, Al Tarique A, Begum YA, Svennerhlm AM. Disease burden due to enterotoxigenic *Esch. coli* in the first 2 years of in an urban community in Bangladesh. *Infect Immun* 2007; 75(8): 3961-8.
17. Nweze EI. Virulence Properties of diarrheagenic*E.coli* and etiology of diarrhea in infants, young children and other age groups in Southeast, Nigeria. *American-Eurasian J Sci Res* 2009; 4(3): 173-9.
18. World Health Organization. Position Paper: Rotavirus Vaccines. Weekly Epidemiological Record, Geneva: *WHO*.2007; 82(32): 285-95.
19. Seigel RR, Santana E Sant'anna C, Salgado K, De Jesus P. Acute Diarrhea among Children from High and Low Socioeconomic Communitiesin Salvador, Brazil. *Int J Infect Dis* 1996;1: 28-34.
20. Al-Gallas N, Bahri O, Bouratbeen A, Ben-Haasen, Ben-Aissa R. Etiology of acute diarrheain children and adults in Tunis, Tunisia, with emphasis on diarrheagenic*Escherichia coli*: prevalence, phenotyping and molecular epidemiology. *Am J Trop Med Hyg* 2007; 77: 571-82.
21. Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhea. *J Med Microbiol* 2006; 55: 1159-63.
22. Arif SK, Salih IFL. Identification categories of diarheagenic *Escherichia coli* in stool samples by using multiplex PCR technique. *Asian J Med Sci* 2010; 2(5): 237-43.
23. Galane PM, Roux ML. Molecular epidemiology of *Escherichia coli* isolated from young South African children with diarrheal diseases. *J Health PopulNutr*2001; 19(1): 31-38.
24. Yang JR, Wu FT, Tsai JL *et al.* Comparison between O serotyping method and multiplex real time PCR to identify diarrhoeagenic*E.coli* in Taiwan. *J ClinMicrobiol* 2007; 45(11): 3620-25.
25. Mandomando IM, Macete EV, Ruiz J, *et al.* Etiology of diarrhea in children iyounger than 5 years of age admitted in a rural hospital of Southern Mozambique. *Am J Trop Med Hyg* 2007; 76(3): 522-7.
26. Bueris V, Sircili PM, Taddei CR *et al.* Detection of diarrheagenic*Escherichia coli* from children with and without diarrhea in Salvador, Bahia, Brazil. *MemInstOswaldo Cruz* 2007; 102(7): 839-844.
27. Vilchez S, Reyes D, Paniagua M, Bucardo F, Mo"llby R, Weintraub A. Prevalence of diarrheagenic *Escherichia coli* in children from Leo'n, Nicaragua. *J Med Microbiol* 2009; 58: 630-7.
28. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli.ClinMicrobiol Rev* 1998; 11(1): 142-201.
29. ICDDR,B 2002, Trends in Aetiologies for Diarrhoeal Diseases, Health and Science Bulletin, Vol 1, no. 1, pp 12-15.
30. Prats G, Mirelis B, Miro E. Cephalosporin-resistant *Escherichia coli* among summer camp attendees with salmonellosis, *Emerg Infect Dis*, 2003; 9: 1273-80.
31. Rappelli P, Folgosa E, Solinas ML, DaCosta JL, Colombo MM. Pathogenic enteric *Escherichia coli* in children with and without diarrhea in Maputo, Mozambique, *FEMS Immun and Medical Microbiol*, 2005; 43: 67-72.