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Prevalence of Multi-Drug Resistant Bacteria Associated with Diarrhoea among Infants in Ado Ekiti, Nigeria.

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Abstract

Bacteriological investigations were carried out on faecal samples of 76 patients, less than one year of age, attending paediatric clinic of Ekiti State University Teaching Hospital Ado Ekiti and two Primary Health Centres in Ado Ekiti, on diarrhoea related illnesses; in the year 2013. The bacteria isolated were *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Corynebacterium accolens*, *Morganella morgani*, *Aeromonas popoffii*, *Citrobacter freundii*, *Leteococcus sanguinis*, *Branchiibius cervicis*, *Aeromonas bestiarum*, *Vibrio minicus*, *Aeromonas caviae*, *Proteus mirabilis*, *Serratia marcescens*, *Enterobacter aerogenes*, *Leminorella grimontii*, *Citrobacter youngae*, *Bacillus cereus*, *Citrobacter koseri*, *Enterobacter intermedius*, *Yersinia enterocolitica*, *Providencia stuartii*, *Pantoea agglomerans*, *Vibrio fluvalis*, *Vibrio natiensis*, *Salmonella enterica*, *Citrobacter sedlakii*, *Klebsiella variicola*. The bacterial isolates showed high resistance to many of the antibiotics tested. High prevalence of multidrug resistant bacteria was recorded. The Gram positive bacteria showed high resistance to most of the antibiotics used. The Gram negative bacterial isolates were 100% susceptible to Levofloxacin and ofloxacin, with a 100% resistant to amoxicillin and varied resistance to other antibiotics. In general, for the gram negative bacterial isolates, the penicillins are the most ineffective group of antibiotics, while the quinolones are the most efficacious. Resistance to high concentrations of penicillins was obtained. The prevalence of multidrug reported in the study could lead to its failure of antibiotic therapy and prolong hospitalization of diarrhoeic infants.

Key words: Bacteria, diarrhoea, infant, multi drug resistance

Introduction

There are many causes of diarrhoea in infants which include viruses, bacteria and parasites (Navaneethan and Giannella, 2008), but the focus of this study is diarrhoea caused by bacteria infections,. The bacterium *Campylobacter* is a common cause of bacterial diarrhoea but infections by *Salmonella*, *Shigella* and some strains of *Escherichia coli* are frequent (Viswanathan *et al* .,2009). Although diarrheagenic *E. coli* pathotypes are of public health relevance, they are not routinely sought as enteric pathogens in clinical laboratories worldwide; thus, their incidence in children less than 2 years of age and their importance in community-acquired diarrhoea are generally unknown, particularly in areas of endemicity.

Diarrhoea can alter the normal balance of water and salts (electrolytes). When too much water is lost in diarrhoea, babies can become dehydrated. Dehydration can happen very quickly in babies within a day or two after the diarrhoea starts and it can be very dangerous, especially in newborns. (Alli, 2012).

Antibiotics have revolutionized the treatment of common bacterial infections and play a crucial role in reducing mortality. Antimicrobial therapy should be used in severe cases of diarrhoeal diseases to reduce the duration of illness and may be used to prevent traveller's diarrhoea (Nataro and Kaper 1998). However, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. In addition, the overuse and misuse of antibiotics in the treatment of diarrhoea could lead to an increase of antibiotic resistance (Chuc *et al*., 2002). Many people in developing countries could easily buy antibiotics without doctor's prescription. As a result of this, many infants with symptoms of illnesses such as diarrhoea may have been empirically treated with antibiotics without advice from medical personels (Doung *et al*.,1997), which might have resulted in the resistance of the diarrhoea causing bacteria to antibiotics (Chuc and Tomson, 1999).

This study is designed to determine the drug resistance pattern of diarrhoea causing bacteria among the infants in Ado Ekiti, while using the infant patients of Ekiti State University Teaching Hospital as case study.

Materials and Methods

Study Area and Population

The study was conducted at Ekiti State University Teaching Hospital, Ado Ekiti, Nigeria and two primary health centres in Ado Ekiti, over a six month period (Feb-July, 2013). Ethical clearance was obtained from Ethical Review Committee of the teaching hospital prior to carrying out this study. Investigations were carried out on patients in the paediatric ward and faecal samples were obtained with sterile universal bottles, from seventy six patients, made up of 32 males and 44 females, attending the hospital/health centres on diarrhoea related illnesses. Information was obtained from each patient as regards age, sex, clinical signs and previous treatment pattern. Microbiological investigations were carried out on the faecal samples at the Microbiology Laboratory, Afe Babalola University, Ado Ekiti, Nigeria.

Collection and processing of samples

The faecal samples were collected before starting antibiotic therapy in the hospital/clinics; this helped in the determination of antibiotic resistant pattern of diarrhoeal bacteria. Small quantities of faecal samples were collected in sterile universal bottles, the bottles were labelled appropriately and the specimens were taken to the laboratory they were processed within two hours of collection.

Inoculation, isolation, characterization and identification

The collected samples were cultured in duplicates on Nutrient agar plates and incubated aerobically at 37^0C for 24hrs. Each representative colony of bacteria was selected from each plate and purified by sub-culturing into plates of nutrient agar and thereafter subcultured into slants which were stored at 4^0C isolates. Cultures were Gram-stained and morphologies of the organisms observed under the microscope.
Biochemical tests were carried out on the bacterial isolates as described by Barrow and Feltham (1993). Identification of microorganisms, based on cultural, microscopic and biochemical characteristics, was determined using an online bacteria identification system, the Gideon Informatics (1997-2011), with reference to Barrow and Feltham (1993) and Garrity et al (2005).

Antibiotic Susceptibility Test

Susceptibility test was determined using antibiotic disc after due sub-culturing. Briefly, the isolates were inoculated in Muller Hinton agar plates by streaking evenly on the agar surface. Antibiotic discs were placed on the set agar plates, allowed to equilibrate at room temperature for 15 minutes and finally incubated at 37^0C for 24h. Thereafter, the plates were observed for obvious zone for clearing. The zones of inhibition were measured and recorded according to Clinical Laboratory Standards Institute (CSLI, 2013).

Assay for susceptibility of bacterial isolates to high concentrations of penicillins.

Mueller-Hinton agar plates were prepared and bored aseptically to create wells in the plates. The plates were streaked with the test organisms adjusted to McFarland standard, after which the different dilutions of the penicillins tested were introduced in the wells (each dilution to each well). Ceftazidine, a cephalosporin, was equally tested for comparison with the penicillins. The plates were incubated for 24hrs at 35^0c and the zones of inhibition were measured and recorded as described by Clinical Laboratory Standards Institute (CLSI, 2012 & 2013).

Statistical analysis

Paired *t* test was used to test for significant difference in the distribution of organisms along gender, using SPSS 16.0 window.

Results

Bacteriological investigations were carried out on faecal samples of 76 patients less than one year of age, 32 males and 44 females, with diarrhoea related cases. All the samples collected showed presence of mucor while only 2% showed presence of blood. Presence of mucor and blood in faeces often indicate a gastrointestinal bacterial infection. In the study group,the isolation of *Escherichia coli* was 15.38%, *Proteus vulgaris* (19.2%), *Klebsiella varicola* (3.85%), *Pseudomonas aeruginosa* (3.85%), *Corynebacterium accolens* (19.2%), *Morganella morganii* (3.85%), *Aeromonas popoffii* (7.69%), *Citrobacter freundii* (11.5%), *Luteococcus sanguinis* (7.69%), *Bacillus cereus* (15.38%), *Aeromonas bestiarum* (19.20%), *Vibrio minicus* (11.5%), *Aeromonas caviae* (7.69%), *Proteus mirabilis* (11.5%), *Serratia marcescens* (3,85%), *Enterobacter intermedius*

(3.85), *Yersinia enterocolitica* (3.85), *Providencia stuartii* (3.85%), *Pantoea agglomerans* (3.85%), *Vibrio fluvalis* (3.85%), *Vibrio natiensis* (7.69%), *Salmonella enterica* (3.85%) and *Citrobacter sedlakii* (3.85) (Figure 1). The distribution of the bacterial isolates along gender is presented in Figure 2. No significant difference in distribution of the bacteria along gender was determined (t = 0.284: p = 0.779).

High resistance to multiple drugs were recorded among the bacteria isolated (Table 1 & Figure 3). The Gram positive bacteria were highly resistant to all the antibiotics used; 100% each for cotrimazole, cloxacillin and erythromycin and 96.15, 76.92, 73.08, 69.25 and 61.54 respectively for Tetracycline, augumentin, Streptomycin, Chloramphenicol and gentamycin (Figure 3).

The gram negative bacterial isolates were 100% susceptible to Levofloacin and ofloacin, 100% resistant to amoycillin with varied resistance to other antibiotics. The gram negative bacteria are resistant to multiple drugs. The penicillin group of drugs were the most ineffective antibiotics, while the quinolones are the most efficacious (Table 1).

For Gram negative bacteria were resistant to high concentrations of penicillins. While most of the penicillins did not produce any zone of inhibition, Ceftazidine, a cephalosporin, gave MIC of 3µg/ml (the least concentration used) for 11 out of the 20 bacteria (Table 3).

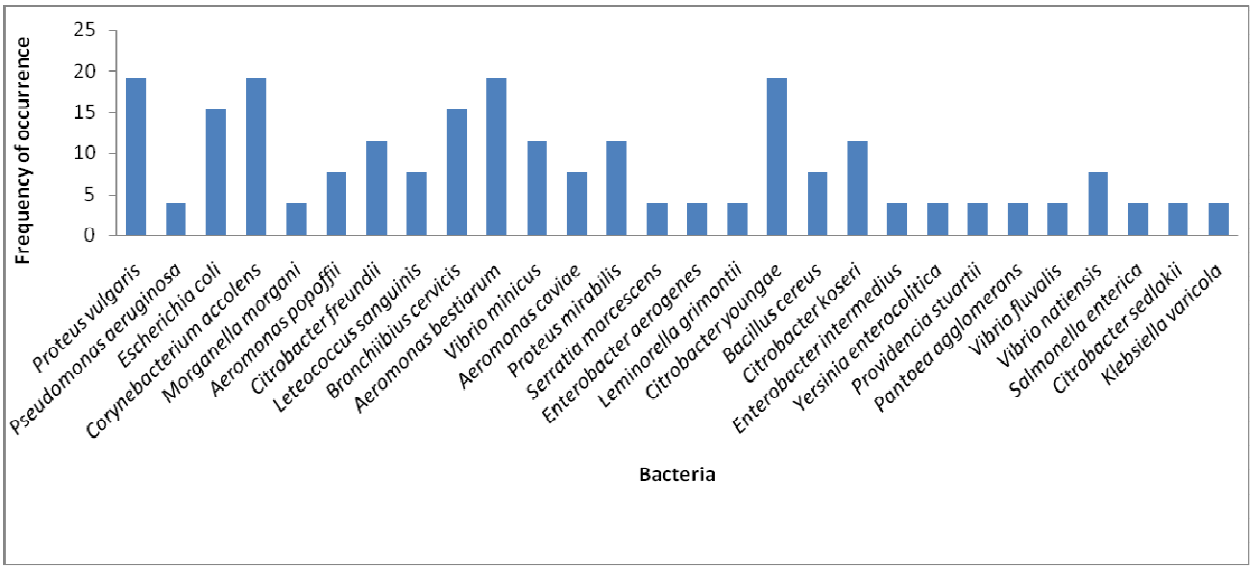

Figure 1: Frequency of isolation of bacteria from stool samples

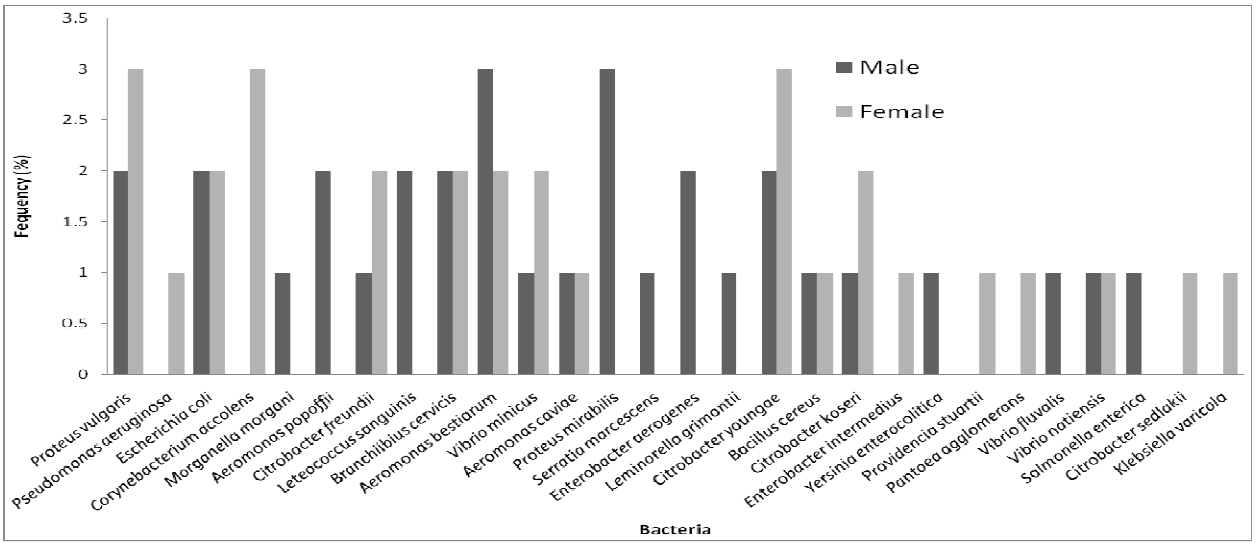

Figure 2: Distribution of bacterial isolates from stool along gender (t = 0.284, P = 0.779).

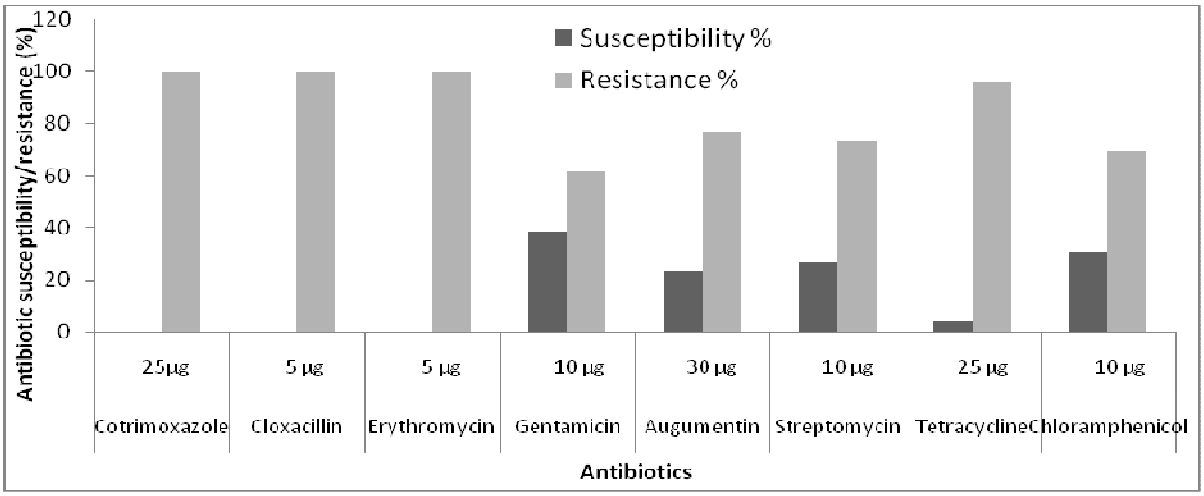

Figure 3: Susceptibility of Gram positive bacteria isolated from stool to antibiotics

Table 1: Resistance of gram negative bacteria isolated from stool to antibiotic groups

Group	Antibiotics	Resistance(%)
A	PENICILLINS	
	Ampicillin, AMP 10µg	95
	Augumentin (Amoxycillin/clevulanic acid), AUG 20/10µg	99
	Amoxycillin, AMX 20µg	100
B	CEPHALOSPORINS	
	Ceftriazone, CRO 30µg	32
	Ceftazdine, CAZ 30µg	37.5
C	AMINOGLYCOSIDES	
	Gentamycin, GEN 10µg	57
	Clarithomycin, CLR	62
	Tetracycline, TET 30µg	91
D	QUINOLONES	
	Nalidixic acid, NAL 30µg	42
	Ciprofloxacin, CIP 5µg	8
	Levofloxacin, LEV 10µg	0
	Perfloxacin, PEF	3
	Ofloxacin, OFL 5µg	0
E	ERYTHROMYCIN, ERY 15µg	91
F	TRIMETHOPRIM/ SULPHAMETHAZOLE, COT 1.25/23.75µg	77.5
G	NITROFURANTOIN, NIT	
	NIT 100µg	77
	NIT 200µg	26
H	CHLORAMPHENICOL, CHL 30µg	37

Table 2: Cluster of antibiotic resistant exhibited by bacteria isolated from stool

S/N	Drug combination	Frequency
	Gram positive	
1	COT/CLO/ERY/GEN/AUG/STR/TET/CHL	6
2	COT/CLO/ERY/GEN/AUG/STP/TET	3
3	COT/CLO/ERY/AUG/STR/TET	1
4	COT/CLO/ERY/TET/CHL	2
	Gramm negative	
1	AMP/AMX/NIT/COT/GEN/AUG/CHL/CLR/TET/CAZ	1
2	AMP/AMX/NIT/CRO/COT/GEN/AUG/TET/CAZ	1
3	AMP/AMX/NIT/COT/GEN/AUG/CLR/TET	1
4	AMX/ COT/GEN/AUG/CLR/TET/CAZ	1
5	AMP/AMX/NIT/COT/GEN/AUG/CLR/TET	1
6	AMP/AMX/NIT/COT/AUG/CLR/TET	1
7	AMP/AMX/NIT/COT/AUG/CHL/CLR/TET	1
8	AMP/AMX/NIT/CRO/COT/GEN/AUG/CHL/CLR/TET/CAZ	1
9	AMP/AMX/CRO/COT/GEN/AUG/TET	1
10	AMP/AMX/NIT/COT/GEN/AUG/TET/CIP	1
11	AMP/AMX/CRO/COT/GEN/AUG/TET/CAZ	1
12	AMP/AMX/NIT/COT/AUG/CHL/CLR/TET/CAZ	1
13	AMP/AMX/NIT/COT/GEN/AUG/CHL/CLR/TET/CAZ/NAL	1
14	AMP/AMX/NIT/COT/AUG/TET/CIP	1
15	AMP/AMX/NIT/CRO/GEN/AUG/PEF/CAZ/NAL/CIP	1
16	AMP/AMX/ COT/ AUG//TET/CAZ	1
17	AMP/AMX/NIT/CRO/COT/AUG/CLR/TET	1
18	AMP/AMX/NIT/CRO/AUG/TET/CIP	1
19	AMP/AMX/NIT/CRO/GEN/AUG/CLR/TET/NAL	1
20	AMP/AMX/NIT/CRO/COT/GEN/AUG/CHL/CLR/TET/NAL	1

Table 3: Susceptibility of selected drug resistant Gram negative bacterial isolates to high concentrations of penicillins compared to ceftazidine

S/N	Bacteria	PENICILIN G						AMPICILIN						AMOXICILIN						AMPICLOX						C EFTAZIDINE					
		100 µg	50 µg	25 µg	12.5 µg	6.25 µg	3.13 µg	100 µg	50 µg	25 µg	12.5 µg	6.25 µg	3.13 µg	100 µg	50 µg	25 µg	12.5 µg	6.25 µg	3.13 µg	100 µg	50 µg	25 µg	12.5 µg	6.25 µg	3.13 µg	100 µg	50 µg	25 µg	12.5 µg	6.25 µg	3.13 µg
1	*Proteus mirabilis*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	26	25	21	19	11
2	*Aeromonas bestiarum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	*Enterobacter aerogenes*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	21	13	12	1	0
4	*Vibrio mimicus*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	12	10	9	3	0
5	*Yersinia entercolitica*	0	0	0	0	0	0	0	0	0	0	0	0	6	4	0	0	0	0	0	0	0	0	0	0	21	18	5	12	8	3
6	*Aeromonas bestiarum*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	5	3	0	0	0	22	19	15	8	9	6
7	*Pseudomonas aeruginosa*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	6	1	0	0	0
8	*Aeromonas papoff*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	*Cirtobacter freundi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31	26	23	13	13	6
10	*Proteus vulgaris*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24	20	19	19	14	5
11	*Citrobacter youngae*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23	22	20	15	13	7
12	*Pantoea agglomerans*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	*Escherichia coli*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	24	19	16	27	11
14	*Citrobacter koseri*	0	0	0	0	0	0	0	0	0	0	0	0	7	3	0	0	0	0	0	0	0	0	0	0	20	16	12	7	6	4
15	*Enterobacter intermidius*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	23	20	17	14	11
16	*Liminorella grimontii*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	19	17	15	14	11
17	*Serratia mascesens*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	6	6	4	0	0	0	0	0	0	0
18	*Proteus mirabilis*	0	0	0	0	0	0	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0	0	0	31	27	24	22	20	18
19	*Cirtobacter freundii*	0	0	0	0	0	0	0	0	0	0	0	0	6	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	*Enterobacter intermidius*	0	0	0	0	0	0	0	0	0	0	0	0	10	7	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	*Escherichia coli* ATCC 25922	29	26	24	22	15	10	25	20	19	14	12	9	22	21	19	15	9	4	23	20	19	14	10	5	33	29	25	21	15	10

Values are in mm

Discussion

Diarrhoea is a common cause of death in developing countries and the second most common cause of infant deaths worldwide (WHO, 2009). The progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical issue of concern. In addition, the overuse and misuse of antibiotics in the treatment of diarrhoea could lead to an increase of antibiotic resistance (Chuc *et al*., 2002). Many people in developing countries can easily buy antibiotics without doctor's prescription due to the privatization in the market economy of the country including drug provision. As a result of this, many infants with symptoms of illnesses such as diarrhoea may have been treated with antibiotic preparations without advice from medical personels (Doung *et al*.,1997), which might have resulted in the resistance of the diarrhoea causing bacteria to antibiotics (Chuc andTomson, 1999).

In the present study, 76 diarrhoeal cases of both sexes, under one years of age were studied. The male: female ratio was 52 %: 48 % in this study indicating only a slight male preponderence which is in agreement with the previous workers (Joshi *et al*, 1980). In our study, all the samples collected showed presence of mucor while only 2% showed presence of blood. Presence of mucor and blood in faeces often indicate a gastrointestinal bacterial infection. The *Escherichia coli* and *Klebsiella* rate of isolation (15.38 & 3.58% respectively) obtained in this study correlates well with another study which shows an isolation of *Escherichia coli 21.1%* and *Klebsiella* (2.8%). (Khanna *et al.*, 1977). *Echerichia coli* isolation rate of 15.38% in this present is in contrast with earlier report of Joshi and co-workers (1980) which had an *Escherichia coli* isolation rate of 82%. This contrast indicates that many other bacteria species apart from *Escherichia coli* can also cause diarhoea in infants, although the pathogenicity of organisms other than *Escherichia coli*, *Salmonella enterica*, *Yersinia enterocolitica* and *Vibrio species* in diarrhoea cases is controversial. However when these suspected pathogens are isolated in pure culture or in significant numbers and in the absence of other definite pathogens, their presence cannot be ignored. All these organisms were isolated in pure culture.

The organisms were 100% resistant to Amoycillin, Cloxacillin and Erythromycin. There was also a high degree of resistance to Ampicillin (95%) ,Cotrimoxazole (87%), Augumentin (87.96 %), Tetracycline (93.57%), Gentamicin (59.27) and 100ug Nitrofurantoin (86%) exhibited by all the bacteria isolated in this study, although gram positive organisms showed more resistance to the antibiotics tested. The organisms showed high level of susceptibility to some antibiotics such as Ofloxacin (100%), Pefloxacin (97%), Ciprofloxacin (92%), Levofloxacin (100%), 200ug Nitrofuratoin (74%), Cetriazone (68%) and Nalidixic acid (58%). The antimicrobial resistant pattern of the bacteria isolated in this study agrees with the work Teresa and co-workers (Teresa *et al.*, 2005) which showed 65 % resistance to Cotrimoxazole, 75 % resistance to Ampicillin and no resistance to Ciprofloxacin. In another study (Bartelesi & Bartolona, 2006), high resistance rates to Ampicillin (95%) and,Cotrimoxazole (84 %) were seen which correlates with our study. Some of the gram positive bacteria isolated in this study were resistant to all the antibiotics tested.

Conclusion

High level resistance to first line antimicrobials in diarrhoeal cases is due to unselected use of these drugs in patients with a mild presentation with low risk for complications. The choice of antimicrobial agent has to be made empirically; it should consist of the narrowest antimicrobial spectrum that covers the most likely pathogens. Also, routine use of antibiotics for infectious diarrhoea in children must be avoided as it brings little benefit in most cases. Further, periodic monitoring of drug resistance in enteric pathogens should be carried out in each geographical area so that an appropriate agent can be chosen for empiric therapy. This could lead to not only control of drug resistance but also decrease the financial burden on the community.

References

Barrow, G.I. and Feltham, R.K.A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge University Press, London. 331pp.

Chuc, N. T. and Tomson,G . (1999). "Doi moi" and private pharmacies: a case study on dispensing and financial issues in Hanoi, Vietnam. *European Journal Clinical of Pharmacology*, 55:325-332.

Chuc, N. T., Larsson M., Diwan,V.K., Tomson, G.B. and Falkenberg, T. (2002). Improving private pharmacy practice: a multi-intervention experiment in Hanoi, Vietnam. *Journal of Clinical Epidermiology,* 55:1148-1155.

Clinical and Laboratory Standards Institutes, CLSI (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – 9th Edition, CLSI, Wanye, USA.

Clinical and Laboratory Standards Institutes, CLSI (2013). Performance Standard of Antimicrobial Susceptibility Testing; 23rd Information Supplement, CLSI, Wanye, USA.

Garrity, G.M., Brenner, D.I., Krieg, E.R. and Stanley, J.T.(2005). *Bergey's Manual of Systemic Bacteriology*, 2nd edition, volume 2. Springer-Verlag, New York.

Gideon Informatics. (1994-2013). *Gideon- Microbiology- Identify Bacteria*. Web. www.gideononline.com.

Isenbarger, D. W., Hoge,C.W., Srijan,A., Pitarangsi,C ., Vithayasai, N., Bodhidatta, L., Hickey, K.W. and Cam, P.D. (2002). Comparative antibiotic resistance of diarrhoeal pathogens from Vietnam and Thailand, 1996-1999. *Emerging Infectious Diseases,* 8:175-180

Joshi, C.K., Bhardwaj, A.K. and Vyas, B.L. (1980). *Indian Journal of Pediatrics,* 47: 307 – 310.

Khanna, K.K., Ramanathan, A.L.and Puri, R.K. (1977). *Indian Journal of Pediatrics*, 44(354): 169-175.

Mims, C., Dockrell, H.M., Goering, R.V., Wakelin, D and Zuckerman, M. (2005). *Medical Microbiology* 3rd Edition Elsevier Mosby Spain 660pp.

Navaneethan, U. and Giannella, R.A. (2008). Mechanisms of infectious diarrhoea. *Nature Clinical Practice; Gastroenterology & Hepatology,* 5 (11): 637–47.

Teresa, E.S., Jorge, F. and Cema, C. (2005). Drug-resistant Diarrhoeagenic Esch. coli, Maxico" *Emerging Infectious Diseases*. 2005 Aug;:1–5

Viswanathan, V.K., Hodges, K. and Hecht, G. (2009). Enteric infection meets intestinal function: how bacterial pathogens cause diarrhoea. *Nature Reviews in Microbiology,* 7(2): 110–9.

World Health Organization**.** (February 2009). Diarrhoeal Diseases. W.H.O. Geneva Available at: http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf