Study of Microscopy, culture and Immuno Assays in the laboratory Diagnosis of Acute Diarrheal Diseases

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ABSTRACT: Diarrheal diseases are a major public health concern and are the leading cause of child mortality & morbidity & mostly results from contaminated food & water sources. Acute infectious diarrhea results from various viral, bacterial & parasitic infestations. Acute diarrhea presents with two basic syndromes- watery diarrrhea and blood and mucous diarrhea (dysentery). Rotavirus infection accounts for 20% to 60% of all diarrhoeal episodes and is the major cause of acute diarrhoea in <5 years of age.

AIMS & OBJECTIVES :
1. Isolate and identify bacterial, viral & parasitic agents causing acute diarrheal disease.
2. Perform Antibiotic susceptibility of bacterial isolates.
3. Perform ELISA for Rotavirus antigen detection.

MATERIALS & METHODS : 100 Fecal samples from patients suffering from acute diarrheal disease admitted to SRRIT&CD from March 2015 to August 2015 constituted the material of the study. They were examined macroscopically and microscopically & were processed as per standard guidelines. Elisa for Rotavirus antigen detection was performed.

RESULTS : Out of 100 samples processed, Shigella species were isolated in 19%, Salmonella in 3%,Vibrio cholera in 4%, Escherichia coli in 6% of cases. Among Parasitic agents, Entamoeba histolytica in 22% , Cryptosporidium oocyst in 7%, Rotavirus detected in 14.5 % of cases

CONCLUSION: Diarrheal diseases are mostly of infectious origin. The most important factors contributing are low socio economic status, poor sanitation and lack of awareness. Hence, prompt identification of causative agent helps in controlling the diarrheal illness & in preventing spread of the disease in the community.

I. INTRODUCTION:

Diarrheal diseases are a major public health concern for both developed and developing countries. They are a leading cause of childhood morbidity & mortality in developing countries & an important cause of malnutrition[1]. Diarrheal diseases are the second leading causes of death in children under 5 years old & are responsible for killing around 760,000 children every year[2]. Diarrhea is defined as the passage of loose, liquid or watery stools. These liquid stools are usually passed more than three times a day. However, it is the recent change in consistency & character of stools rather than the number of stools that is more important. The term 'Diarrheal Diseases' should be considered only as a convenient expression not as an epidemiological entity- for a group of diseases in which the predominant symptom is diarrhea[3]. It should be distinguished from 'Pseudodiarrhea' which is the frequent passage of small volume of stools, which is often associated with rectal urgency & accompanies IBS or Proctitis[4]. Diarrhea may be further defined as acute if < 2 weeks, persistent if 2-4 weeks & chronic if > 4 weeks in duration[4]. Diarrhea can be further recognized into 4 clinical types depending on underlying pathology & altered physiology- Acute watery Diarrhea, Acute Bloody Diarrhea, Persistent Diarrhea, Diarrhea with severe malnutrition[3].

Acute infectious diarrhea results from various viral, bacterial & parasitic infections & it is most frequently of infectious origin[1].Rotavirus infection accounts from 20% to 60% of all diarrhoeal episodes in developing and developed countries and is the major cause of acute diarrhoea in young children under five years of age. Although rotavirus infections are usually mild, they may lead to more pronounced watery stool loss, which may sometimes lead to severe dehydration. The WHO has estimated that rotavirus infection was responsible for 453,000 deaths in children under 5 years in 2008, accounting for 37% of diarrhoea-related deaths[1]. Side effects from medications are probably the most common non infectious causes of acute diarrhea. Some are NSAIDS, Antibiotics, Anti hypertensives, Antacids & Laxatives[4]. Nonetheless in about 40% of the cases, no causative agent can be detected. The relative contribution of the different pathogens may vary depending on the specific geographical location &

on the season with acute diarrhea being predominantly of viral originin winter & of bacterial origin in summer. Bacterial pathogens are relatively more important in developing countries & viral pathogens relatively more important in developed countries[1].

Judicious use of antibiotics is appropriate in selected instances of acute diarrhea & may reduce its severity & duration[4]. Due to overuse of antibiotics & change in epidemiology & Antimicrobial resistance of bacteria agents, a study on local epidemiology of bacterial diarrhea & Antimicrobial susceptibility plays an important role in choosing the appropriate antibiotic for empirical treatment[5].

II. MATERIALS & METHODS

Fecal samples from patients suffering from acute diarrheal disease admitted to SRRIT&CD constituted the material of the study. 100 samples were processed as per standard guidelines during the period from March 2015 to August 2015. History of diarrhea was elicited from patients & details regarding number of stools passed per day, colour & consistency, whether bloody/mucus/watery/ricewater, presence of any foul smell, any passage of worms/ segments & other details like H/O fever, vomiting, tenesmus, pain abdomen, intake of outside food, history of travel & antibiotic intake.

Specimen Collection :-

Stool samples two in number were collected on admission in clean, non greasy wide mouth containers. At the time of collection of material special emphasis was laid on the following points :
-   Fresh stool samples were collected.

-   Samples were collected before antibiotic therapy.
-   The specimens collected were transported and processed immediately.
-   One sample stored at -20^{0}c for performing ELISA.

Naked eye examination was done & colour, consistency, odour, presence of mucus, blood were noted down.

Microscopic examination :-

Wet mount of stool samples was done with 0.9% normal saline & Lugol's Iodine for looking fecal leukocytes, RBC's, presence of parasitic ova, trophozoites or cysts. Modified Acid Fast staining was also performed. Samples were then cultured on enrichment, selective, non selective media. Faeces was directly inoculated on Blood Agar, Sorbitol MacConkey Agar. 2ml of faeces was inoculated in to enrichment broth like Alkaline peptone water, Selenite F broth for 6-8 hours of enrichment at 37^{0}c.

Heavy inoculum was inoculated from Selenite F broth on to Wilson & Blair Bismuth Sulfite, Deoxycholate Citrate Agar, Xylose Lysine Deoxycholate Agar. Simultaneously, a heavy inoculum from Alkaline Peptone Water was inoculated on to Thiosulphate Citrate Bilesalt Sucrose, Bile Salt Agar & inoculated overnight aerobically at 37^{0}c.
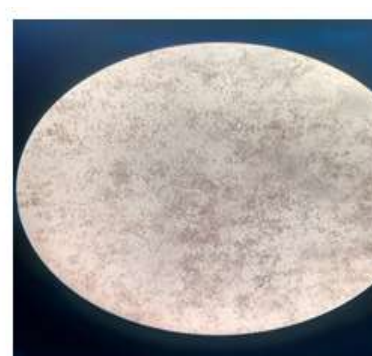
Inoculated plates were examined for colony morphology, any pigmentation, metallic sheen & fermentation of lactose in media. 2 or 3 colonies of same morphology or single colony is selected for further preliminary testing & biochemical reactions for identification of bacteria up to genus/species level.
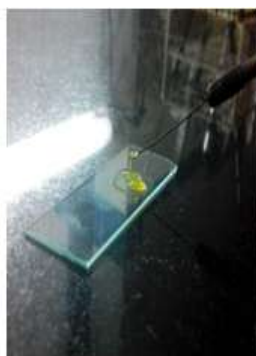
String test | Yellow coloured colonies on TCBS agar | Chick cell Agglutination

III. RESULTS:

Stool samples were collected from 100 patients admitted in gastroenteritis ward at Sir Ronald Ross Institute of Tropical & Communicable Diseases during the period from March 2015 to August 2015, to study the bacterial, viral, fungal & parasitic agents causing Acute Diarrhoeal Disease & to understand the antibiotic susceptibility pattern of bacterial isolates. These samples were collected and processed as per standard protocol. Out of 100 samples processed, 44(44%) samples were from males whereas 56(56%) samples were from females. The age of the patients ranged from one month old child to 79 years old adult. Highest positivity was seen in the age group >15 – 50 years.

Table 1. Symptom wise distribution of samples

Symptoms	Percentage
Fever	50%
Vomiting	51%
Pain Abdomen	22%

Table 1 shows the symptom wise distribution of samples. All the patients presented with loose stools.

Table 2. Macroscopic Appearance of stool samples

Number of Samples	Watery stools	%	Bloody stools	%	Mucous stools	%
100	51	51	30	30	19	19

Table 2 showed Watery stools (51%), Bloody stools(30%) and Mucous stools(19%)..

Table 3. Microscopic Appearance

Microscopy	Positives
RBCs	30
Pus cells	49
Entamoeba histolytica cyst	22
Cryptosporidium oocyst by Modified Acid Fast Staining	07

Table 4. Incidence of Enteric Pathogens isolated

Enteric Pathogens	Number
Single infection :-	
Shigellaspp	18
Salmonella spp	03
Vibrio cholera	04
Escherichia coli	06
Entamoeba histolytica cyst (Microscopy)	21
Cryptosporidium oocyst (Modified Acid Fast Staining)	06
Candida albicans (Culture)	02
Rota virus (ELISA)	07
Mixed infections :-	
Eh + Shigella	01
Candida + Cryptosporidium	01

Table 4 showed the incidence of enteric pathogens in the present study. Mixed infections of Entamoeba histolytica + Shigella and also Candida + Cryptosporidium had been observed.

CHART1.ISOLATION PATTERN OF ENTERIC BACTERIAL PATHOGENS

a
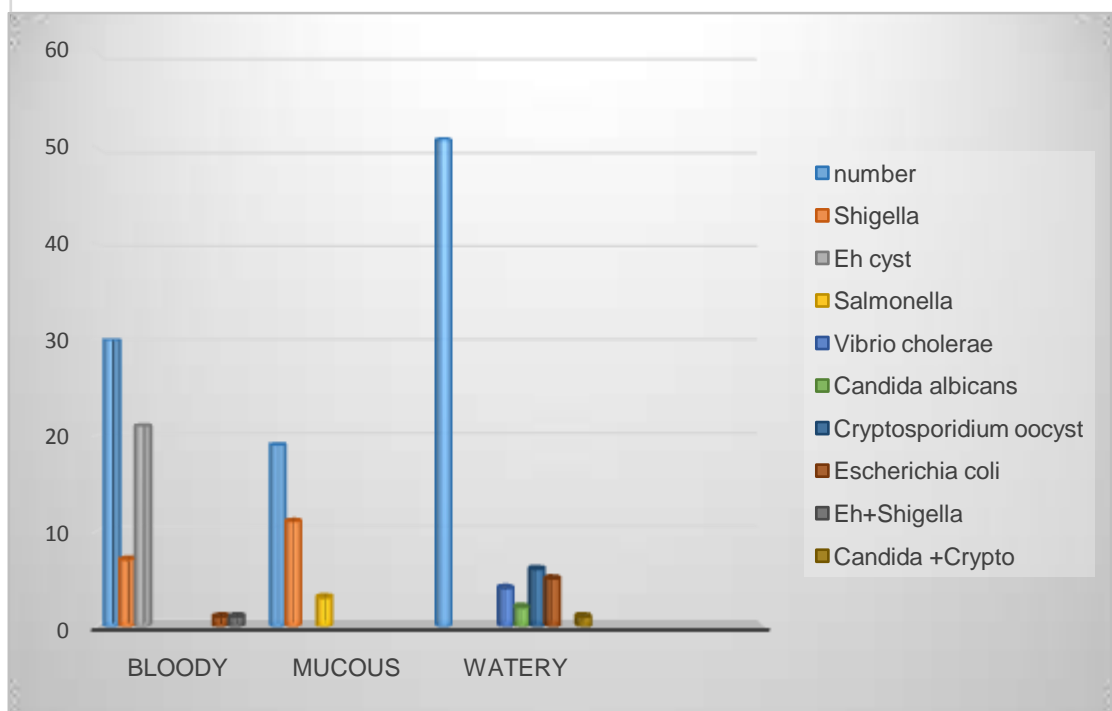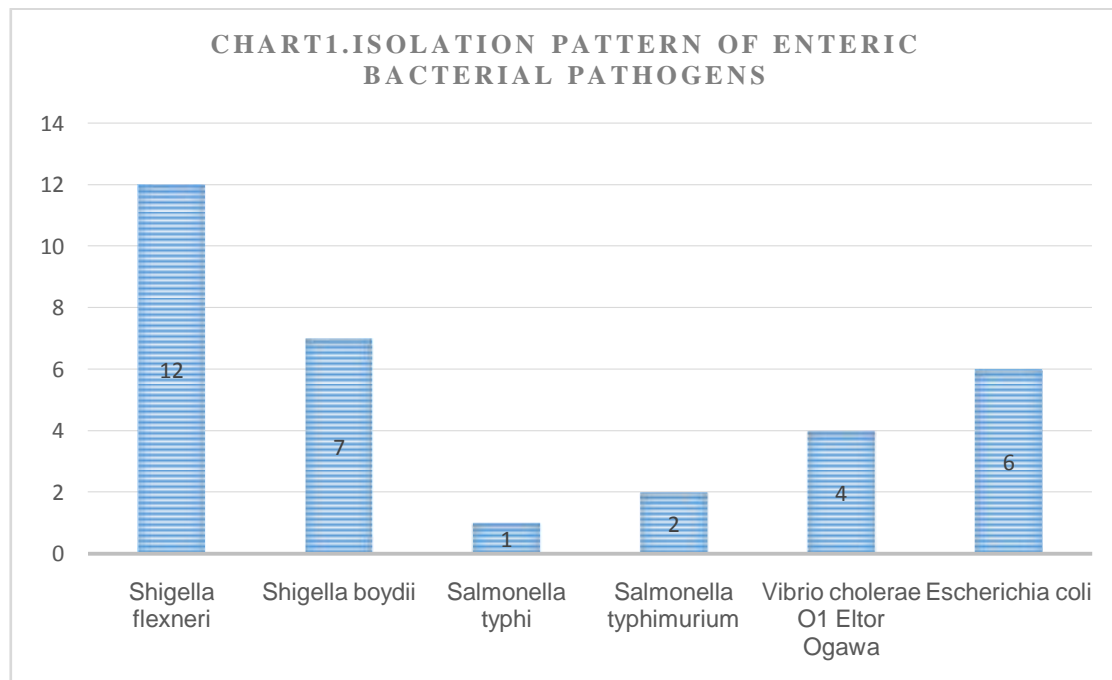
The above chart 2 shows isolation pattern in stool samples.
Antibiotic susceptibility was done to the isolates obtained according to standard guidelines and depited in the charts below.

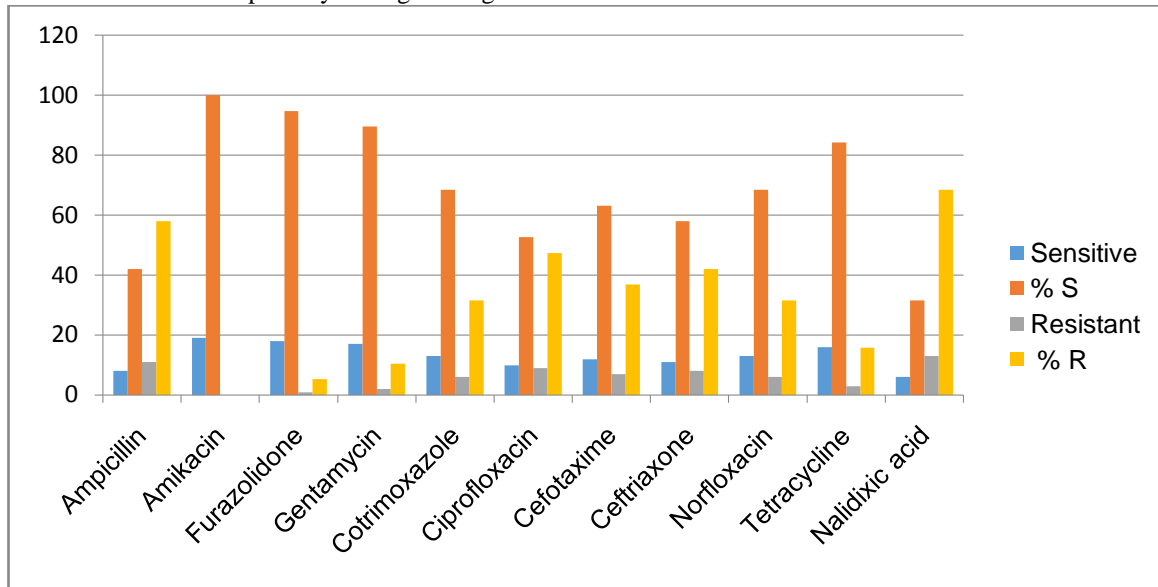Chart 3. Antibiotic susceptibility testing of Shigella isolates

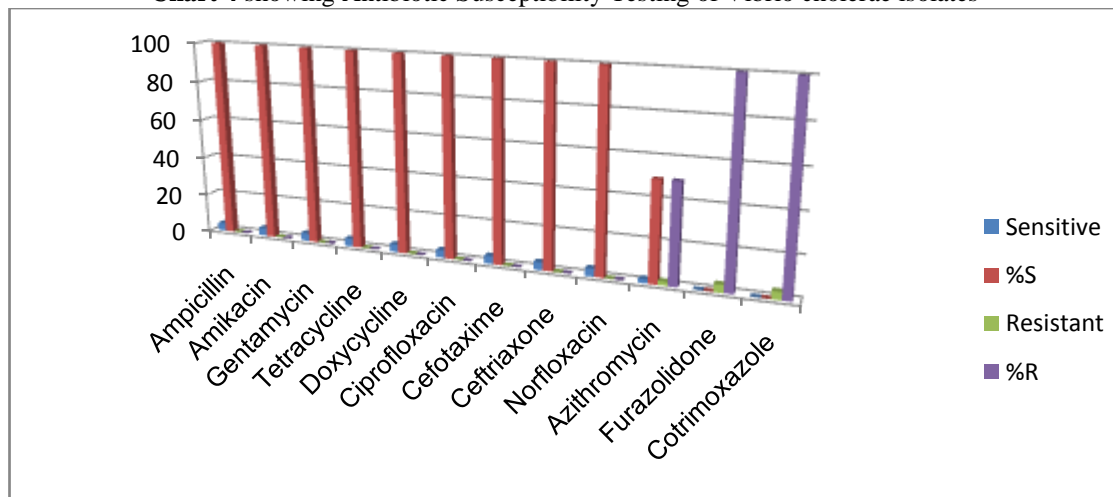**Chart 4** showing Antibiotic Susceptibility Testing of Vibrio cholerae isolates

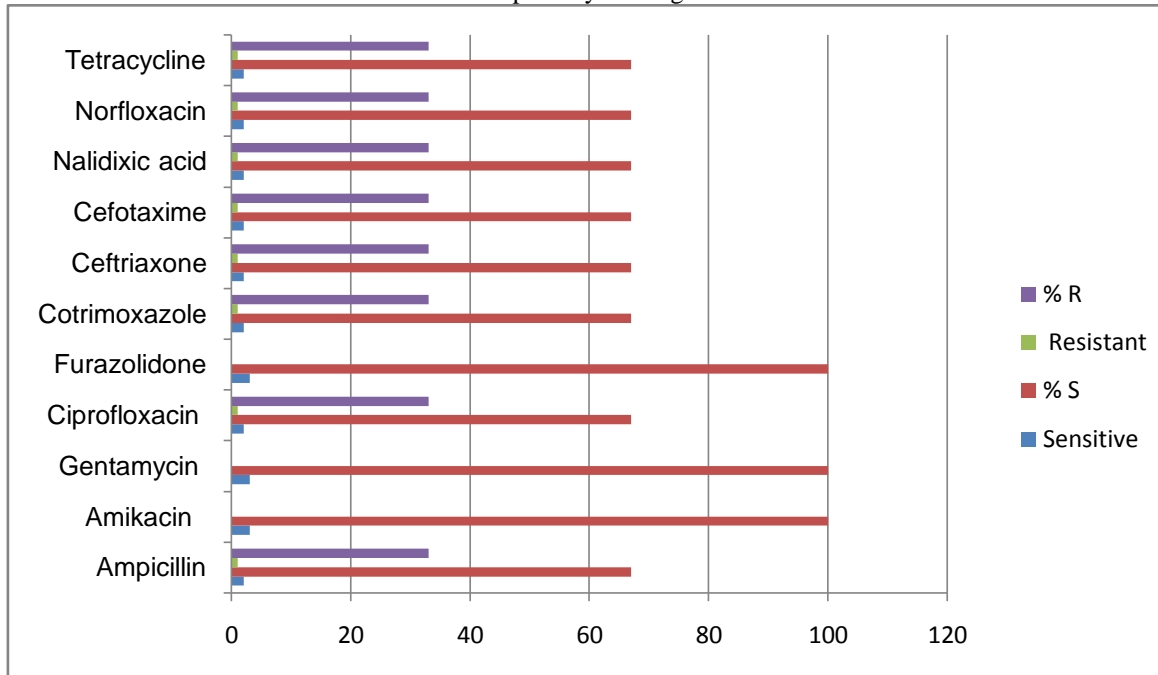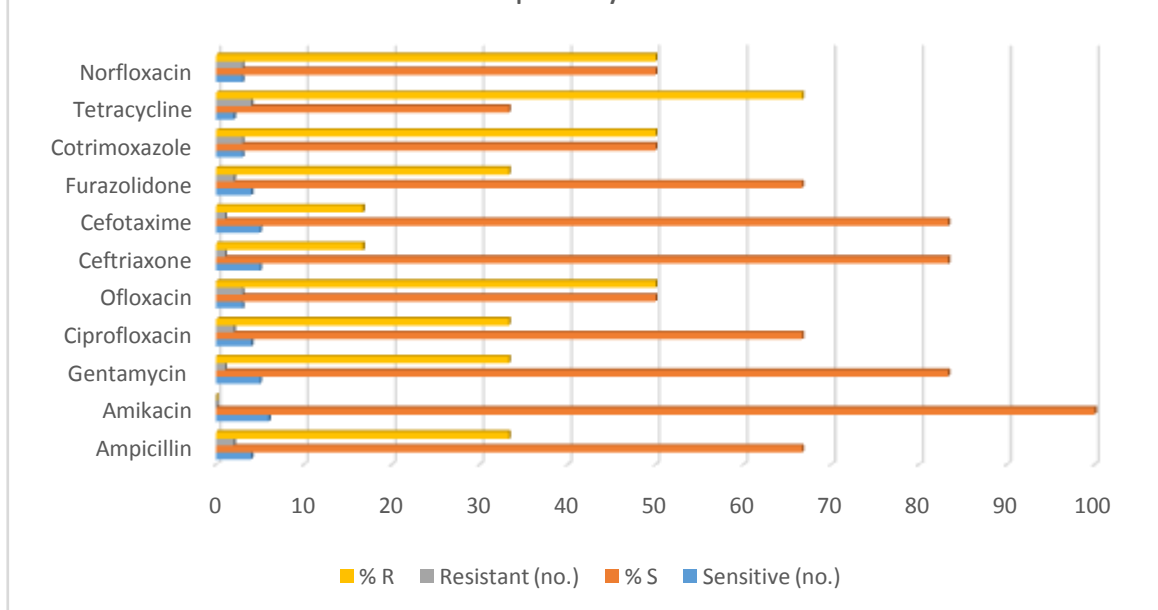Chart 5. Antibiotic Susceptibility Testing of Salmonella Isolates

Chart 6. Antibiotic Susceptibility of Escherichia coli isolates

Rota virus Elisa was performed for detection of VP6 antigen in suspected viral diarrhoeal samples.

Elisa was performed with RIDASCREEN ® Rotavirus kit supplied by R – Biopharm company. The Sensitivity and Specificity of the kit was 95.6% and 99.1% respectively. The Rotavirus antigen was positive in 7 cases out of 42 samples tested in the age group of < 5 years constituting for 16.6% positivity.
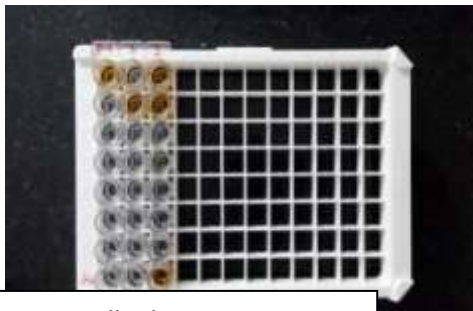
Micro titre wells showing positivity

RIDASCREEN Elisa kit

IV. DISCUSSION :

Gastro enteritis is recognised as a serious public health problem in India. S.Das et al in 2007 quoted that fluid & electrolyte replacement is the treatment of choice for acute diarrhoea, however antibacterial agents are often administered for treatment of Salmonella gastroenteritis & Shigellosis or even diarrhoea due to Escherichia coli or Vibrio cholera. Indiscriminate use of these agents have resulted in development of multi drug resistant organisms[6]. According to a study by N.Kaminski et al 1994 [7], the patients presented with Fever (49.7%), Vomiting (35.8%), Pain Abdomen (45.4%). The present study reported Fever in 50%, Vomiting in 51%, Pain Abdomen in 22% and loose stools in 100% of cases. Out of total 100 samples, macroscopically stools resembling watery (51%), bloody (30%), mucous (19%). These results were in correlation with studies of NeelamTaneja et al , quoting watery (65%), Bloody(27%), Mucous(54%)[8]. M. Youssef et al quoting Watery(91.7%), Bloody(10.6%), Mucous (55.8%)[9]. Out of 100 samples, enteric bacterial pathogens have been identified in 32% which is correlating with studies by Germani et al[10] 1994 quoting 40.6%, N. Kaminski et al[7] 1994 quoting 37%, Somily Ali M et al[11], 2014 quoting 2% positivity. Isolation rates of Salmonella, Shigella, diarrhoeagenic Escherichia coli were significantly different by age. Shigella & Salmonella strains were predominantly identified in samples from adults. Diarrhoeagenic Escherichia coli was a predominant pathogen in children. Infections with Salmonella, Shigella have peak occurrence in March & April which was similar to that quoted by Germani et al[12] 1994.

S.Das et al[6] 2007 reported that Shigella isolates were susceptible to Norfloxacin, Gentamycin, Cefotaxime but resistant to Furazolidone and Cotrimoxazole. The study also stated that resistance to Nalidixic acid has been increasing. The present study showed 68.4% resistance to Nalidixic acid. The treatment of Shigellosis with Nalidixic acid to which it had developed resistance would be a matter of concern for physicians as this drug was highly approved for use. The study stated that Nalidixic acid resistant strains readily acquired resistance to Ciprofloxacin.

S.Das et al, 2007 quoted that Salmonella typhimurium & Salmonella typhi were the commonest serotypes associated with diarrhoea with acute presentation of nausea & vomiting[6].

All the E.coli isolates were confirmed by Antisera. They were found to be Entero Pathogenic Escherichia coli. Jafari et al[13] 2012 reported that EPEC was common in children in the developing world. Recent studies dealing with diarrhoea in children under 5 years of age have reported varying rates of isolation for EPEC ranging from 12.6 – 44.9% showing a significant association between EPEC isolation and diarrhoea. A noticeable reduction in isolation rate of EPEC was reported in 2005 in which 200 children suffering from acute diarrhea and EPEC was isolated from 6% of patients.These studies were in correlation to the present study.

The present study isolated Vibrio cholerae in 4 % of samples. All the patients presented with Rice watery stools. This was correlating with the study of Adkins et a[14] 1987 of 4.1% & 3.6% in 1983 & 1984 respectively.

All the four isolates were Vibrio cholerae O1 Eltor Ogawa (100%), which was similar to studies of Rajoor UG[15] et al(92.3%). The present study isolated Candida albicans in 3% which was in correlation to the study by Gopal Nath et al[16] 1999, of 11.8%. Parasitic yield from examination of ova & cyst showed Entamoeba histolytica in 22% of cases in the present study, while Das et[6] al in 2007 reported 4.8%, Adkins etal [14] 0.1% . Das et al[6], 2007 recovered Cryptosporidium oocysts in 8(1.4%) cases detected by Modified Ziehl Neelsen

technique. Of these, three patients were HIV seropositive, & 5 were identified in children of six months to two years of age. The present study isolated Cryptosporidium oocysts in a similar way in 7(7%) cases. Of these, 3 were identified in children, one patient was HIV seropositive and 3 were identified in elderly people.

Rota virus was the most common cause of severe diarrhoea requiring hospitalization among infants and young children worldwide.[17] Adkins et [14]al stated that children under 5 years were most often infected with Rotavirus.

Germani et[12] al reported that Rotavirus was identified only in samples from infants under 3 years old which was in correlation to the present study.

V. CONCLUSION:

Constant Antibiotic Surveillance is warranted as bacteria were highly resistant to various antimicrobial agents which are currently recommended for empiric treatment of diarrhoea. An improvement in the sanitation & hygiene is essential in decreasing the morbidity due to enteric pathogens. A periodic laboratory monitoring of drug susceptibility & a formulation of antibiotic policy in the hospital should become mandatory to prevent further difficulty & apprehension in treating diarrhoeal illness. The information about the prevalence of wide range of enteropathogens should facilitate the control & management of diarrhoeal diseases among infants & children. The prevalence of Rotavirus infection suggests that these organisms are important causes of diarrhoea in children. The use of vaccine   to control Rotavirus infection is now recommended in developing countries.

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