ORIGINAL RESEARCH PAPER

Microbiology

KEY WORDS: Acute diarrhoea, Dysentery, antibiogram.

ANTIBIOGRAM OF ACUTE DIARRHEA IN UNDER FIVE CHILDREN-A STUDY OF CENTRAL INDIA

Dr. Madhurendra Rajput Rajput, Associate Professor, Department of Microbiology, Amaltas institute of medical science, Dewas-455001

Dr Vijay Tiwari* Assistant Professor, Department of microbiology, S.S. Medical College, Rewa. *Corresponding Author

ABSTRACT

Introduction: Dysentery accounts for a significant proportion of all diarrhea cases. India alone looses 0.6 million children each year due to diarrhea. Effective antimicrobial therapy can reduce both the duration and severity of dysentery.
AIM: To study the antibiogram of acute diarrhea caused by aerobic bacteria in faecal samples of infants and children.
Methodology: The present study of bacteriology is a hospital based descriptive, cross sectional study, on aerobic bacteria causing watery diarrhea (< 15 days) in infants>1 month and children under 5 years of age. Total 120 samples were processed for direct microscopy and culture growth for identification of enteropathogen. Organism identification and antibiogram of study population was analyzed (IBM SPSS version 21).
Results: Among *Escherichia coli* isolates majority of the strains were sensitive to chloramphenicol (98.1%) amikacin (96.2%), gentamycin (92.4%) and cefotaxime (79.2%). Majority of *Shigella* strains (92.9%) were found to be sensitive to ciprofloxacin, gentamicin and amikacin. 50% of strains were resistant to amoxicillin and majority to nalidixic acid (57.1%).Among the salmonella sp. isolated, amikacin was sensitive in all cases, most of the strains were sensitive to ciprofloxacin (87.5%), Furazolidone (87.5%), gentamicin and chloramphenicol (62.5%).
Conclusion: For future control of antimicrobial resistance, some degree of regulation of antimicrobial use is necessary, but obviously this regulation will require administrative and political commitment and support.

INTRODUCTION

Acute diarrheal diseases rank 2nd among all infectious diseases, as a killer in 0 to 5 years age group[1]. India alone looses 0.6 million children each year due to diarrhea. Morbidity due to diarrheal diseases is also very high, amounting to 6 to 12 episodes of diarrhea/year/child[2]. Dysentery accounts for a significant proportion of all diarrhea cases. It is characterised by the passage of loose stools mixed with blood and mucus, fever, abdominal cramps and tenesmus.

The World Health Organization (WHO) and UNICEF estimates that almost 2.5 billion episodes of diarrhea occur annually in children <5 yr of age in developing countries, with more than 80% of the episodes occurring in Africa and South Asia (46% and 38%, respectively)[3] . In developing countries, on an average every child suffers 3.3 episodes of diarrhea per year, but in some areas the average exceeds 9 episodes per year [4]. It causes about 14% of child deaths worldwide[5]. In India acute diarrheal diseases accounts for about 8% of deaths under-5 years of age group[6]. During the year 2011, about 10.6 million cases with 1,293 deaths, were reported in India[7]. In India, children under 5 years of age suffer from 2-3 episodes of diarrhea annually [8].

Effective antimicrobial therapy can reduce both the duration and severity of dysentery. Emergence of resistance to ampicillin and co-trimoxazole in the 1980s led to the use of nalidixic acid as the first line drug for shigellosis. However, increasing number of isolates are showing resistance to nalidixic acid and quinolones, leading to therapeutic problem which needs to be studied in detail. There is a paucity of data in this regard from central India. In this study, we present the clinico-etiologic spectrum of dysentery in children at our tertiary care centre with an attempt to define the causative organisms and their sensitivity pattern to various antimicrobials.

Aim: To study the antibiogram of acute diarrhea in infants and children.

Objectives:

1. To find the etiology of acute diarrhea in infants and children.
2. To find the antibiogram of bacterial isolates.

MATERIAL AND METHODS

The present study of bacteriology is a hospital based descriptive, cross sectional study, on aerobic bacteria causing diarrhea and their antibiogram from stool samples of infants>1 month and children under 5 years of age, conducted in the Department of Microbiology and Pediatrics, Index Institute of medical science, Indore (MP) from September 2011 to September 2014.

All the Children of age group >1 month to 5 years attending the OPD and admitted in IPD from September 2012-September 2013, with watery diarrhea (<15 days) and those whose parents/guardians gave an informed consent were included in the study. Diarrheal cases with > 15 days duration, passage of frequently formed or pasty stools, diarrhea other than watery diarrhea, food poisoning and systemic infections where excluded. All study samples (120) taken only under aseptic precautions were processed. Ethical clearance was obtained. The results and names were kept confidential.

The collected sample was processed as below:

a) Direct microscopic examination of the specimen collected.
b) Inoculation of the samples onto different culture media for isolation of organisms.
c) Preliminary identification on culture media as required
d) Bio-chemical tests
e) Antibiotic sensitivity

The data was entered in excel sheets, expressed in percentages and fractions and analysed by IBM SPSS 21ST version.
As this study is a part of a big study (clinical spectrum of acute diarrheal diseases in tertiary care centre of central India),hence bacterial isolates and their antibiogram related details are mentioned here.

Antibiotic sensitivity testing was done by modified Kirby -Bauer disc diffusion method as per the Clinical Laboratory Standards Institute guidelines.

Using a sterile inoculating loop, four or five isolated colonies of the organism to be tested were picked and suspended in 2 ml of sterile saline. The turbidity of this suspension was adjusted to a 0.5 McFarland standard by adding more organism if the suspension was too light or diluting with sterile saline if the suspension was too heavy. This suspension was used within 15 minutes of preparation.

A sterile swab was dipped into the inoculum tube and with the process of streaking 3 time and rotating at 90 degrees every time on the dried surface of Mueller Hinton agar plate, the plate was

subjected to incubation and later antibiotic susceptibility discs were applied. Discs were applied evenly and no closer than 24 mm from each other and 15 mm from the plate margin. A maximum of 5 discs were placed in a 90 mm plate.

The plates were incubated at 37°C. After 16-18 hours incubation the diameter of each zone was measured with a scale, recorded in mm and interpreted as sensitive or resistant, in accordance to the indications by the disc manufacturer.

OBSERVATION AND RESULTS:

Stool culture for bacteria was carried out in all the study cases, and was positive in 50.66% of the cases. Escherichia coli was found to be the predominant organism, found in 44.2% of the cases. Although Escherichia coli is a commensal of the gut but on occasions when there is an exacerbation it may become pathogenic specially the diarrhea causing species. Among the isolates Salmonella was 6.7% ,Klebsiella 8.3% and Shigella 11.6% of the cases

Amongst the culture positive cases 39 patients (45.9%) had fever at the time of admission. Pus cells > 10/HPF in stools were present in 27(31.8%) of the culture positive cases.

Among *Escherichia coli* isolates majority of the strains were sensitive to chloramphenicol (98.1%) amikacin (96.2%) and gentamycin (92.4%) followed by cefotaxime (79.2%). The isolates were resistant to amoxycillin (92.5%) followed by cotrimoxazole (41.5%) ciprofloxacin, ampicillin and nalidixic acid.

Majority (92.9%) of *Shigella* strains were found to be sensitive to ciprofloxacin, gentamicin and amikacin. 50% of strains were resistant to amoxycillin. The isolates were mostly resistant to nalidixic acid (57.1%) followed by amoxicillin and co trimoxazole 50% each.

Among the salmonella sp. isolated, amikacin was sensitive in all cases, most of the strains were sensitive to ciprofloxacin (87.5%). Furazolidone is also sensitive in 87.5% followed by gentamicin and chloramphenicol (62.5%). In the present study resistance to amoxycillin, nalidixic acid, cotrimoxazole and cefotaxime was 62.5, 62.5, 25 and 50 per cent respectively.

DISCUSSION

E. coli isolates in the present study showed less susceptibility to first line antibiotics, such as amoxycillin, nalidixic acid, cotrimoxazole and ampicillin, which tend to be more affordable and accessible to families.

Majority of strains were susceptible to aminoglycosides, gentamycin (92.4%) and Amikacin (96.2%). High degree of resistance to commonly used antibiotics was also seen by Das et al[9], who reported 33% of E. coli strains to be resistant to norfloxacin, 33% were resistant to gentamycin, 44% were resistant to cefotaxime and 77% were resistant to nalidixic acid. Thus local susceptibility patterns should be assessed periodically to guide antimicrobial therapy.

Ansari Set al[10] reported 41.7% of the isolates were resistant to ampicillin followed by 33.3% were resistant to nalidixic acid, cotrimoxazole and amikacin. According to this same study[10] chloramphenicol showed 100% efficacy while fluoroquinolones, gentamycin and 3rd generation cephalosporin showed better efficacy 91.7%.

Krishnan R et al[11] reported greater degree of resistance to amoxycillin (95%) and cefotaxime (56%). Majority of strains were sensitive to gentamycin and amikacin 88.8% and 100% respectively. 66.7% of strains were resistant to norfloxacin and 88.9% were resistant to nalidixic acid.

In the present study the resistance pattern of shigella species to the following drugs is cotrimoxazole (50%), Chloramphenicol (28.6%), Amoxycillin (50%), Cefotaxime (21.4%), Ceftriaxone (69.2%), Gentamycin (7.1%), Nalidixic acid (57.1%), Ciproflaxacin (7.1%), Furazolidone (14.3%), and some of these findings correlates with other studies [10,12].

Resistance to nalidixic acid has increased whereas resistance to furazolidone has decreased over the years. This is due to widespread use of nalidixic acid as the first line agent for empirical treatment of infectious diarrhoea in children and ever decreasing use of furoxones[12]. Amikacin was most effective and an overall resistance of amikacin was 3.1%.

In a study conducted by Bhattacharya S et al[13] of the 1396 routine stool samples, 53 yielded Shigella species. 42 (79%) were isolated from children aged less than five years. Amongst Shigella species (Shigella dysenteriae, Shigella flexneri, Shigella boydii, Shigella sonnei) an overall resistance of 88.7%, 86.8%, 69.8%, 49% and 28.3% was observed for Co-trimoxazole, Ampicillin, Nalidix acid and ciprofloxacin respectively.

Krishnan R et al [11] reported majority (95.6%) of Shigella strains were sensitive to cefotaxime, gentamycin and amikacin. 56.5% of strains were resistant to amoxycillin, 87% were resistant to norfloxacin and 95.7% of strains were resistant to nalidixic acid

Majority of salmonella strains isolated in this study were found to be sensitive to ciprofloxacin and gentamicin ,amikacin is sensitive in all the isolates (100%). 7 out of 8 (92.9%) strains isolated were found to be sensitive to these drugs. 50% of strains were resistant to amoxycillin. The isolates were mostly resistant to nalidixic acid (57.1%) followed by amoxycillin and co trimoxazole 50% each.

Ansari S, et al[10] found that Salmonella species were sensitive to chloramphenicol and tetracycline in 90.0% of the isolates followed by amikacin, cotrimoxazole in 80.0% and fluoroquinolones in 70.0% isolates whereas 70.0% isolates were resistant to ampicillin followed by 60.0% isolates resistant to nalidixic acid.

Multi-drug resistant non-typhoidal Salmonella spp. (*S. typhimurium, S. enteritidis, S. hidelberg* etc) are widespread all over the world[14]. Neelam Taneja et al[12] reported that resistance to amoxycillin, nalidixic acid, cotrimoxazole, cefotaxime, chloramphenicol and ciprofloxacin was 62.5, 66.7, 34.6, 48.1, 37 and 18.5 per cent respectively.

Dahifar et al[15] from Tehran found 67% of Salmonella isolates were resistant to nalidixic acid which is inconsistent with our study.

CONCLUSION AND IMPLICATIONS:

At present, the continued development of newer antimicrobials, particularly those for the treatment of shigellosis in children, is critically important. For future control of antimicrobial resistance, some degree of regulation of antimicrobial use is necessary, but obviously this regulation will require administrative and political commitment and support.

Other more indirect but highly important aspects are safe water supply and proper sanitation, so that transmission of these enteric organisms are diminished; development of vaccines to decrease the incidence of the diseases, and improving nutrition, an important risk factor for the under 5 children in the developing world, in which diarrheal diseases are most common and a major cause of morbidity and mortality.

Table 1: Table showing prevalence of bacterial isolate in faecal study samples.

CULTURE POSITIVE - ORGANISM ISOLATED	NO. OF CASES	PERCENTAGE (%)
ESCHERICHIA COLI	53	44.2
SHIGELLA SPP.	14	11.6
SALMONELLA SPP.	8	6.7
KLEBSIELLA SPP.	10	8.3
TOTAL	85	70.8
CULTURE NEGATIVE	35	29.2

Table 2: Table showing antibiogram of bacterial isolates from faecal sample of study population.

	Escherichia coli (53)		Shigella spp(14)		salmonella spp(8)	
Sensitivity pattern	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Antibiotic	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
Amikacin	51 (96.2%)	2(3.8%)	13(92.9%)	1(7.1%)	8(100%)	0
Amoxycillin	4 (7.5%)	49(92.5%)	7(50%)	7(50%)	3(37.5%)	5(62.5%)
Cefotaxime	42 (79.2%)	11(20.8%)	11(78.6%)	3(21.4%)	4(50%)	4(50%)
Co-Trimoxazole	31(58.5%)	22(41.5%)	7(50%)	7(50%)	6(75%)	2(25%)
Chloramphenicol	52(98.1%)	1(1.9%)	10(71.4%)	4(28.6%)	5(62.5%)	3(37.5%)
Gentamicin	49(92.4%)	4(7.6%)	13(92.9%)	1(7.1%)	5(62.5%)	3(37.5%)
Ciprofloxacin	32(60.4%)	21(39.6%)	13(92.9%)	1(7.1%)	7(87.5%)	1(12.5%)
Nalidixic acid	37(69.8%)	16(30.2%)	6(42.9%)	8(57.1%)	3(37.5%)	5(62.5%)
Furazolidone	32(60.4%)	21(39.6%)	12(85.7%)	2(14.3%)	7(87.5%)	1(12.5%)

REFERENCES

1. Centers for Disease Control and Prevention(CDC), [INTERNET]Global Water, Sanitation, & Hygiene (WASH), Diarrhea: Common Illness, Global Killer . December 17, 2015 accessed on 20-1 2016 AVAILABLE AT https://www.cdc.gov/ healthy water/ global/diarrhea-burden.html
2. Christopher Troeger, Mohammad Forouzanfar, Puja C Rao, Ibrahim Khalil ,Alexandria Brown ,Robert C Reiner Jr et al "Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015", Volume 17, ISSUE 9, P909-948, September 01, 2017,THE LANCET Published:June 01, 2017 DOI:https:// doi.org/ 10.1016/S1473-3099(17)30276-1
3. Bhan MK. Accelerated progress to reduce under-5 mortality in India. Lancet Glob Health. 2013;1:e172–3
4. Fischer Walker, C. L., Perin, J., Aryee, M. J., Boschi-Pinto, C., & Black, R. E. (2012). Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. BMC public health, 12, 220. doi:10.1186/1471-2458-12-220
5. WORLD HEALTH ORGANIZATION "Countdown to 2015 2010 Report" Child mortality Millenium Development Goal (MDG) 4 Updated September 2011.accessed on 21-1-2015 Available at https://www.who.int/pmnch/media/press_materials/fs/fs_mdg4_childmortality/en/
6. Centre for Global Health Research "Causes of Death in India-report on causes on death in 2001-2003,india ministry of home affairs , accessed on 21-1-2015 Available at www.cghr.org/wordpress/wp-content/uploads/Causes_of_ death_ 2001- 03.pdf
7. Park K. Acute Diarrhoeal Disease. In : Textbook of Preventive and Social Medicine. Chapter 5, Part II, 22nd edn., Banarsidas Bhanot Publishers, India. 2013;200-206.
8. Reddiah VP, Kapoor SK. Epidemiology of Diarrrhea and it's Implications for Providing Services. Indian J Pediatr 1991; 58: 205 -8.
9. Das S, Saha R, Singhal S (2007). Enteric Pathogens in North India Patients with Diarrhea. Indian J. Community Med. 32(1):27-31
10. Ansari S Sher chand JB Parajul K, bacterial etiology of acute diarrhoea in children under five years of age, J Nepal Health Res Counc 2012 Sep; 10(22):218-23
11. Krishnan Rajeshwaril*, Madhumita Mishral, A. P. Dubeyl, Beena Uppal2 and S. Anuradha, Spectrum of dysentery in children presenting to a tertiary level teaching hospital in New Delhi Vol. 5(4), pp. 158-162, April 2013.
12. Neelam Taneja, Balvinder Mohan, Sumeeta Khurana & Meera Sharma: Antimicrobial resistance in selected bacterial enteropathogens in north India: Indian J Med Res 120, (July 2004); 39-43.
13. Bhattacharya S, Khanal B, Bhattarai NR, Das ML. Prevalence of Shigella species and Their Antimicrobial Resistance Patterns in Eastern Nepal. J Health Popu 1 Nutr 2005;23(4):339-342.
14. Niyogi SK, Dutta D, Bhattacharya MK, Bhattacharya SK. Multi-drug resistant non-typhoidal Salmonella spp. associated with acute diarrhoeal disease. India J Med Res 1999; 110 : 183-5.
15. Dahifar H, Ghorbani A, Ghods M. Isolated and non-isolated enteric A, Ghods M. Isolated and non-isolated enteric pathogens in children with diarrhoea and related laboratory characteristics. Medical J Islamic Rep Iran. 2008;22(l):40-6.