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Bacteria Associated with Diarrhoea in Children in Khartoum State, Sudan 2006

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Abstract :

This study was carried out to identify enterobacteria in children suffering from diarrhoea in Khartoum State (Khartoum and Khartoum North) . Samples of diarrhoeal stool were collected during the period from May to June 2006 from 60 children aged between 4 months to 5 years suffering from diarrhoea .

Isolated bacteria were identified on the basis of their cultural and biochemical characteristics and included the following species : *Escherichia coli* and *Salmonella typhi* from 5 specimens , *S. paratyphi* from 5 specimens , *S. typhimurium* from 4 specimens , *Shigella dysenteriae* from one specimen , *Sh. Flexneri* from 2 specimens, *Sh.boydii* from one specimen , *Proteus vulgaris* from 2 specimens , *Klebsiella pneumoniae* from 4 specimens , *K. rhinoschermatis* from 2 specimens and *Pseudomonas aeruginosa* from 6 specimens .

The susceptibility of isolated bacteria to some antibiotics using the multi disk method was variable.

Introduction:

A wide variety of causes result in chronic diarrhoea in children around the world. The causes range from developmental and dietary to infectious particularly in immunocompromised patients (Binder,H.J 2006).

In the developing world, chronic diarrhoea is typically associated with serial enteric infections and malnutrition (Gibbons and Fuchs, 2007).

Acute diarrhoea is frequent among travelers in whom enterotoxigenic *E. coli* is particularly common (Black, 1986). In practice, most episodes of acute diarrhoea that are assumed to be caused by an infectious agent are treated without the causative agent being identified. Major causes of acute infectious diarrhoea are mainly depend on local factors such as availability of clean water and sanitation (Walker and smith, 1993).

The disease course of most viral and bacterial diarrhoeas lasts less than 1 week; therefore, infectious diarrhoea lasting more than 7 days is more likely to be caused by protozoa (Guerrant et al ., 2001) .

Microorganisms cause diarrhoea by one of four mechanisms: (1) enterotoxins that subvert the regulatory mechanisms of enterocytes, (2) cytotoxins that destroy enterocytes, (3) adherence to the mucosa by organisms (so-called enteroadherent organisms) that alter enterocyte function as a result of physical proximity to the mucosa, and (4) invasion of the mucosa by organisms that provoke an inflammatory response by the immune system. In general, patients with cytotoxin-mediated diarrhoea and those with invasive organisms experience more toxicity and have more abdominal pain than patients with enterotoxin-mediated diarrhoea or enteroadherent infections (Vazquez and Fang, 2000) .

The public health laboratories in Germany have been isolating *Salmonella* species in general in increasing numbers during the last three years and there was a steep increase in the isolation rate of *S. enteritidis*. (Sander, 1993).

A study made of 159 infants (including 30 neonates) suffering from diarrhoea who were admitted to the Diarrhoea Treatment Center in Dacca, Bangladesh revealed that the infections were caused by *Sh. boydii* and *Sh.sonnei* whereas *Sh. dysenteriae* type 1 , was less common in infants than in older children;

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the proportion of sh.flexneri infections was smaller in the two groups (Huskins et al., 1994)

Diarrhoeal diseases are noted to be a major cause of morbidity in children bellow five years of age in Sudan. Most cases are due to bacterial infections. The most common bacterial pathogens are E. coli strains , Shigella strains and Salmonella strains. Mixed infection with these organisms also occur. Other potential bacterial pathogens are enterococci, Proteus spp , and Pseudomonas aeruginosa (Erwa , 1969) .

Materials and Methods

Materials :

Collection of samples:

Faecal specimens were collected from 60 children 4 months to 5 years of age admitted to hospital suffering from diarrhoea. Samples were collected in sterile containers and transported directly to the laboratory.

Samples were collected from both sexes during the period from May to June 2006 from Ibraheem Malik hospital (Khartoum), Khartoum Teaching Hospital and Ahmed Qasim Hospital for children (Khartoum North).

Culture media :

Culture media were obtained from Oxoid Ltd U.K. and they were prepared as instructed by the manufacturer, these were: Blood agar , MacConkey's agar, Muller- Hinton agar, Nutrient agar, Selenite F broth, Semi-solid medium, Koser's Citrate Medium, Peptone water, SS agar, Kligler Iron Agar (K.I.A) and Urea agar,

Methods:

Identification of Clinical Isolates:

Primary Identification:

Primary identification of isolates was based on colonial morphology , lactose-fermenting and motility. The smell of the culture was also noted. This was followed by microscopic examination of Gram-stained smears.

Secondary Identification:

Secondary identification was done using biochemical tests such as : Catalase test, Oxidase test, Citrate Utilization test, Urease production test, Indole test , Motility test and reaction in Kligler Iron Agar (K.I.A) Cowan and Steel(1975).

Antibiotic Susceptibility pattern:

Antibiotic susceptibility of isolates was made by Kirby-Bauer disc diffusion method using commercially prepared discs (Oxoid).

Results

Identification of the clinical isolates:

It was found that out of the 60 faecal samples , clinical isolates , 60 were Gram-negative rods which included : E. coli associated with 5 Salmnella typhi isolates (8.3%) , 5 Salmonella paratyphi isolates (8.3%) , 4 Salmonella typhymurium isolates (6.7%), one Shigella dysenteriae isolate (1.7%) , 2 Shigella flexneri isolates (3.3%) , one Shigella boydii isolates (1.7%) , 28 Escherichia coli isolates (46.7%) as pure culture , 2 proteus vulgaris isolates (3.3%) , 4 Klebsiella preumoniae isolates (6.7%) ,2 were Klebsiella rhinoscleromatis isolates (3.3%) and 6 isolates of Pseudomonas aeruginosa (10%). (**Table 1 and 2**):

Kirby – Bauer disc diffusion method:

Bacterial isolates were subjected to antibiotic sensitivity testing using different anti-microbial agents discs ,the results are presented in Table 3:

Discussion:

In developing countries like Sudan , infections often occur via faecal-oral transmission and are thus more common in situations where sanitation is poor and water supply is contaminated. Motarjemi et al

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(1993) noted that diarrhoea among infants and children under 5 years of age was associated with malnutrition , water supply and sanitation.

In this study Faecal samples were collected from 60 children with diarrhoea results showed the presence of different species of Salmonella and Shigella associated with diarrhoea in infants . In a similar study of diarrhoeal patients during the period from May to December in Hong Kong, Ling and Cheng (1993) found that , salmonellae were the most common pathogens followed by shigellae. Salmonellae were isolated more often from young children aged 1-4 years whereas shigellae commonly affected young adults . They noted that salmonellae were more common in the hotter season months , but there was no seasonal predominance for shigellosis . In this study we found that salmonellae and shigellae were both prevalent in the summer season , but the rate of salmonellae was higher than that of shigellae in diarrhoeal patients.

In this study other organisms in less abundance included klebsiellae , Proteus vulgaris and Pseudomonas aeruginosa and found associated with diarrhoea . The presence of klebsiellae in acute diarrhoea was previously reported by Bernard et al(1980). The same authors noted Proteus spp frequently in normal human faeces , but often in much increased numbers in individuals receiving antibiotics therapy or during diarrhoeal diseases due to other organisms.

E. coli is a normal inhabitant of the intestinal tract . Infants less than 6 months of age are most commonly infected with enterobacteria mainly E. coli (Huilan, 1999) . The association of E. coli with diarrhoeal disease has been reported by many authors in different countries including the Sudan (Erwa, 1969) , Ruska and vesikari (1991) , Kmetova et al ., (1991) , Robin et al .,(1993) and Huilan et al ., (1991)

Blank et al,(2000) noted that many serotypes of E. coli caused diarrhoea in 550 children in Sao Paulo, in 1978 and 1979; serotypes O111ab:H⁻, O111ab:H₂, and O119:H₆ were significantly associated with diarrhoea in children from birth to 5 months old and were the most frequent agents of diarrhoea in this age group as compared with enterotoxigenic and enteroinvasive E. coli . It was not possible to serotype isolates of E. coli in this study.

Acquired antimicrobial resistance is a world wide problem . The situation in developing countries , however , is serious because antibiotics are taken without medical authorization or supervision .

Enteropathogenic species show variation in their susceptibility to most antibiotics commonly used for the treatment of diarrhoea :

Blank et al., (2000) found that 53% of E. coli strains from diarrhoeic children were resistant to ampicillin, 47% to chloramphenicol, 30% to co-trimoxazole 67% to tetracycline. In this study all E. coli strains were resistant to ampicillin , chloramphenicol, co-trimoxazole but sensitive to tetracycline.

Of seven shigella isolates , three were resistant to chloramphenicol and four to tetracycline (Blank et al., 2000) . In this study shigella showed susceptibility to chloramphenicol and resistance to tetracycline

Pickering (1991) recommended ampicillin, chloramphenicol, ceftriaxone, and cefotaxime for treatment of salmonella. In this study salmonellae were resistant to ampicillin and cefotaxime and sensitive to chloramphenicol.

Engel and Schaeffer (1998) recommended gentamicin, amikacin and piperacillin/tazobactam for treatment of any infection caused by klebsiella, variable results were noted in this study , K. pneumoniae was sensitive to gentamicin , but resistant for amikacin whereas K. rhinoscleromatis isolates were resistant to gentamicin , but sensitive for amikacin . The two species were sensitive to piperacillin/tazobactam

Fisman and Kaye , (2000) recommend ampicillin and gentamicin for treatment of proteus , In this study , both antibiotics were effective against proteus.

Gentamicin and ciproflaxin were recommended by Abuqaddom et al, (2003) for treatment of Ps. aeruginosa . In this study Ps. aeruginosa was sensitive to gentamicin , but resistant to ciproflaxin .

Table 1 :

Growth of isolated bacteria from diarrheic children stool specimens on different culture media :

		Media			

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		N. A	Mac. A	SS . A	S. F.B	B. A
1	Salmonella typhi (5)	+	N. L	+	+	+
2	S. paratyphi (5)	+	N.L	+	+	+
3	S. typhimurium (4)	+	N. L	+	+	+
4	Shigella dysenteriae (1)	+	N.L	+	+	+
5	Shi. Boydii (1)	+	N.L	+	+	+
6	Shi. Flexneri (2)	+	N.L	+	+	+
7	Proteus vulgaris (2)	+	N.L	-	-	+
8	Escherichia coli (29)	+	L.F	-	-	+
9	Klebsiella pneumonia (4)	+	L.F	-	-	+
10	K.rhinoscleromatis(2)	+	L.F	-	-	+
11	Pseudomonas aeruginosa (6)	+	+	-	-	+

N.A : Nutrient Agar, Mac. A: MacConkey Agar, SS.A: Salmonella Shigella Agar, S.F.B: Selenite F Broth, B.A : Blood Agar, N.L: Non Lactose fermenting (white colonies), L.F: Lactose Fermenting (pink colonies), + : Growth, - : No growth

Table 2:

biochemical Characteristics of the bacteria from faecal specimens of diarrheic children:

Serial No.	Organism	Slope	K.I.A			Fermentation of sugars						Urea			
			But t	H2 S	G sa	L ac	M an	Glu	Suc	O x	Cat		Cit	Mot	In d
1	S. typhi	R	Y	+	-	-	+	+	-	-	+	-	+	-	-
2	S.paratyphi	R	Y	-	+	-	+	+	-	-	+	-	+	-	-
3	S.typhimurium	R	Y	+	+	-	+	+	-	-	+	-	+	-	-
4	Sh. dysenteriae	R	Y	-	-	+	+	+	-	-	+	-	-	-	-
5	Sh. boydii	R	Y	-	+	+	+	+	-	-	+	-	-	+	-
6	Sh. flexneri	R	Y	-	+	+	+	+	-	-	+	-	-	-	-
7	Proteus vulgaris	R	Y	+	D	+	+	+	-	-	+	D	+	+	+
8	E. coli	Y	Y	-	+	+	+	+	-	-	+	-	+	+	-
9	K. pneumonia	Y	Y	-	+	+	+	+	+	-	+	+	-	-	+
10	K.rhinoscleromatis	Y	Y	-	-	+	+	+	+	-	+	+	-	-	+
11	Ps. aeruginosa	R	R	-	+	-	-	D	-	+	+	+	+	-	D

R: Red, Y: Yellow, K.I.A: Kligler Iron Agar, Lac : lactose, Man: Mannitol, Glu: Glucose, Suc: Sucrose, Ox: Oxidase test, Cat: Catalase test Cit: Citrate utilization test, Mot: Motility test, Ind: Indole test, + : Positive test., - : Negative test., D: Variable.

Table 3:

Antibiotics susceptibility of isolated bacteria from faecal specimens of diarrheic children :

Microorganism	Aantimicrobial agents											
	AS 20 mcg	BA 25 mcg	CF 30 mcg	TZP 100/10 mcg	CH 30 mcg	CP 5 mcg	CI 30 mcg	TE 30 mcg	OF 5 mcg	GM 10 mcg	AK 30 mcg	PF 10 mcg
S. typhi	R	R	R	S	S	S	R	R	S	S	S	S
S. paratyphi	R	S	R	R	S	S	R	R	S	S	S	S
S.typhimurium	R	R	R	S	S	S	R	R	S	S	S	S
Sh.dysentriae	R	R	S	S	I	S	S	R	R	S	S	S
Sh. flexneri	R	S	R	S	S	S	R	I	S	S	S	S
Sh. boydii	R	R	S	S	R	S	S	R	S	S	S	S
K. pneumonia	R	R	R	S	R	R	R	R	S	S	R	S
K. rhinoscleromatis	R	R	R	S	R	R	R	R	R	R	S	R
Proteus vulgaris	R	R	S	S	S	S	S	R	S	S	S	S
E. coli	R	R	R	R	R	R	R	R	S	R	S	R
Ps. aeruginosa	R	R	R	I	R	R	R	R	R	S	R	R

S: Susceptible I : Intermediate R: Resistant.

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References:

- Abuqaddom ,A.I., Darwish, R.M., Muti, H (2003).** The effects of some formulation factors used in ophthalmic preparations on thiomersal activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *J Appl Microbiol*; 95(2): 250-5
- Bernard, D., Davis,M.D., Renato,M.D., Hermann,N., Eisen,M.D.and Harold,s. (1980).** Microbiology including immunology and molecular genetics, 3 edition. Harper and Row, Publisher. New York., 659-660.
- Binder HJ.** (2006). Causes of chronic diarrhea. *N Engl J Med.* 2006 Jul 20;355(3):236-9.
- Blank, T. E., Zhong, H., Bell, A. L., Whittam, T. S and Donnenberg, M. S. (2000).** Molecular Variation among Type IV Pilin (bfpA) Genes from Diverse Enteropathogenic *Escherichia coli* Strains. *Infect. Immun.* 68: 7028-7038
- Cohen ,M.B (1991).** Etiology and mechanisms of acute infectious diarrhea in infants in the United States. *Journal of Pediatrics* 118 (4, Part2): S34-S39.
- Cowan,S.T., and Steel,K.J.,(1975).**Manual for the Identification of Medical Bacteria. Cambridge University Press. Cambridge.
- Engel, J.D and Schaeffer ,A.J (1998).** Evaluation of and antimicrobial therapy for recurrent urinary tract infections in women . *J. Urol Clin North Am .* , 25(4): 685-701.
- Erwa,H.H.(1969).** Bacterial disease in Sudanese children .*J. Trop.Med. Hyg,* 72-261.
- Fine, K.D and Schiller, L.R (1999).** AGA technical review on the evaluation and management of chronic diarrhea. *J. of Gastroenterology .*,116: p.1464
- Fisman , D.N and Kaye, K.M, (2000).** Once-daily dosing of aminoglycoside antibiotics. *J. Infect Dis Clin North Am .* , 14(2): 475-87.
- Fitzgerald JF. (1989).** Management of acute diarrhoea. *Pediatric Infectious Disease Journal* 8 (8): 564-9.
- Gibbons, T and Fuchs, G.J (2007).** Chronic enteropathy: clinical aspects. *Nestle Nutr Workshop Ser Pediatr Program.* 2007;59:89-101; discussion 102-4.
- Guerrant , R.L.,Van Gilder, T and Steiner T.S.(2001).** Practice guidelines for the management of infectious diarrhea .*J. Clin Infect Dis* 32: 331
- Huilan , S ., Zhen , L.G ., Mathan , M M ., Mathew , M.M ., Olarte ,J and Espejo , R. (1991) .** Etiology of acute diarrhoea among children in developing countries: a multicentre study in developing countries. *Bulletin of the World Health Organization .* , 69(5):549-55.
- Huskins,W.C., Griffiths, J.K., Faruque, A.S and Bennish, M.L.(1994).** Shigellosis in neonates and young infants ., *J- Paediatr .* , 125(1) : 14-22
- Kidd, A.H., Esrey, S.A.and Ujfalusi, M.J.(1989).** Shedding of coronavirus-like particles by children in Lesotho. *J Med Virol* ,27: 164-9.
- Laney DW, and MB Cohen. (1993).** Approach to the pediatric patient with diarrhea. *Gastroenterology Clinics of North America* 22 (3): 499-516.
- Motarjemi ,Y ., Kafersstein, F .,Moy, G and Quevedo,F. (1993).** Contaminated weaning food : a major risk factor for diarrhoea and associated malnutrition .*J. Bull- World – Org .* , 71(1) : 79-92.
- Pickering , L., and M.M ,Levine.(1991).** Management of acute diarrheal disease: Discussion VII. *Journal of Pediatrics* 118 (4, Part 2): S137-S138.
- Robin-Brown , R.M., Yam, W.C., O Gorman, L.E and Bettelheim, K.A . (19993) .** Examination of archetypal strains of enteropathogenic *Escherichia coli* for properties associated with bacterial virulence .*J- Med- Microbiol.*, 38(3): 222-6
- Ruuska T, Vesikari T.(1991).** A Prospective Study of Acute Diarrhoea in Finnish Children from birth to 2 ½ Years of Age. *J. Acta Pædiatr Scand.*, 80:500-507.
- Sander , J . (1993) .** Pathogenesis of salmonella infections in humans ., *DTW-Dtsch – Tierarztl- Wochenschr.*, 100(7): 283-5
- Talley, N.J., Weaver, A.L .and Zinsmeister, A.R. (1994).** Self-reported diarrhea: what does it mean? *Am. J. Gastroenterol.*, 89: 1160,
- Thielman, N.M.and Guerrant, R.L (2004).** Clinical practice. Acute infectious diarrhea., *N Engl .J. Med,* 350: 38.
- Vazquez-Torres , A and Fang ,F.C (2000) .** Cellular routes of invasion by enteropathogens. *J. of Curr Opin Microbiol .* , 3: 54
- Walker-Smith , J.A (1993).** Diarrhoeal disease: current concepts and future challenges., *Malnutrition and infection. Transactions of the Royal Society of Tropical Medicine .* , 87 (3): 13-5.