Epidemiological and antibiotic susceptibility profiles of infectious bacterial diarrhoea in Juba, South Sudan

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BACKGROUND: Diarrhoeal diseases have remained a major health problem in South Sudan where they accounted 45% prevalence in under five-year olds. Between 2006 and 2007, the country reported a morbidity of 8,337 cases and 176 deaths due to diarrhoeal outbreaks.

METHODOLOGY: We investigated causative agents of diarrhoeal diseases and their antibiogram in persons presenting with diarrhoea to selected health facilities in Juba.

RESULTS: Bacterial agents were prevalent in 20 (6.9%) of the 286 patients with 5.7% (4/70) in under five-year olds alone. *S. dysenteriae* 50% (10/20) accounted for the majority of the identified pathogens followed *S. flexneri* 25% (5/20) and *S. typh* 25% (5/20). Antibiotic testing showed that *S. flexneri* (5/5) and S. typhi (5/5) were all 100% sensitive to ceftriaxone, and gentamicin while *S. dysenteriae* had varying sensitivity to ciprofloxacin (70%), nalidixic acid (90%), and ceftriaxone(100%). These pathogens had 100% resistance to amoxicillin, ampicillin, tetracycline and cotrimoxazole. No difference existed in isolation rates among different age groups, educational status, gender, water drank, use of chlorine, toilet use, exposure at home to diarrhoea patient, hand washing with soap and location of residence. However, diarrhoeagenic bacteria isolation was higher for participants with no source of income (OR=6.08, p<0.05).

CONCLUSION: With emerging menace of resistance to commonly used antibiotics in South Sudan we recommend antibiotic resistance monitoring and regulation of antibiotic use.

Introduction

Diarrhoeal diseases are a major health problem in developing countries [1] and accounts for an approximate global mortality of two million people annually [2]. Globally, 88% of diarrhoea cases are attributable to unsafe drinking water, inadequate sanitation or insufficient hygiene. In Africa, an average morbidity rate of 912.9 million diarrhoeal episodes per year in children has been reported with four out of 10 deaths annually caused by diarrhoeal disease[3]. Diarrhoeal diseases are also common among travelers to tropical areas [4]. *Escherichia coli,* Rotavirus, *Salmonella* spp., Shigella spp., *Campylobacter jejuni*, *Entamoeba histolytica,* and *Giardia lamblia* are the common diarrheogenic pathogens [5, 6, 7]. Ample fluid and electrolyte replacement and maintenance is the cornerstone for management of diarrheal illness [8]. Suitable antimicrobial treatment shortens bacterial excretion and clinical periods, but the incidence of multidrug-resistance is rising [9].

South Sudan, with a reported 45% prevalence of diarrhoea in children aged under five years of age, has experienced outbreaks in the recent past. In 2009 and 2010 there were 68,983 and 147,071 diarrhoeal cases with 0.09 and 0.08 case fatality rates (CFRs) respectively. In April 2006 alone, there were 5,108 diarrhoea cases and 98 deaths (CFR: 1.92%) in Juba City [10]. From January-June 2007, there were 3,157 diarrhoeal cases with 74 deaths (CFR: 2.34%) [11]. These outbreaks could be due to the influx of people from neighboring countries following the comprehensive peace agreement (CPA). Movement of people is associated with spread of disease and antibiotic resistance [12]. To our knowledge, no diarrhoea-causing bacterial pathogens were identified in these outbreaks due to facilities for bacterial culture and isolation being unavailable. This study determined the prevalence, distribution and antibacterial susceptibility profiles of enteropathogenic bacteria causing diarrhoea in patients attending selected health facilities in Juba City.

Materials and methods

Study design, site, target population and sample size

A cross-sectional study was conducted in Juba Teaching Hospital (JTH), Al Sabah children's hospital, and Malakia, Kator, Munuki and Nyakuron primary health centres (PHC). JTH is the main teaching and referral health facility in South Sudan with 516 beds. A total of 286 stool specimens or rectal swabs from randomly selected adults and children with diarrhoea respectively were collected between September and December 2012,

Table 1. Distribution and bacterial culture results for diarrhoeagenic bacterial among study participant attending selected health facilities in three payams of Juba City, South Sudan.

Payam (District)	Participants n (%)		Bacterial Isolation	
			(-) n (%)	(+) n (%)
Juba	32 (11.18)		29	3
Kator	111 (38.81)		106	5
Munuki	143 (50)		131	12
Education level and employment				
Higher certificate and above	12 (4.2)		12 (4.5)	0 (0.0)
Secondary	57 (19.93)		50 (18.9)	7 (35.0)
Primary	84 (29.37)		81 (30.6)	3 (15.0)
Uneducated	132 (49.15)		122 (46.0)	10 (50.0)
Unemployed	216 (75.5)		198 (74.5)	18 (90.0)
Employed	70 (24.5)		68 (25.)	2 (10.0)
Health facilities		**Sex**		
Al sabah	47 (16.43)	female male	26 18	2 1
JTH	55 (19.23)	female male	32 20	1 2
Kator	6 1(21.33)	female male	28 29	4 0
Melikia	26 (9.09)	female male	13 11	2 0
Munuki	58 (20.28)	female male	28 27	3 0
Nyakuron	39 (13.64)	female male	26 8	2 3
All participants (286)		**female**	**153 (53.49%)**	**14 (4.89%)**
		male	**113 (39.52%)**	**6 (2.09%)**

(+)=culture positive and (-) = culture negative for diarrhoeagenic bacteria.

inoculated into Cary Blair media (Oxoid®, UK) and transported in a triple package container at 2^{0}C-8^{0}C for laboratory analysis. Diarrhoea was defined as the passage of at least three loose or liquid stool in 24 hours. Patients who could not consent or had known underlying causes other than bacteria were left out.

Isolation and identification of bacterial pathogens

Stool specimens were emulsified in peptone water, selenite F broth, Alkaline Peptone Water (APW) and incubated at 35-37^{0}C for 6-8 hours. Peptone water-emulsified specimens were inoculated into Karmali medium, Sorbitol MacConkey (SMAC) and Deoxycholate Citrate agar (DCA) and Hektoen enteric agar (HE) for Campylobacter, *E. coli* O157 and *Shigella* spp isolation respectively, using sterile plastic loops. Selenite F broth emulsified specimens were inoculated into DCA and HE for *Salmonella* isolation. APW-emulsified specimens were inoculated into Thiosulfate Citrate Bile Salts sucrose agar for *Vibrio cholerae* isolation and sub-cultured in Heart infusion agar (HIA). Inoculated plates were incubated at 35-37^{0}C except Karmali plates that were incubated at 42^{0}C in 5% carbon dioxide atmosphere for 18-24 hours. The plates were examined for growth and pure isolates with colonial characteristics of the target diarrhoeagenic bacteria were sub-cultured and identified using specific agglutinating antisera (Remel® UK) and API20E® (Biomerierux, UK).

Antibacterial susceptibility testing

Antibacterial susceptibility was determined by Kirby Bauer disc diffusion method using Mueller–Hinton (MH) agar and results interpreted according to Clinical Laboratory Standards Institute (CLSI, 2011). Commonly used antibacterials as per South Sudan treatment guidelines were selected for sensitivity testing.

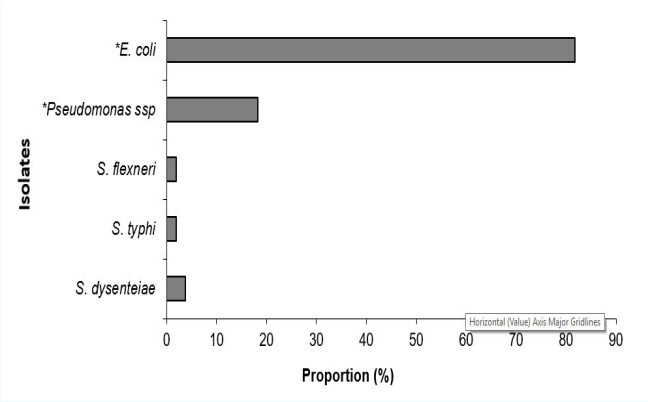
Ethical consideration, data collection and analysis

Ethical approval and specimen shipment clearance was obtained from the Ministry of Health. Data were collected on standard structured questionnaire, entered, cleaned and analyzed by Epi Info® 7 (CDC, Atlanta, USA). Generated data were kept in a password protected computer. Personal identifiers were removed to ensure confidentiality.

Results

Demography and characteristics of the participants

We recruited 286 participants, 167 (58.4%) females and 119 (41.6%) males, mean age 19.81±14.8; range 2-71 years, between August and November 2011 from selected health facilities (HFs).



*These were normal gut flora, although there could be pathogenic E. coli among the E. coli isolates, there was no further analysis for pathogenic strains, a limitation in this study

Figure 1. Proportionate Isolation rate of bacteria among the study participants attending selected health facilities in Juba City.

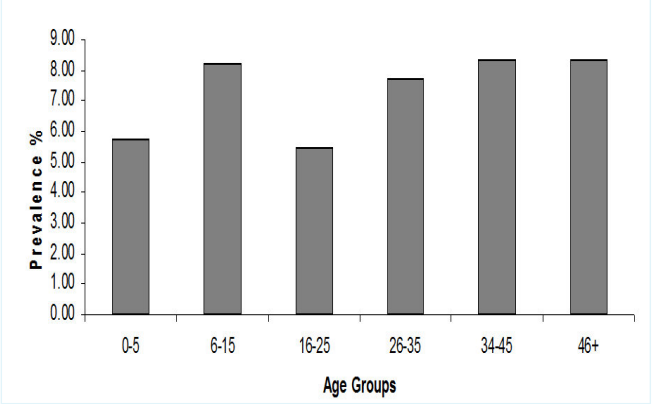


Figure 2. Age group specific prevalence of diarrhoeagenic bacterial among study participant attending public health facilities in Juba City.

The majority of the participants were from Munuki 143/286 (50.0%), followed by Kator and Juba (11.2%). Only 12 (4.2%) participants reached higher education with certificates or higher, and 70 (24.5%) were employed – see Table 1. The participants presented with either diarrhoea with blood or other diarrhoea.

Bacterial isolates and isolation rates

A total of 286 stool specimens were analyzed and diarrhoeagenic pathogens isolated from 6.99% (20/286) of the specimens. The prevalence in under five-year olds was 5.7% (4/70) and adults 7.4% (16/216). The pathogen specific prevalence were *S. typhi* (1.7%), *S. flexneri* (1.7%) and *S. dysenteriae* (3.5%). Nonpathogenic gut flora grew in 93.01% (266/286) of total specimens. E. coli 18% (48/266) and Pseudomonas ssp 82% (216/266) were isolated (see Figure 1 No E. coli O157 was isolated from SMaC and other *E. coli* isolates were not analyzed further for presence of other pathogenic strains. The isolates of enteric bacteria pathogens had similar distribution trends across all ages as shown in Figure 2.

Antibiotic phenotypic susceptibility profiles

Antibiotic resistance in the three (3) different species of pathogenic bacteria of 30-100% was found to amoxicillin, ampicillin, chloramphenicol, tetracycline and co-trimoxazole. Although all isolates were however ceftriaxone susceptible, quinolone resistance was identified with intermediate susceptibility to ciprofloxacin in the majority of Shigella isolates and nalidixic acid resistance in all of the *S. typhi* isolates.

Bivariate analysis

A bivariate analysis for association of the most plausible factors with stool culture results was done but did not yield any independent significant association - see Table 3. Reporting either having any other diarrhoea or diarrhoea with blood by the participants did not have any association with their education level, employment status and sex. Having a source of income was significantly associated (OR=6.08, p<0.05) with stool culture results - see Table 3.

Discussion

Infectious diarrhoea is a major public health problem in South Sudan. This study established the prevalence and antimicrobial resistance profiles of bacterial pathogens isolated from stool of persons with diarrhoea. *S. typhi* (1.7%), *S. flexneri* (1.7%) and *S. dysenteriae* (3.5%) were the enteric pathogens isolated with an overall prevalence of 6.99%. This prevalence was lower than that of Ifeanyi [13] in Nigeria probably due to difference in socioeconomic indices and the exclusion of *E. coli* for determination of our prevalence. The prevalence of diarrhoeagenic bacteria in under five-year olds was 5.7% (4/70) and in adults 7.4% (16/216), demonstrating that everyone is at risk. This concurred with the findings of Samie et al [14] in South Africa and Sabrina et al [15] in Tanzania.

The distribution and isolation rates observed in this study were lower for *S. flexenri* but similar for *S. typhi* and *S. dysenteriae* compared to a related study by Jafari et al [16] in Tehran. These could be due to seasonal differences and geography [13]. Little difference existed in pathogenic bacteria isolation rates among different age groups, educational status, gender, the water drank, use of chlorine, use of toilet, exposure at home to diarrhoea patient, use of water and soap for washing hands, and location of residence. However, odds of diarrhoeagenic bacteria isolation was higher for participants with no source of income as compared to those with some income (OR=6.08, p<0.05).

Evaluation of antibiotic susceptibility profiles revealed that *S. flexneri* 5(100%) were all sensitive to ceftriaxone, nalidixic acid, gentamicin and resistant to amoxicillin, ampicillin, tetracycline and co-trimoxazole. This is not unusual as Ruslan et al [17] also found out that S. flexneri was resistant to ampicillin, co-trimoxazole, chloramphenicol and tetracycline. *S. dysenteriae* isolates were sensitive to ciprofloxacin (70%), nalidixic acid (90%), ceftriaxone (100%), while resistance was observed to amoxicillin (100%), ampicillin (100%), co-trimoxazole (90%), chloramphenicol 7 (70%) and tetracycline 8 (80%). A similar result was obtained in a related study in Kenya [18,19]. *S. typhi* 5(100%) were sensitive to ciprofloxacin, and ceftriaxone but 100-90% resistant to tetracycline, amoxicillin, ampicillin, cotrimoxazole, gentamycin nalidixic acid although Willie et al [18] reported a lower resistance to nalidixic acid at 44%.

Conclusion

This study has established enteropathogenic bacteria as a significant cause of diarrhoea among children and adults irrespective of their ages as evident by 6.99% prevalence. Although it is difficult to draw firm conclusions about antibiotic susceptibility patterns from the small numbers of pathogenic bacteria isolated in this study, profiles were generally consistent with other studies. The high rates of resistance to ampicillin, co-trimoxazole, tetracycline and chloramphenicol indicate they should not be given as empirical treatment for these infections. Further study of antimicrobial resistance trends in the area, including during outbreak periods, would help to inform antibiotic choices. There is an emerging menace of resistance to commonly used antibiotics in South Sudan with ceftriaxone being the only antibiotic currently reliably active against the enteropathogenic bacteria isolated in this study. Antibiotic use needs to be monitored and regulated in South Sudan to prevent outbreak of multidrug resistance.

Limitation

In this study we could not analyze *E. coli* isolates for pathogenic strains due to financial constraints, however presence of E. coli O157 is ruled out since there was no growth on SMaC agar media. Other pathogens (virus) and physiological factors could be responsible for much of the uncharacterized diarrhoea in the study but which are difficult to identify by methods deployed in this study.

Table 2. Antibiotic and bacterial strain specific distribution of resistance in bacteria isolated from patients attending selected health facilities in Juba City, South Sudan

	S. Dysenteriae			*S. Flexneri*			*S. Typhi*		
Antibiotics	I	R	S	I	R	S	I	R	R
CIP	7	3	-	-	5	-	5	-	-
NAL	9	1	-	-	5	-	-	-	5
AMP	-	-	10	-	-	5	-	1	4
CRO	10	-	-	5	-	-	5	-	-
CHL	2	1	7	-	-	5	2	-	3
GEN	3	4	3	5	-	-	5	-	-
TET	-	2	8	-	-	5	-	-	5
SXT	-	1	9	-	-	5	-	-	5
Age groups									
5-0		3			0			1	
15-6		2			2			1	
25-16		1			1			1	
35-26		1			2			1	
45-34		2			0			1	
+46		1			0			0	

R=Resistant, S=sensitive, I=intermediate CIP=ciprofloxacin, NAL=nalidixic acid, AMP=ampicillin, CRO=ceftriaxone, CHL=chloramphenicol, GEN=gentamicine, TET=tetracycline, SXT=co-tremoxazole

Acknowledgment

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Table 2. Antibiotic and bacterial strain specific distribution of resistance in bacteria isolated from patients attending selected health facilities in Juba City, South Sudan

Variables (exposures)	Culture Results (+v)	(-v)		OR (%95 CI)	P-Value
Payam of Residence					
Juba	(+) (-)	3 17	152 116	0.71(0.19, 2.57)	0.84
Kator	(+) (-)	5 15	106 160	0.50(0.17, 2.57)	0.28
Munuki	(+) (-)	12 8	131 135	1.55(0.61, 3.90)	0.48
Female Male		14 6	153 113	1.95(0.72, 5.24)	0.39
Toilet facility	(+) (-)	9 11	82 184	1.84(0.73, 4.60)	0.22
Drink tank water	(+) (-)	17 3	199 67	1.91(0.54, 6.71)	0.42
Use chlorine	(+) (-)	10 10	123 143	1.16(0.47, 2.89)	0.82
Use soap & water	(+) (-)	2 18	18 248	1.53(0.33, 7.21)	0.92
Employment	(+) (-)	2 18	68 198	0.32(0.07, 7.21)	0.19
No income Some income		16 2	152 116	6.08(1.56, 39,77)	<0.05
Education	(+) (-)	10 10	143 122	1.17(0.47, 2.90)	0.91
Husehold exposure	(+) (-)	14 16	51 207	0.98(0.32, 3.07)	0.79
Female Male		15 84	112 35	0.84(0.51, 1.41)	0.61

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DR PETER NEWMAN APPOINTED ROYAL COLLEGE OF PHYSICIANS ASSOCIATE INTERNATIONAL DIRECTOR FOR SUB-SAHARAN AFRICA

Royal College of Physicians

Dr Peter Newman has been appointed Royal College of Physicians London (RCP) Associate International Director for Sub-Saharan Africa.

A consultant neurologist based in Middlesbrough, he takes over from Professor Simon Taylor-Robinson who has stepped down to focus on his role on the RCP Council.

In his first term in the post, Dr Newman led the RCP's work in Africa between 2005 and 2009 and was integral in implementing its first projects in the region including a series of 'Doctors as Educators' courses attended by several doctors from South Sudan. He is a regular PACES examiner in the UK and overseas, and member of the interview panel for the RCP's Medical Training Initiative – a scheme which enables international medical graduates to undertake a 2-year training fellowship in the UK.

He joins a team of seven RCP associate international directors, each of whom is responsible for a different region of the world and leads on developing strategy and implementing projects in that region. Dr Newman will continue to work with Dr Eluzai Hakim , RCP Adviser for South Sudan and looks forward to the rekindling in due course of the intercollegiate initiative in postgraduate medical training.