

Etiology and antimicrobial resistance of bacterial bloody diarrhea in rural Babylon provinces⁺

مسببات الاسهال ومدى مقاومة المضادات الحياتية للبكتريا المسببه للاسهال الدموي في قرى محافظة بابل

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Abstract:

From January 2007 to April 2008, 500 stool samples were collected from persons with bloody diarrhea presenting to rural clinics to study the Etiology and antimicrobial resistance of bacterial bloody diarrhea in rural Babylon provinces.

Cultural of 210 (42%) samples yielded 217 bacterial pathogens: 180 belong to which included species Shigella (98 *S. flexneri*, 31 *S. dysenteriae* type 1, 32 *S. dysenteriae* type non-1, 12 *S. boydii*, 8 *S. sonnei*), 23 *Campylobacter*, 13 non-typhoidal *Salmonella*, and 1 *Vibrio cholerae* O1. More than 90% of the isolates were resistant to trimethoprim-sulfamethoxazole and tetracycline, and more than 80% were resistant to ampicillin. Bloody diarrhea was most common among persons less than five years of age (98) and young adults 20–29 years old(110)

المستخلص:

تم جمع ٥٠٠ نمذج براز للفترة من كانون الثاني ٢٠٠٧ الى نيسان ٢٠٠٨ من مرضى راجعوا مستشفيات خارج مركز محافظة بابل (قرى) وكانوا يعانون من اسهال دموي لدراسة مسببات ومقاومة المضادات الحياتية للبكتريا التي تسبب الاسهال الدموي في المحافظة . ٢١٠ نمذج (٤٢%) اظهر الزرع البكتريولوجي لها وجود ٢١٧ مسبب

بكتيري ١٨٠ تعود لجنس الشيكلا شملت الانواع (98 *S. flexneri*, 31 *S. dysenteriae* type 1, 32 *S. dysenteriae* type non-1, 12 *S. boydii*, 8 *S. sonnei*), 23 *Campylobacter*, 13 non-typhoidal *Salmonella*, و 1 *Vibrio cholerae* O1.

ان اكثر من ٩٠% من العزلات كانت مقاومة للمضادات الحياتية تراميثبرم – سلفاميثوكزازول والتتراساكيلين كذلك وجد ان اكثر من ٨٠% مقاومة للامبيسلين . كذلك بينت الدراسة ان الاسهال الدموي كان شائعا بين الاطفال دون سن الخامسة من العمر (٩٨) و البالغين من عمر ٢٠ الى ٢٩ سنة (١١٠)

Introduction

Bloody diarrhea, a source that kills as many as 700,000 people worldwide every year [1]. Most of the victims live in developing areas with poor sanitation, but sporadic cases can pop up anywhere in the world [2]. Bloody diarrhea causes property greater in morbidity and mortality. [3]. Antibiotics are recommended for

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treating bloody diarrhea to shorten the duration of illness, decrease morbidity and mortality, and reduce the duration of bacterial shedding [4]. Antimicrobial resistance among the major bacterial causes of bloody diarrhea is increasing worldwide [5].

*Large outbreaks of bacillary dysentery have occurred in communities where drinking water contaminated with sewage [6]. Contaminated Fruits and vegetables were another common source of disease [7]. Infections can also spread through households when people don't wash their hands after using the bathroom, after changing diapers, or before handling food [8] [9]. Causative pathogens such as *Shigella dysenteriae* type 1 have developed resistance to locally affordable and available antibiotics [10]. Treatment of dysentery with antibiotics to which the etiologic agent is resistant may prolong illness and increase risk of hemolytic uremic syndrome and death [11], [12].*

MATERIALS AND METHODS

Sampling or patients:

The study took place in the Babylon Province occurring generally from January 2007 to April 2008, conducted laboratory-based surveillance for diarrheal illness at clinics. All clinics treated adults and children and defined diarrhea as bloody if blood was visible in the specimen or if the patient reported seeing blood in their stool. After each participant provided written informed consent, (demographic information; symptoms, and therapy history).

Laboratory procedures:

3 gram of stool collected from each person , 1 gm was inoculated in to 10 ml of selenit F-broth and 1 gm inoculated into Tetrathionate broth for enrichment, culture broth were inoculated at 37 °c for 24 hours and subculture were then made on to XLD agar and S.S agar . All stool specimens in additions to being inoculated in to alkaline peptone water and sub culture in TCBS agar. Stool specimens were cultured for *Shigella*, *Salmonella*, *Vibrio*, and *Campylobacter*; media, reagents, bacterial isolation technique; (Isolation & identification of pathogens standard methods were used to isolate and final identification by API 20 E Kit (Biomérieux) and serological test in central laboratory [13]. Were determined antimicrobial susceptibilities of 150 bacterial pathogens except *Campylobacter* ,antibiotics used was ampicillin, amoxicillin-clavulanate, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole by the Kirby-Bauer disk diffusion method; [14].

Case-control study

Between May 2007 to April 2008 conducted a case-control study to determine risk factors for bloody diarrhea. For each healthy controls matched by age group and sex. After obtaining written informed consent, local health workers administered an initial questionnaire to cases and controls in local dialect asking about drinking water sources and food eaten during the past seven days, and personal hygiene (e.g., use of latrines, hand washing). Within 14–30 days after enrollment, village health workers visited both cases and controls at home (repeat visits were made to compound-based controls) to conduct follow-up interviews and collect observational data about the household.

Results

During period of my study 500 specimens with bloody diarrhea were collected and cultured, the results indicate that there is a highly significant difference P value ($P < 0.05$), 10(2%) of which no growth. The median age of persons with bloody diarrhea was 26 years and 60% were male. Reported symptoms included abdominal cramping (88%), fever (70%), nausea (43%), vomiting (51%), coincident mucous diarrhea (88%), and coincident watery diarrhea (56%). also isolated bacterial no pathogen from 280 (56%) specimens, at least one pathogen from 210 (42%) specimens, and more than one pathogen from 7 (Table 1). *Shigella* was most common and accounted for 180 isolates (36% of all specimens): 98 (19.6%) *S. flexneri*, 31 (6.2%) *S. dysenteriae* type 1, 32 (6.4%) other *S. dysenteriae*, 12 (2.4%) *S. boydii*, and 8 (1.6%) *S. sonnei*. *Campylobacter* (23 isolates, 4.6%), non-typhoidal *Salmonella* (13 isolates, 2.6%), and a single *Vibrio cholerae* O1 (2%) were also not isolated *E. coli* O157:H7 from specimens from persons with bloody diarrhea.

(Table1) Bacterial enteric pathogens isolated from 500 persons with bloody diarrhea

	All specimens (%)* n = 500	Specimens from case-control study (%) (n = 100)
No growth	10 (2)	3 (3)
Normal growth	280 (56)	60 (60)
Yielded an enteric pathogen	210 (42)	37 (37)
Total pathogens isolated	* 217	39 (39)
<i>Shigella</i>, all	180 (36)	30 (30)
<i>S. flexneri</i>	98 (19.6)	11 (11)
<i>S. dysenteriae</i>, type 1	31 (6.2)	8 (8)
<i>S. dysenteriae</i>, other	32 (6.4)	5 (5)
<i>S. boydii</i>	12 (2.4)	5 (5)
<i>S. sonnei</i>	8 (1.6)	2 (2)
<i>Campylobacter</i>	23 (4.6)	4 (4)
<i>C. jejuni</i>	15 (3)	2 (2)
<i>C. coli</i>	4 (0.8)	2 (2)
<i>C. jejuni/coli</i>	1 (0.2)	-
Non-<i>jejuni</i>, non-<i>coli</i> <i>Campylobacter</i>	3 (0.6)	1 (1)
<i>Salmonella</i>, non-typhoidal	13 (2.6)	2 (2)
Group B	6 (1.2)	-
Group C1	-	-
Group C2	4 (0.8)	1 (1)
Group D	2 (0.4)	1 (1)
Group E	1 (0.2)	-
<i>Vibrio cholera</i> serotype O1	1 (0.2)	-

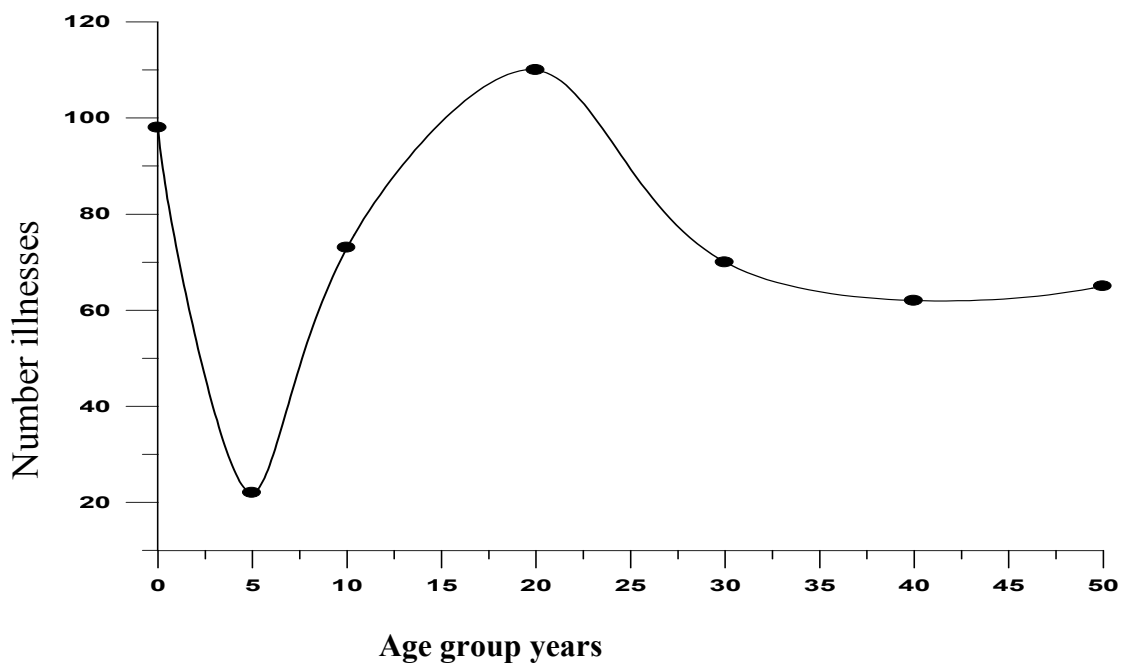
* Calculated as the percentage of isolates among all specimens 500

S. Typhimurium, 3 *S. Enteritidis*, 2 *S. Heidelberg* and *S. Aberdeen*, 1 each *S. Chailey*, *S. Newport*, *S. Uganda*, and *S. Zanzibar*.

Highly significant difference P value ($P < 0.05$)

Bloody diarrhea was most common among persons less than five years of age (98) and young adults 20–29 years old (110) (Figure 1). Among 98 specimens from children less than five years of age that yielded 40 isolates. In comparison, among 402 specimens from persons greater

than or equal to five years old that yielded 177 isolates. Also the results indicate that there is a no significant association between the age groups and number illnesses ($P > 0.05$).



No Significant difference $P > 0.05$
(Figure 1) : Cases of bloody diarrhea illness by age

100 cases controls were older than other persons with bloody diarrhea in my surveillance to study the risk factors for bloody diarrhea (median age = 26 - 33 years, Only five (5%) cases were less than five years old, compared with 15.4% of all persons with bloody diarrhea identified through surveillance. Cases did not differ from other surveillance patients by sex, village of residence, bacterial isolation rates, or the proportional distribution of bacterial species isolated.

The antimicrobial susceptibilities of isolated pathogens are shown in table 2 . Less than 10% of the isolates were susceptible to tetracycline, trimethoprim-sulfamethoxazole, sulfisoxazole, and streptomycin, 19% were susceptible to ampicillin, 33% to chloramphenicol, and 34% to amoxicillin-clavulanate. More than 95% remained susceptible to nalidixic acid, kanamycin, and gentamicin, and 100% to ciprofloxacin and ceftriaxone.

Pathogen (number tested)	Chl	TMP-SMZ	Tet	Cpfx	NA	Amp	SSZ	Stm	Km	Gm	Ctri	Amox-CA
All isolates (150)	33	8	6	100	97	19	7	6	97	98	100	34
<i>Shigella</i> spp. (139)	30	5	2	100	98	17	7	4	98	99	100	32
<i>S. dysenteriae</i> type 1 (21)	0	7	0	100	100	2	7	7	100	100	100	22
<i>S. dysenteriae</i> (other (10)	74	0	5	100	97	13	10	0	97	97	100	56
<i>S. flexneri</i> (90)	9	6	0	100	97)	15	7	4	98	100	100	19
<i>S. boydii</i> (10)	100	8	8	100	100	46 (8)	0	0	100	100	100	62
<i>S. sonnei</i> (8)	100	0	12	100	100	88	0	0	100	100	100	88
<i>Salmonella</i> , nontyphoidal (10)	82	64	64	100	82	54	9	54	73	73	100	54
<i>Vibrio cholerae</i> serotype O1 (1)	100	0	100	100	0	100	0	0	100	100	100	100

(Table 2) : Antimicrobial susceptibility patterns among 150 bacterial isolates tested from persons with bloody diarrhea

* Chl = chloramphenicol; TMP-SMZ = trimethoprim-sulfamethoxazole; Tet = tetracycline; Cpfx = ciprofloxacin; NA = nalidixic acid; Amp = ampicillin; SSZ = sulfasoxazole; Stm = streptomycin; Km = kanamycin; Gm = gentamicin; Ctri = ceftriaxone; Amox-CA = amoxicillin-clavulanic acid.

Among risk factors identified 8 that were significantly or nearly significantly associated with bloody diarrhea, all of them increased risk factors (Table 3). Exposures that alter risk for bloody diarrhea included drinking boiled water from any source, presence of soap or a latrine at the home compound, frequency of latrine use, distance of latrines from the home compound's food preparation area, presence of human feces in the home compound yard, eating at a funeral within seven days of illness onset, density of buildings and rooms in the home compound, methods for water storage, and methods for removing water from storage vessels.

(Table 3): Frequency of selected exposures among controls study

Exposure	Controls, n/N (%)
Used as a source of drinking water during the last 7 days	
Borehole (well)	77/100 (77)
Stream/river	50/100 (50)
Purchased water from the following sources in the last 7 days	
Rain water	62/100 (62)
Regarding general hygiene	
Washed hands after defecating	90/100 (90)
Washed hands before preparing food	72/100 (72)
Allowed other families to use their latrine	52/100 (52)
Ate food from the following places in the last 7 days	
Another compound (family or friend)	70/100 (70)
Restaurant	51/100 (51)

Discussion :

Similar to previous studies in Africa, found that *Shigella* predominated as a cause of sporadic bloody diarrhea, [16.15.17]. In my study found that *Campylobacter* and *Shigella* genus were isolated with similar frequency from children less than five years old with bloody diarrhea. In comparable studies of semi-urban Bolivian children less than five years old [18]. With bloody diarrhea [19]. *Campylobacter* was isolated at least half as frequently as *Shigella*. Although *Campylobacter* infections are among the most common causes of childhood bacterial diarrhea in developing countries, young children in these circumstances also exhibit high rates of asymptomatic *Campylobacter* carriage. [20]. [21].

Also found a high level of resistance to the antibiotics most commonly prescribed; 74% of persons with bloody diarrhea received antibiotics to which their isolate was not susceptible. Our data were inadequate to assess the clinical impact of these findings (e.g., duration of bloody diarrhea, mortality, or bacterial shedding). Nonetheless, strategies to improve prescription practices that use surveillance data to rationally guide more judicious antibiotic use warrant consideration. And identified no resistance to ciprofloxacin and minimal resistance to nalidixic acid. Other studies have also found little resistance to nalidixic acid and/or no resistance to ciprofloxacin among *Shigella* in East Africa [22]. And in other African countries [23]. However, in areas where nalidixic acid has been introduced as the drug of choice to treat presumptive shigellosis, a marked increase in corresponding resistance has been observed [24].

The case-control study identified several opportunities for primary prevention of bloody diarrhea that could decrease the overall need for antibiotics. Drinking water from Lake increased risk. Rainwater and borehole water used for bathing, washing clothes, and watering livestock, leading to contamination with human and animal waste. During dry seasons when rainwater and borehole water are less available, disinfecting drinking water from available surface sources may substantially reduce illness.

Sharing latrines has been previously associated with an increased risk of infection with epidemic *S. dysenteriae* type 1[25]. In this study, sharing latrines increased risk for sporadic bloody diarrhea. This practice may increase community exposure to infected feces; building more latrines could potentially reduce cases of bloody diarrhea.

Washing hands after defecating was protective, a finding also consistent with other studies. [26]. It has been estimated that the attributable risk for dysentery from not washing hands before preparing food in rural African communities is as high as 30%[29]. Hand washing, especially if soap is used, substantially reduces both primary *Shigella* infections and secondary transmission [27].

The observation that street vendor foods were protective contradicts the findings of numerous previous investigations. [28]. [29]. these studies were all conducted in urban or peri-urban settings.

In summary, *Shigella* causes most sporadic bloody diarrhea in Babylon province although *Campylobacter* may account for a large fraction of illnesses in children less than five years old. There is substantial resistance among *Shigella* to the antibiotics most frequently prescribed for bloody diarrhea, and many persons receive medication to which their infecting bacteria are not susceptible. Providing access to safe drinking water and to latrines, and promoting hand

washing could substantially reduce the incidence of bloody diarrhea, the resultant need for antibiotics, and the pressures favoring increased antimicrobial resistance.

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