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Bacterial Agents of Enteric Diseases of Public Health Concern in Benghazi City, Libya

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ABSTRACT

Background: *Globally, infectious diarrhea (also known as gastroenteritis) estimated as a major public health concern. Fortunately, such diseases are self-limiting, but the diagnosis of the infecting microbe is important for the prevention and control of the disease (food-borne disease). Infectious diarrhea is a major cause of childhood mortality, with estimated 1.9 and 5.6 million deaths yearly. In the last few decades, several enteric pathogens including bacteria (such as, Campylobacter spp., Escherichia coli, Salmonella and Shigella), viruses (e.g., norovirus, adenovirus, and astrovirus), and parasites (e.g., Cryptosporidium spp.) have been identified as important causes of diarrhea in humans, particularly in children. However, these pathogens either have not yet or have rarely been reported from pediatric diarrhea in Libya and other countries of the North Africa region.*

Objectives: *The aim of this study was to assess the presence of bacterial agents of enteric disease in the study area.*

Methods: *Stool samples collected from three hundred Libyan children with diarrhea, attending to Benghazi pediatric hospital in Benghazi, were examined for bacterial agents, the specimens were cultured and antibiotic sensitivity done using standard microbiological techniques. The study was carried out between April 2016- and April 2017.*

Results: *Of the 300 examined stool samples, Salmonella was detected in 13 (4.3%). gender (53.85%) were from male the remaining (46.15%) were from female .The majority of isolates (84.6%) were from patients less than 5 years old. All isolates were in the summer season. Antibiotic resistance was low in our study. All isolates were sensitive to nalidixic acid, ciprofloxacin and chloramphenicol. The result reveals statistically significant association between diarrhea and patients' age, seasonal variation and Antibiotic sensitivity.*

Conclusion: *The present work has clearly demonstrated that Salmonella is the causative agents of diarrhea. In the future, studies are needed on larger groups of patients from major cities in Libya to determine the exact role of these bacteria in children diarrhea in the country.*

1. Introduction

Ingestion of pathogens can cause many different infections. These may be confined to the gastrointestinal tract or are initiated in the gut before spreading to other parts of the body. A wide range of microbial pathogens are capable of infecting the gastrointestinal tract. They are acquired by the fecal- oral route, from fecally contaminated food, fluids or contaminated hands. For an infection to occur, the pathogen must be ingested in sufficient numbers or possess attributes to elude the host defenses of the upper gastrointestinal tract and reach the intestine. Here they remain localized and cause disease as a result of multiplication and / or toxin production. Diarrhea is the most common outcome of gastrointestinal tract infections (Richared, *et al*., 2007). Diarrhea is one of the most frequent diseases, with attack rates estimated to be from 2 to 12 or more diarrheal episodes per person annually worldwide (Bern, *et al*., 1992; Gue rrant, *et al*., 1990).

Globally, among children aged less 5 years old diarrheal infections remain the leading cause of mortality and represent nearly 10% of child deaths annually (Kotloff, *et al*., 2013). In addition, about 3.3 million children younger than 5 years die each year from diarrhea (Bern, *et al*., 1992). *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. are known to cause mainly community-acquired diarrhea, but they rarely cause nosocomial enteritis (Barbut, *et al*., 1995; Fan, *et al*., 1993; Siegel, *et at*., 1990 and Telzak, *et al*., 1990). Considerable savings may be achieved if cultures for bacterial enteric pathogens are restricted to samples from patients hospitalized for 3 days (Morris, *et al*., 1996), (Valenstein, *et al*., 1996). Cytotoxigenic *Clostridium difficile* and rotaviruses may cause

both community-acquired and nosocomial diarrhea, with the latter being a particular cause of diarrhea in children (Haffejee and I.E, 1995 and Hirschhorn, *et al*., 1994).

In this study, results obtained with fecal specimens from the included ambulatory and hospitalized patients were retrospectively analyzed with the aim of adjusting our recommendations for requesting culture so as consequences the yield of enteric pathogens could be improved. In particular, charts of patients hospitalized for 3 days with enteric pathogens in the stool cultures were reviewed in order to determine the possible reasons for this rare event, as well as the clinical significance of these results.

2. Material and Methods

Prospective randomized cross sectional study of (300) stool samples obtained from patients with diarrhea admitted to different hospitals at Benghazi city. Study was conducted in the period from April 2016- April 2017. However, all data that concern about enteric diseases were not available at these Centers with the exception of Benghazi pediatric hospital.

The study populations were individual of all age groups who attended to different Medical Centers in Benghazi city during the study period. Personal data were collected via questionnaire (Annex 4). Questionnaire contained demographic data (Age, sex, symptoms of gastroenteritis, medical diseases etc.). Isolates were tested for antibiotic susceptibility test using standard microbiological techniques (Mackie and Mc Carteny, 1996).

Stool specimens were inoculated into Campy-blood agar plates, Sorbitol Mac-Conkey agar (SMAC agar), Xylose lysine desoxycholate agar (XLD agar) plates and Selenite F broth as fluid enrichment (subculture after 24-h onto XLD). The inoculated plates were incubated aerobically for 24 to 48-h at 37°C and in micro-aerophilic atmosphere for 48-h at 42°C for Campy - blood agar plates.

2.1 Identification of isolated bacteria

2.1.1. Culturing and identification

The collected stool specimen was processed for bacteriological analysis. The stool sample was inoculated into MacConkey (Oxoid Ltd.) and Xylose Lysine Deoxycholate agar (Oxoid Ltd.) by using sterile wire loop. The inoculum was incubated under aerobic condition at 37°C for 24-h. Cultural characteristics were examined for growth and colonial morphology as well as any visible changes in the medium such as color, shape, size, etc. Liquid media were examined for turbidity and sediment.

2.1.2 Gram staining

Gram stain was used to study the morphology, shape and gram reaction of each isolate. A smear was prepared from the isolate by emulsifying apart of colony in a drop of normal saline on clean glass slide. The smear was allowed to dry in air, fixed by flaming, placed on rack and flooded with crystal violet (basic stain) for 2 minutes. Slide was washed in running tap water. Then the slide was covered with Gram's Iodine for 60 seconds, and washed in tap water. Decolourization was made with a few drops of alcohol for seconds and the slide was washed thoroughly in water. The smear counterstained with safranin for 30 seconds, washed and blotted to dry with filter paper.

Microscopy viewing

The prepared slides were examined under the microscope using oil-immersion objective lens (×100).

2.1.3 Biochemical identification

Suspect colonies were inoculated into a biochemical testing system (Analytical Profile Index API 20 E). API 20E is a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae. In, the plastic strip holds twenty mini-test chambers containing dehydrated media having chemically-defined compositions for each test.

Negative result

Positive result

Figure 2.1. (API 20E Strip)

2.2 Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility test was carried out for identified both *Salmonella* and *Shigella* species. The test performed on Muller Hinton agar (MHA) by using Kirby–Bauer disc diffusion technique. Precisely, pure identified colonies from the overnight culture was suspended in nutrient broth and incubated for 4-h at 37°C.

Turbidity of broth culture was checked against 0.5 McFarland standards. By using sterile swab the organism in broth was uniformly inoculated into MHA. The antibiotic discs were applied on the surface of the inoculated agar. Antimicrobial disc were selected according to committee for clinical laboratory standard (CCLS). Different antimicrobial agents suggested by WHO (Ampicillin 10µg, Ciprofloxacin 5µg, Trimethoprim-Sulfamethoxazole with 1.25/ 23.75µg (Cotrimoxazole), Chloramphenicol 30µg, Nalidixic acid 30µg) [42]. Antibiotic discs were added and incubated at 37°C for 24-h. Susceptibility and resistance to each antibiotics was determined on basis of the size of growth inhibition zone, according to the chart of interpretive standards for disc susceptibility testing (CLSI).

Statistical analysis

All outcome data were analyzed by using Statistical Package for social Sciences (SPSS; version 23.0).

3. Results

A total of 300 stool samples were involved in this study, the isolation rate of bacterial agent was 4.3%. *Salmonella* isolated from 13 patients among 300 gastroenteritis cases at Benghazi pediatric hospital. *Salmonella* was isolated and identified by using morphological and biochemical test as appears in (ANNEX 6). According to the gender,

(53.85%) of isolations were from male (Table 3-1). The majority of isolates (84.6%) were from patients less than 5 years old, and the remaining (15.3%) were from age more than 5 years (Table 3-2). Regarding the type of gastroenteritis, all *Salmonella* cases were isolated from acute infection, there were no chronic infection (Table 3-3). All isolates were in summer months (53.8%), (30.8%) and (15.4%) in June, July, and August, respectively (Table 3-4). The result reveals statistically significant association between (positive culture result) and patient's age, seasonal variation (P. value 0.0003 and 0.01 respectively).

Gender	Frequency	Percentage (%)
Male	7	53.85
Female	6	46.15
Total	13	100.0

Table 3-1 Distribution of *Salmonella* according to patient's gender

Age group (years)	Frequency	Percentage (%)
5	11	84.6
5	2	15.3
Total	13	100.0

Table 3-2 Distribution of *Salmonella* according to patient's age group

Type of infection	Frequency	Percentage (%)
Acute	13	100.0
Chronic	0.0	0.0
Total	13	100.0

Table 3-3 Distribution of *Salmonella* according to the type of infection

Summer months	Frequency	Percentage (%)
June	7	53.8
July	4	30.8
August	12	12.4
Total	23	100

Table 3-4 Distribution of *Salmonella* according to seasonal variation

3.1 Evaluation of the antimicrobial sensitivity profile

3.1.1 Disc diffusion method

Susceptibility testing of the 13 bacterial isolates showed that the most resistance was found against Ampicillin (15.38%) followed by Trimethoprim and Sulfamethoxazole 1.25/23.75 µg {Cotrimoxazole} (7.7%). Regarding susceptibility, bacterial isolates found to be susceptible to Ciprofloxacin, Chloramphenicol and Nalidixic acid (100%), followed by Cotrimoxazole and Ampicillin (76.92% and 53.84%, respectively). The result reveals statistically significant association between (positive culture result) and antibiotic sensitivity (P. value at the 0.01 level).

Bacteria isolates (13)						
Antibiotics	No of isolates	% of isolates	No of isolates	% of isolates	No of isolates	% of isolates
Ampicillin	7	53.84	4	30.76	2	15.38
Ciprofloxacin	13	100.0	0.0	0.0	0.0	0.0
Cotrimoxole	10	76.92	2	15.38	1	7.70
Chloramphenicol	13	100.0	0.0	0.0	0.0	0.0
Nalidixic acid 13	100.0	0.0	0.0	0.0	0.0	0.0

Table 3-5 Antibiotic susceptibility of *Salmonella* by disk diffusion. 1, 2 and 3 according to the CLSI

Distribution a total of specimens according to patients gender, 156 (52%) of specimens were from male, and 144 (48%) were from female (Table 3-6). of all male cases only 149 (95.5%) showed negative result (Table 3-7), and 138 (95.8%) showed negative result in female patients (Table 3-8).

Gender	Frequency	Percentage (%)
Male	156	52
Female	144	48
Total	300	100

Table 3-6 Distribution of specimens according to patient's gender

Male	Frequency	Percentage (%)
Positive	7	4.5
Negative	149	95.5
Total	156	100

Table 3-7 Distribution of specimens in male patients

Female	Frequency	Percentage (%)
Positive	6	4.2
Negative	138	95.8
Total	144	100

Table 3-8 Distribution of specimens in female patients

Age	Frequency	Percentage (%)
Neonate	0.0	0.0
Less than 5 years	254	84.7
More than 5 years	46	15.3
Total	300	100

Table 3-9 Distribution of specimens according to patient's age

Based on age classification, persons with age less than 5 years represented 254 (84.7%) of all cases, and 46 (15.3%) were from age more than 5 years, there were no neonate cases reported in this study (Table 3-9). In age less than 5

years old the negative result were 243 (95.7%), and positive result were 11(4.3%) (Table 3-10), while in age more than 5 years old the negative result were 44 (95.7%) , and positive result were 2 (4.3%) (Table 3-11).

Age less than 5 years	Frequency	Percentage (%)
Positive	11	4.3
Negative	243	95.7
Total	254	100

Table 3-10 Distribution of specimens according to age less than 5 years

Age less than 5 years	Frequency	Percentage (%)
Positive	2	4.3
Negative	44	95.7
Total	46	100

Table 3-11 Distribution of specimens according to Age more than 5 years

Regarding the type of infection, the majority of specimens 287 (95.7%) showed non-bacterial infection, and the remaining showed positive bacterial infection 13 (4.3%) (Table 3-12).

Type of infection	Frequency	Percentage (%)
No infection	287	95.7
Acute infection	13	4.3
Total	300	100

Table 3-12 Distribution of specimens according to type of infection

Nearly 60 (20%) specimens were collected during winter season which showed negative bacterial growth, in spring 93 (31%) were collected without positive bacterial growth, in summer the number of specimens were 68 (22.7%) and there were positive bacterial growth in 13 (4.3%) of cases, in autumn 79 (26.3%) of specimens were collected all of them showed negative bacterial growth (Table 3-13).

Season variation	Frequency	Percentage (%)
Winter	60	20
Spring	93	30
Summer	68	22.7
Autumn	79	26.3
Total	300	100

Table 3-13 Distribution of *Salmonella* infection according to seasonal variation

A total of 287 (95.7%) of specimens showed that there were no growth for *Campylobacter* spp., *Shigella* spp., and *E .coli* O157 as causative agents of enteric disease (Table 3-14).

Pathogenic bacteria	Frequency	Percentage (%)
Salmonella	13	100
Shigella	0	0

Campylobacter	0	0
***E.coli* O157**	0	0
Total	13	100

Table 3-14 Distribution of pathogenic bacteria according to positive result

4. Discussion

This study was performed to investigate the role of bacterial agents in enteric diseases. In the present study, 13 (4.3%) samples were showed positive bacterial growth this is in agreement with research conducted by (Georges, *et al*., 1988). Who found the *Salmonella* rate was 4.5%. As well as, this is matching the results reported in Kenya which established that *salmonella* species were 3.9% of isolates (Shirley, Karambu, *et al.*, 2013).

The National Institute of Health, Korea, reported that 23 of 632 (3.6%) *Salmonella* isolates were of this serovar in 2003 (Heung, *et al*., 2015). In comparison, the isolation rate in the current study was 4.3%, whereas in Oman the bacterial etiology was found in 15.2% of cases 2.1% were due to *salmonella* (Patel, *et al.*, 2008).

In addition, *Salmonella* was isolated from 5.9 % of cases in China (Shenghui, *et al*., 2009). Studies done in Argentina and Bangladesh found that Rotavirus, Shigella species, and ETEC were the most common isolates from diarrheal samples (Youssef, *et al*., 2000). In Libya, rotavirus and *Salmonella* have been documented as major causative agents of childhood diarrhea (Ghenghesh, *et al*., 2008). In the last few decades, several enteric pathogens including bacteria (e.g., *Campylobacter* spp., enterohemorrhagic *E. coli*, and enteroadherent *E. coli*), viruses (e.g., norovirus, adenovirus, and astrovirus), and parasites (e.g., *Cryptosporidium* spp.) have been identified as an important cause of diarrhea in humans, particularly in children (Nataro and Kasper, 1998; Xiao, *et al*., 2000). However, these pathogens either have not yet or have rarely been reported from pediatric diarrhea in Libya and other countries of the North Africa region.

Approximately (95.7%) of diarrhea cases had no bacterial pathogen, suggestive of probable viral origin. More than one-third of salmonellosis cases occur in children younger than 10 years old, and the incidence in children younger than 1 year old are 10 times higher than in the general population (U.S.D.A, 1998). The majority of isolates (84.6%) were < 5 years. This is similar to what was found by (Patel, *et al*., 2008). This is in agreement with research done by (Mustafa, *et al*., 2001). Who found that the major of cases were children younger than 12 years old.

Kalaf and Ghenghesh, 2011, have reported that the aged of children with acute gastroenteritis were from a few days to 60 months attending the Aljalla Pediatric Hospital, Tripoli, Libya. In this study, sample from male patients were (53.85%) and from female patients were (46.15 %). This agrees with the study illustrated in teaching hospital in Nigeria were the number of cases distributed between males and females were (54% and 40%, respectively), found that diarrhea not significantly related to sex (Donald, *et al*., 1993). A study done on the children living in urban slums in Salvador, Brazil on incidence of diarrhea revealed that male children were associated with episodes of diarrhea as compared to their female counterparts (Yilgwan and Okolo, 2012). Similar study done in Denmark found that being a male, one stands a chance of getting diarrhea (Maria, *et al*., 2008) these two studies contradict our finding in this study.

Our finding was also contradicted by a similar study done in India in a pediatric ward of Command Hospital Pune, boys were more likely to be afflicted with a diarrheal disease than girls (63.16% vs. 36.84%) (Kare, *et al*., 1997). Schilling did a similar study in Kenya and found no association between gender and diarrhea disease (Katharine, 2010). Most of bacteria were isolated from acute infection. The present study showed that there was relation between seasonal variation and infection with *Salmonella.* The bacteria were detected in summer season (53.8%), (30.8%) and (15.4%) in June, July, and August, respectively. This is in agreement with study was done by (Lal, *et al*., 2012), who found the bacterial diseases, campylobacteriosis, salmonellosis and VTEC, showed temporal variations in incidence , indicated by the Gini index and a similar spread of seasonal peaks in summer for most regions . In other studies, it was found that increased environmental temperatures were associated with an increase in visits to emergency departments, clinic visits and number of counted cases of diarrheal diseases in children (Chou, *et al*., 2010; Zhou, *et al*., 2013). In addition to these, the relationship between diarrhea incident cases and temperature has been documented in other areas such as Peru, Fiji and Dhaka where a 1℃ increase in ambient temperature could result in 8%, 3% and 6% increases in diarrheal cases, respectively (Checkley,*et al*., 2000 ; Hashizume, *et al*., 2007 ;Singh, *et al*., 2001) . This is equivalent to what we found in our study.

Results of the antimicrobial sensitivity demonstrated that approximately (15.38%) of the *Salmonella* isolates were resistance to Ampicillin (7.7%) and to Trimethoprim/ sulfamethoxazole. Isolates were sensitive to nalidixic acid, ciprofloxacin and chloramphenicol this is in agreement with research done by (El-Ghodban., 2002) who reported that resistance rates for *Salmonella* varied tremendously among the different commonly used antibiotics. High resistance rates for ampicillin were reported in 1979-2008 from Tripoli, during the period 1990-1999, more than 40% of *Salmonella* and *Shigella* from children with diarrhea were resistant to ampicillin and trimethoprim-sulfamethoxazole (El-Ghodban, *et al*., 2002; Ghenghesh, *et al*., 1997). Similar findings were reported for *Salmonella* from Benghazi (Salih, *et al*., 1994). This is matching the study done in Dhahira hospital in Oman by (Patel *et al.*, 2008). Who found approximately 10% of the *Salmonella* isolates were resistance to Ampicillin and 7.1% were resistance to nalidixic acid and Trimethoprim/ sulfamethoxazole. Isolates were sensitive to ciprofloxacin and chloramphenicol (Patel, *et al*., 2008).

Antibiotic resistance was low in this study, in contrast to a Yemen study where more than two-thirds of the *Salmonella* isolates were resistant to nalidixic acid, chloramphenicol, co-trimoxazole, gentamicin, and amoxicillin, while 42% were resistance to cefotaxime (Banajeh, *et al*., 2001). Other studies done by (Mustafa, *et al*., 2001; El-Ghodban, *et al*., 2002) their studies from Libya reported fluoroquinolones susceptibility of all salmonellae isolated from diarrheic children.

5. Conclusion

Salmonella species were the organism responsible for bacterial diarrhea in this study, and the infection are highest in infants. Travel outside the country was associated with illness in infants 3 to 6 and >6 months of age. Attending day care centers with a child with diarrhea was associated with salmonellosis in infants >6 months of age). The impact of environmental temperature increase on the incidence of diarrhea cases observed in the present study, *Salmonella* infection is more common in summer months (June, July, August) than winter . Antibiotic resistance

was low in our study, approximately (95.7%) of diarrhea cases had no bacterial pathogen, suggestive of probable viral origin. Most cases of acute gastroenteritis in children are viral, self- limiting and only supportive treatment. Appropriate fluid and electrolyte therapy, with close attention to nutrition, remains central to treatment. Antibacterial therapy serves as an adjunct to shorten the clinical course, eradicate causative organisms, reduce transmission and prevent invasive complications.

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Consent for publication

We declare that we consented for the publication of this research work.

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