

Antibiotic Susceptibility of *Salmonella* spp., *Shigella* spp. and enteropathogenic *Escherichia coli* strains isolated from diarrheic children in Tripoli, North Lebanon

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Abstract

Background: Enteric infections are a leading cause of diarrheal disease and death in infants and young children, particularly in the developing regions. The aim of this study is to determine the antibiotic susceptibility of three common *Enterobacteriaceae* enteropathogens (*Salmonella* spp., *Shigella* spp. and enteropathogenic *Escherichia coli* (EPEC)) isolated from hospitalized diarrheic children in North Lebanon during the period of 7 years.

Methods: A total of 172 *Enterobacteriaceae* enteropathogens were isolated from fecal and blood specimens of hospitalized children with diarrhea who have been admitted to Nini hospital in Tripoli, North Lebanon. Culture, identification and antibiotic susceptibility of suspected enteropathogens were carried out through conventional tools, API identification and serotyping according to standard protocols proposed by the "Référentiel en Microbiologie Médicale" and the recommendations of the European Committee on Antimicrobial Susceptibility Testing.

Results: Out of 172, 158 (91.9%) enteropathogens were isolated from stool samples and 14 (8.1%) were isolated from blood cultures. EPEC were predominant (78/172; 45.3%), followed by *Salmonella* spp. (64/172; 37.2%) and *Shigella* spp. (30/172; 17.5%). *Salmonella enterica* Typhi was the predominant serotype (32/64; 50%). Susceptibility testing showed that around 50% and 25% of the isolates were resistant

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to ampicillin and extended-spectrum cephalosporins, respectively. Overall, *Salmonella enterica* Typhi isolates were mostly susceptible to antibiotics.

Conclusion: This study revealed a relatively high antibiotic resistance rate of EPEC, *Shigella* spp. and Non-typhoid *Salmonella* isolates among our studied population. The findings of this study provide useful data to health authorities to develop practical effective programs for control of diarrheal disease and antimicrobial resistance in North Lebanon.

Keywords: Enteropathogens, *Salmonella*, *Shigella*, enteropathogenic *Escherichia coli*, diarrhea

Introduction

Infectious diarrhea is the most common cause of disease in young children worldwide and is the leading cause of child mortality and morbidity [1]. It is mostly caused by contamination of food and water sources and unhygienic and unsafe environment [2]. However, a number of non-infectious medical conditions may also cause diarrhea, including hyperthyroidism, irritable bowel syndrome, inflammatory bowel disease, lactose intolerance and a number of medications. Therefore, a major challenge facing the medical doctor is to decide whether an episode of diarrhea is infectious or due to another cause [3].

Gastrointestinal infections have their major impact in the developing countries, particularly in children population. Children under three years old experience on average three episodes of diarrhea every year [2]. A recent study showed that 51.8% (3.257 million) died of infectious causes out of 6.3 million deaths in children younger than 5 years. The three common infectious diseases were pneumonia, diarrhea and measles collectively were responsible for half of these death cases [4].

Furthermore, several investigations searched for

the etiology of infectious diarrhea. A recent evidential data from the Global Enteric Multicenter Study (GEMS) on the burden and etiology of childhood diarrhea in developing countries has shown that interventions targeting five pathogens including rotavirus, *Shigella*, enterotoxigenic *Escherichia coli* producing heat-stable toxin (ETEC), *Cryptosporidium*, and enteropathogenic *E. coli* (EPEC) can substantially decrease the burden of moderate-to-severe diarrhea [1]. The trends of microbial enteropathogens causing gastroenteritis keep on changing following change in standard of living and environmental hygiene [5].

The GEMS has also described that rotavirus and *Shigella* spp. are consistently reported as highly associated with diarrhea cases in young children, while EPEC shows significant association with diarrhea in infants, while *Salmonella* spp. is often found in similar proportions in children with or without diarrhea [1].

Over the past two decades, several reports showed a rising rate of infections and colonization caused by extended-spectrum cephalosporin-resistant *Enterobacteriaceae* due to production of extended-spectrum- β -lactamases (ESBL), plasmid-

mediated AmpC (pAmpC) and/or carbapenemase enzymes in enteropathogens isolates in Arab Mid-east countries [6-8].

In Lebanon, three recent studies indicated that the prevalence of rotavirus (48%), *Campylobacter* spp. (11.1%) and *Cryptosporidium* spp. (11%) was relatively frequent among Lebanese symptomatic hospitalized patients [9-11]. However, information about the epidemiology and antibiotic susceptibility of *Enterobacteriaceae* enteropathogens in Lebanon is limited, even though other microbial infections are prevalent [9-12].

This study aimed to determine the antibiotic susceptibility of three *Enterobacteriaceae* enteropathogens (*Salmonella* spp., *Shigella* spp. and EPEC) isolated from hospitalized diarrheic children in Tripoli, North Lebanon during a period of 7 years.

Materials and Methods

This study was conducted at the microbiology department of Nini hospital in Tripoli, North Lebanon. Fecal and blood specimens were collected from 172 hospitalized children (104 males, 68 females, age ranging from 10 days to 15 years, with a mean age of 2.88 years) during the period from January 2009 through December 2015. All children have been presented with diarrhea or gastrointestinal disorders

Culture of specimens was performed according to standard protocols proposed by the "Référentiel en Microbiologie Médicale" [13]. Identification of *Salmonella* spp., *Shigella* spp. and EPEC was carried out through the use of API-20E (bioMérieux® - France). Confirmation the identity of *Salmonella enterica* Typhi, Paratyphi A, B, and C serotypes and EPEC serotype was performed by agglutination techniques using polyvalent antisera T, A, B, C, Vi (Bio-Rad®, France) and polyvalent antisera for the serotyping of EPEC (*E. coli* I Nonavalent, Bio-Rad®, France)

Antibiotic susceptibility testing was performed by the disk diffusion method on Muller-Hinton agar (Bio-Rad®, France) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The antibiotics tested were ampicillin, amoxicillin + clavulanic acid, ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam, cefalexin, cefuroxime, ceftazidime, cefotaxime, cefixim, cefepime, ceftoxitin, aztreonam, ertapenem, imipenem, meropenem, gentamicin, tobramycin, netilmicin, amikacin, colistin, fosfomicin, tetracycline, minocycline, tigecycline, ofloxacin, ciprofloxacin and trimethoprim-sulfamethoxazole. ESBL production was recorded according to resistance phenotype pattern.

Results

A total of 172 non-duplicated enteropathogenic isolates were recovered from children's stool and blood specimens. Of these isolates, 158 (91.9%) were enteropathogens isolates from stool and 14 (8.1%) were isolated from blood. **Table 1** shows the distribution of these isolates.

EPEC has caused the highest infection rate of diarrhea (78/158), followed by *Salmonella* spp. (50/158) and *Shigella* spp. (30/158) according to recovery from stool cultures, while *Salmonella enterica* Typhi was the only isolated bacteria species (14/14) from blood cultures of children. Serotyping of *Salmonella* isolates showed that 32 of isolates belonged to *Salmonella enterica* Typhi, 3 to *Salmonella enterica* Paratyphi B, 2 to *Salmonella enterica* Paratyphi C and 1 to *Salmonella enterica* Gallinarum. Additionally, 25 of the isolates couldn't be identified at the serotype level.

The rates of antibiotic susceptibility of all *Enterobacteriaceae* enteropathogens isolates are shown in Table 2. Out of all the isolates, 46.8% were resistant to ampicillin, followed by ticarcillin (44.9%), piperacillin (44.9). Less resistance rates were observed

Table 1. Distribution of enteropathogens isolates according to the origin of 172 culture specimens.

<i>Enterobacteriaceae</i> enteropathogens	Origin of specimen	
	No. stool isolates	No. blood isolates
EPEC*	78	0
<i>Shigella</i> spp.	30	0
<i>Salmonella</i> spp.	50	14
Total (100%)	158(91.9%)	14(8.1%)

* Found only in children aged less than 2 years.

to amoxicillin-clavulanic acid (13.3%), ticarcillin-clavulanic acid (15.8%) and piperacillin-tazobactam (5%). Antibiotic susceptibility to first-, second-, third- and fourth- generation cephalosporins was 75.9%, 77.8%, 77.8% and 78.5%, respectively. No isolate was resistant to carbapenems. In addition, a high rate of resistance was observed to tetracycline (45.6%), trimethoprim-sulfamethoxazole (38.0%) and minocycline (33.5%). A low rate of resistance was found against fluoroquinolones (13.9% ofloxacin and 8.2% ciprofloxacin), and to less extent to aminoglycosides (7.6% tobramycin, 7% gentamicin, 5.1% netilmicin and 1.3% amikacin). Low resistance rates were recorded to tigecycline (4.5%), fosfomicin (0.6%) and colistin (0.6%). Overall, 30 out of 172 (17.4%) of isolates were ESBL-producing including EPEC (23), *Shigella* spp. (5) and *Salmonella* spp. (2). Overall, *Salmonella enterica* Typhi isolates were mostly susceptible to antibiotics (**Table 2**).

Discussion

According to our laboratory records, the average number of reported cases of EPEC, *Salmonella* spp. and *Shigella* spp. infections over the last 7 years was 11.1, 9.1 and 4.3 cases per year, respectively. Furthermore, 90% of *Shigella* infections occurred during the fall and summer seasons of June to December. However, no significant difference was found concerning the yearly distribution of *Salmonella* spp. and EPEC infections.

This study shows that EPEC infection is the most common enteric infection occurred among hospitalized diarrheic children aged less than 2-year in Tripoli, North Lebanon. Although many studies have showed a significant association of EPEC with infant diarrhea compared to other children group [14-16].

The routine stool culture in Lebanese laboratories is only designed to detect EPEC, *Salmonella* spp. and *Shigella* spp. The detection of both *E. coli* O157:H7 and O104:H4, serotypes which are responsible for worldwide epidemics causing thousands of gastrointestinal infections is only applied on demand for investigation the cause of public outbreaks of diarrhea. Our previous study in North Lebanon, showed a rare presence of enterohemorrhagic *E. coli* in the stool of Lebanese hospitalized diarrheic patients [8].

Kanj *et al* [17] showed that *Salmonella enterica* Typhi infections continues to be a problem in Lebanon, especially during the summer months. In addition, Naji-Rammal *et al.* [18] reported the occurrence of 6,148 typhoid fever cases in Lebanon between 2000 and 2008, with an annual range from 461 to 891 cases, and with the highest incidence occurring during the months June-August. Moreover, our recent retrospective 8-year surveillance study reported that typhoid is endemic in Lebanon and could cause major outbreaks [19]. In this study, we found a homogenous distribution of the *Salmonella* infection which is consistent with the previous pattern showing that typhoid is endemic in our country [18-20].

The World Health Organization estimated that the yearly incidence of typhoid fever exceeds 21 million

Table 2. Percentage of antibiotic susceptibility of all enteropathogens isolates.

	Total enteropathogens N=172 (%)	EPEC N=78 (%)	<i>Shigella</i> spp. N=30 (%)	<i>Salmonella</i> spp.* N=64 (%)	Non-typhoidal <i>Salmonella</i> N=32 (%)	<i>Salmonella</i> <i>enterica</i> Typhi N=32(%)
AMP	53.2	25.6	76.7	84.4	68.9	100
AMC	86.7	76.9	93.3	98.5	96.9	100
TIC	55.1	28.2	80.0	84.4	68.9	100
TCC	84.2	71.8	93.3	98.5	96.9	100
PIP	55.1	28.2	80.0	84.4	68.9	100
PPT	95.0	92.0	100	98.5	96.9	100
CFX	75.9	60.3	83.3	96.9	93.8	100
CXM	77.8	64.1	83.3	96.9	93.8	100
FOX	96.8	93.6	100	100	100	100
CFM	77.8	64.1	83.3	96.9	93.8	100
CTX	78.5	65.4	83.3	96.9	93.8	100
CAZ	77.8	64.1	83.3	96.9	93.8	100
FEP	78.5	65.4	83.3	96.9	93.8	100
ATM	79.1	65.4	86.7	96.9	93.8	100
ERT	100	100	100	100	100	100
IMP	100	100	100	100	100	100
MEM	100	100	100	100	100	100
GMN	93.0	92.3	86.7	98.5	96.9	100
TMN	92.4	83.3	83.3	100	100	100
NET	94.9	94.9	86.7	100	100	100
AKN	98.7	96.7	96.7	100	100	100
OFX	86.1	85.9	73.3	95.3	90.6	100
CIP	91.8	88.5	93.3	96.9	93.8	100
TET	54.4	52.6	30.0	78.1	62.5	93.8
MNO	66.5	64.1	60.0	79.7	65.6	93.8
TGC	95.5	98.7	90.0	95.3	90.6	100
CS	99.4	98.7	100	100	100	100
FSF	99.4	100	100	98.5	96.9	100
SXT	62.0	59.0	13.3	95.3	90.6	100

* Including *Salmonella enterica* Typhi and non-typhoidal *Salmonella*

AMP, ampicillin (10 µg) ; AMC, amoxicillin/clavulanic acid (20/10 µg) ; TIC, ticarcillin (75 µg) ; TCC, ticarcillin/clavulanic acid (75/10 µg) ; PIP, piperacillin (30 µg) ; PPT, piperacillin/tazobactam (30/4 µg) ; ATM, aztreonam (30 µg) ; IMP, imipenem (10 µg) ; MEM, meropenem (10 µg) ; ERT, ertapenem (10 µg) ; CFX, cefalexin (30 µg) ; CXM, cefuroxime (30 µg) ; FOX, ceftaxime (30 µg) ; CFM, cefixim (5 µg) ; CTX, cefotaxime (5 µg) ; FEP, cefepime (30 µg) ; CAZ, ceftazidime (30 µg) ; GMN, gentamicin (10 µg) ; TMN, tobramycin (10 µg) ; AKN, amikacin (30 µg) ; NET, netilmicin (10 µg) ; OFX, ofloxacin (5 µg) ; CIP, ciprofloxacin (5 µg) ; TET, tetracycline; MNO, minocycline ; TGC, tigecycline (15 µg) ; CS, colistin (50 µg) ;FSF, fosfomycin (200 µg) ; SXT, trimethoprim/sulfamethoxazole (1,25/23,75 µg).

cases with over 200,000 deaths [21]. The estimated incidence of reported *Salmonella* infection in Lebanon is 13.34/100 000 which is higher than that reported in some neighboring Arab countries [22]. This result can be attributed to differences in the reporting systems and the accuracy of the surveillance system in those countries as compared to Lebanon.

Furthermore, according to our results, the mean number of reported cases of *Shigella* spp. infections in Tripoli was 4.3 cases per year over the last 7 years. Ekdahl *et al.* [23] reported that Lebanese people living in Sweden had high incidence rates of traveler-associated shigellosis after visiting Lebanon. They suggested that *Shigella* spp. are highly prevalent in Lebanon and its association with resistance can cause very serious infection and public health problem [23]. In 2007, we reported the first case of ESBL-producing *Shigella* spp. in Lebanon, found that 16.7% of *Shigella* spp. isolates are ESBL-producers and were associated with high level of resistance rate (86.7%) to trimethoprim-sulfamethoxazole [24].

Interestingly, the highest rate of antibiotic resistance was seen among EPEC isolates, with more than 70% resistance to ampicillin, ticarcillin and piperacillin. Moreover, 29.5% of EPEC strains produced ESBL enzymes. However, no resistance was detected to carbapenem among our EPEC isolates. Our previous study, which was also performed in Nini hospital, showed the emergence of carbapenemase-producing *E. coli* with increasing resistance rate from 0.4% in 2008 to 1.6% in 2012 [25]. For this reason, continued surveillance is necessary to control the emergence of carbapenemase-producing EPEC.

In this investigation, *Salmonella* spp., and particularly *Salmonella enterica* Typhi showed lowest rates of resistance among all detected enteropathogens from stool cultures.

The present study showed that two strains of Non-typhoidal *Salmonella* were ESBL-producers. The first case report of ESBL-producing *Salmonella* in Lebanon was published in 2005 [26]. Non-typhoidal *Salmonella* showed an average susceptibility rate

of 69% to ampicillin, ticarcillin and piperacillin, while all *Salmonella enterica* Typhi isolates were susceptible to appropriate members of the beta-lactam family, but were resistant to tetracycline (93.8%) and minocycline (93.8%).

Aminoglycosides, tigecycline, fosfomycin and colistin are still have excellent activity against all ESBL-producing enteropathogen isolates.

In conclusion, this study indicates that the antibiotic resistant rates of EPEC, *Shigella* spp. and Non-typhoidal *Salmonella* isolates are relatively high among hospitalized diarrheic children in Tripoli, North Lebanon. Therefore, stool cultures and antibiotic susceptibility testing of enteropathogen isolates are very important to determine bacterial causes and to guide appropriate treatment and control of diarrheal disease in Lebanon.

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Competing Interests

The authors declare that they have no competing interests.

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