Antimicrobial Resistance for Enteric Pathogens Isolated from Acute Gastroenteritis Patients

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Abstract

The antibiotic resistance of enteropathogenic bacteria has profound clinical implications. This review paper is attempt for isolation of enter pathogenic bacteria and determines their antimicrobial susceptibility patterns. Inappropriate prescription of antibiotics prompted resistance and increased infectious disease mortality not only in developing countries but also in developed countries. Aging populations, changes in behavior and a decline in the development of new antibiotics exacerbated a deteriorating situation. Poor sanitation and restriction to water access may favour the spread of communicable diseases, especially infectious diarrhea, which is one of the leading causes of morbidity in the world. Uncontrolled use of antibiotics due to the lack of antibiotic prescribing policy in diarrheal infections and for any kind of suspected infection has lead to the emergence of bacterial resistance, which has become a major concern. Copyright © WJNAS, all rights reserved.

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1. Introduction

Antibiotic resistance was reported very early in the development of these wonder drugs. Sir Alexander Fleming’s original report in 1929 noted that some bacteria, including the microbe now called Escherichia coli, were resistant to the effect of penicillin. In 1940, Edward Abraham and Ernst Chain reported the presence of an enzyme in E. coli that destroyed penicillin, this was several years before the drug became widely used to treat patients. In the subsequent decades, bacterial antibiotic resistance has become a widespread and well-known phenomenon [1].

The antibiotic resistance of enteric bacteria has profound clinical implications because it threatens the life and causes many of serious diseases such as acute gastroenteritis [3]. Acute gastroenteritis is a severe infection of the gastrointestinal tract (GI). Sometimes people refer to it as “stomach flu” which is characterized by
diarrhea, stomach pain, nausea, vomiting, fever or feeling unwell. Symptoms may start quite slowly or come on suddenly. Gastroenteritis usually passes in less than 24 hours but can continue for several days [4].

Diarrhea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual. Though most episodes of childhood diarrhea are mild, acute cases can lead to significant fluid loss and dehydration, which may result in death [5]. Acute gastroenteritis or infectious diarrhea is one of the leading causes of illnesses and death in infants and children throughout the world, especially in developing countries. This is so in Asia, Africa and Latin America, where an estimated 2.5 million deaths occur each year in children less than 5 years of age [6,7].

Worldwide, the most common pathogens that cause acute gastroenteritis are: Salmonella spp., Shigella spp., Campylobacter spp., E. coli O157:H7, Listeria monocytogenes, Vibrio cholerae, Yersinia enterocolitica, Rotavirus, Cryptosporidium spp., Entamoeba histolytica, and Giardia lamblia. These pathogens can cause potentially serious diseases, which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks [9].

2. Literature Review

2.1. Acute gastroenteritis

Acute gastroenteritis is a common childhood disease and causes significant economic burden to developed countries. Children in developing countries are at particular risk of both morbidity and mortality. Worldwide, gastroenteritis affects 3 to 5 billion children each year, and accounts for 1.5 to 2.5 million deaths per year or 12% of all deaths among children less than 5 years of age [17]. In developed countries, such as the United States, acute gastroenteritis seldom causes deaths, however, it still accounts for 300 deaths per year. Moreover, it puts a heavy burden on the health care system. Acute gastroenteritis causes 1.5 million visits to primary care providers each year and 220,000 hospital admissions for children under the age of 5 years; that is 10% of all the hospital admissions of children in the United States [18]. In general, developing countries have a higher rate of hospital admissions as compared to developed countries. This may be due to the facts that children in developed countries have a better nutritional status and better primary care. The difference can also be explained by the fact that, the incidence of acute gastroenteritis is significantly higher in developing countries than in industrialized countries [21].

2.2. Antimicrobial resistance

Antimicrobial resistance is one of the world’s most serious public health problems, many of the microbes (bacteria, viruses, protozoa) that cause infectious disease no longer respond to common antimicrobial drugs. The problem is so serious that unless concerted action is taken worldwide, we run the risk of returning to the pre-antibiotic era when many more children than now died of infectious diseases. The major infectious diseases kill over 11 million people per year. The prevalence rate of antimicrobial resistance all overall the world of diarrheal shigellosis is 10-90% for ampicillin and 5-95% for trimethoprim/sulfamethoxazole [22].

2.3. Diarrheal disease is the most common cause of illness

In 2004 diarrheal disease affects far more individuals than any other illness, even in regions that include high-income countries. About 4620.4 million people affected with diarrheal disease [25].

2.3.1. Common bacterial causes of diarrhea

2.3.1.1. Salmonella spp. history
Salmonella first identified in 1885. It was named for one of the two men who discovered it, Daniel Elmer Salmon (the other was Theobald Smith). They first found *Salmonella* in dogs that were ill with a disease called dog cholera [26]. *Salmonella* is a gram-negative rod, facultative anaerobic, flagellated bacterium, has a growth rate, or division rate, of 40 minutes, prefers to grow at 37°C, but has the ability to grow at a wide range of temperatures, from 6 to 46°C. This provides *Salmonella* with many opportunities to grow. Optimum growth occurs at a pH of 6.5-7.5. It is the pathogenic agent of salmonellosis, a major cause of enteric illness and typhoid fever, leading to many hospitalizations and a few rare deaths if no antibiotics are administered. *Salmonella* outbreaks are linked to unhygienic food preparation, cooking, reheating and storage practices. The bacterium can be isolated from raw meat and poultry products as well as from milk and milk-based products [26, 27].

*Salmonella* is a ubiquitous human and animal pathogen. This genus contains > 2,500 recognized serotypes and is divided into two species, *Salmonella bongori* and *Salmonella enterica*, *Salmonella enterica* consists of six subspecies (i.e., enterica, salamae, arizonae, diarizonae, houtenae, and indica) [28]. Serovars of the bacterium *Salmonella enterica* subsp. *enterica* are known to be a significant cause of foodborne illness in humans.

*Salmonella enterica* subsp. *enterica* serotypes can also be divided into subdivisions according to their host adaptation. For example *Salmonella* serotypes can be divided into (i) host-restricted *Salmonella* serotypes (i.e., serotypes exclusively associated with one particular host, e.g., *Salmonella Typhi* and *Paratyphi A*); (ii) host-adapted Salmonella serotypes (i.e., serotypes prevalent in one particular host species, but able to cause disease in other host species, e.g., *Salmonella choleraesuis*); and (iii) unrestricted Salmonella serotypes (i.e., serotypes capable of causing self-limiting gastroenteritis and, less commonly, systemic disease in a wide range of host species, e.g., *Salmonella typhimurium*). *Salmonella paratyphi* A also causes typhoid fever, although the symptoms are typically milder than those caused by *Salmonella typhi* [30].

Non-typhoidal Salmonella serotypes are responsible for gastroenteritis in humans and other animals. These serotypes are mainly transmitted by ingestion of food, or water contaminated with infected feces [31], but can also be transmitted by direct contact [32,33]. Disease caused by non-typhoidal Salmonella is one of the most common bacterial foodborne diseases worldwide [34]. *Salmonella typhimurium* is one of the most common non-typhoidal Salmonella serotypes, is found worldwide, and can cause disease, predominantly self-limiting gastroenteritis, in a large number of animal species [30]. Because of the abuse of antimicrobial drugs, the antibiotic resistance of Salmonella strains from humans is expected to increase. It has been reported that there has been an increase in antibiotic resistance of Salmonella isolates from humans [35]. The detection of Salmonella therefore remains a highly important issue in microbiological analysis for food safety and standards [36], and to guide clinicians in the diagnosis of enteric pathogens [37].

### 2.3.2. *Shigella* spp. history

Kiyoshi Shiga discovered shigella during an outbreak in Japan in 1897, which investigated 36 shigellosis patients and identified the bacilli as the etiology agent in 111898 [38]. To acknowledge him for his discovery, the 1930 Edition of Bergey’s Manual of Determinative Bacteriology formally renamed the genus as ‘Shigella’[39]. Shigella is gram-negative rods, non-motile, belonging to the family *Enterobacteriaceae*. The genus *Shigella* includes four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* designated groups A, B, C and D, respectively. All species of *Shigella* cause acute bloody diarrhea by invading and causing patchy destruction of the colonic epithelium. This leads to the formation of micro-ulcers and inflammatory exudates, and causes inflammatory cells (polymorph nuclear leucocytes, PMNs) and blood to appear in the stool. The diarrheal stool contains $10^9$ to $10^8$ *Shigellas* per gram. Environmental conditions like dryness or direct exposure to sunlight may lead to the death of the organism due to its sensitivity. The first three species include multiple serotypes. *S. sonnei* and *S. boydii* usually cause relatively mild illness in which diarrhea may be watery or bloody. *S. flexneri* is the chief cause
of endemic shigellosis in developing countries [40], while *S. sonnet* is the most commonly isolated species in developed countries, representing over 70% of the total isolates in the United States of America [41] and 87% in occupied Palestine [42].

Shigellosis is an ongoing global public health problem. Due to the fecal-oral transmission route of the organisms, the overwhelming burden of shigellosis is found in resource-poor settings with inadequate sanitation [43, 44]. With an estimated number of episodes exceeding 90 million per annum in Asia alone, shigellosis represents a significant proportion of the total number of bacterial gastrointestinal infections worldwide [45]. Unlike other related bacteria which can cause a particular disease syndrome in specific locations (e.g. Salmonella Typhi) [46], it is a disease which "bridges the gap" between industrialized and developing countries. A report from the National Centre for Infectious Diseases in the United States of America found the incidence of shigellosis to be 7.6 cases per 100,000 persons in 1993 [41].

Shigella species the causative agent of bacillary dysentery (shigellosis) are highly adapted human pathogens that are capable of invading and colonizing the intestinal epithelium, which results in severe inflammatory colitis [47]. Shigellosis symptoms includes fever, headache, malaise, anorexia and occasionally vomiting, followed by excretion of profuse watery diarrhea proceeding bloody and/or mucoid diarrhea [48]. All the members of the genus *Shigella* are pathogens restricted to infecting humans and exert their effects on the gastrointestinal mucosa via the production of a multitude of virulence factors, including enterotoxins and effector proteins [49,50].

Prompt treatment of shigellosis with appropriate antimicrobial agents not only shortens the duration and severity of the illness but also reduces microbial carriage and thus spread of infection in the community, but unfortunately emergence of antimicrobial resistance has complicated the empirical therapy for treatment of shigellosis, due to the prevalence of antimicrobial resistance to some antibiotics used to treat *Shigella* and this resistance differs within *Shigella* serogroups [51,52].

The antibiotics recommended by the World Health Organization (WHO) include ciprofloxacin ceftriaxone and pivmecillinam, which are effective in reducing the clinical and bacteriological signs and symptoms of dysentery, and thus can be expected to decrease diarrhea mortality attributable to dysentery [53]. *Shigella* can be isolated from stool samples by culturing it on specific media, and simple conventional biochemical's tests are used to identify *Shigella* species, which grouped serologically by slide agglutination with specific antisera. Antibiotic susceptibility is usually determined in all samples by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) [54,55].

2.3.3. *Campylobacters* spp. history

The first description of *Campylobacter* in the stools of diarrheic children was observed by *Escherichia* in 1886 in Germany and their first isolation was performed by McFadyean and Stockman in 1913 [56]. Up until the middle of the 1940’s, these bacteria were named *Vibrio fetus* for those isolated from some animals (ovines, bovines and porcines) abortions, or *Vibrio jejuni* and *Vibrio coli* for those isolated from feces of these animals [57]. The probable implication of these microbes in infectious disease of food origin was established in 1946 when Levy described a gastroenteritis epidemic in a prison population in Illinois in the United States [58]. Until 1963, these microaerophilic vibrios were included in the *Vibrio* genus. At this time, Sebald and Veron proposed the creation of the *Campylobacter* genus (etymologically from the Greek kampulos = curved, bacter = rod) [59].

*Campylobacter* is gram-negative, spiral-shaped, has a single polar flagellum, motile, non spore forming, sometimes capsulated, microaerophilic bacterium i.e. grow in the presence of an atmosphere poor in oxygen,
generally between 5 and 10%. Many authors find that Campylobacter is more capnophilic than microaerophilic, i.e. that it requires an atmosphere enriched in CO₂ to proliferate (generally 10%). Most often, but not exclusively, the gaseous mixture appropriate for culture of the microbe is: O₂ = 5%; CO₂ = 10%; N₂ = 85%. All species of the Campylobacter genus can proliferate at 37°C and are true mesophiles, but grow best from 37°C to 41.5°C (Campylobacter coli, jejuni, lari grow best at 41.5 but usually not Campylobacter fetus) and its optimal pH zone is from 6.5 to 7.5, presence of 0.5 % sodium chloride (NaCl) in the medium is recommended for culture, whereas concentrations exceeding 1.5 % tend to inhibit growth [57,60].

Worldwide, Campylobacter is recognized as the major cause of gastroenteritis and diarrhea disease in humans [61-64]. The majority (approximately 90%) of cases of Campylobacter gastroenteritis in humans is caused by Campylobacter jejuni, and most of the remainder is caused by Campylobacter coli [65]. Poultry, particularly chickens, account for the majority of human infections caused by Campylobacter [66,67]. Campylobacter jejuni and Campylobacter coli are the most prevalent species [62, 65, and 68].

Campylobacteriosis, acute bacterial enteritis which is a major problem worldwide, mostly caused by Campylobacter coli and Campylobacter jejuni. These pathogens live in the intestinal tract of most avian species [69]. Outbreaks of campylobacteriosis are relatively rare; most infections seem to be sporadic [70]. However, the epidemiology of sporadic campylobacteriosis, especially the routes of transmission, is to a great extent unclear [71,72]. Internationally it is estimated that handling, preparation and consumption of broiler meat may account for 20% to 30% of the human cases, however, it has been suggested that chicken as a reservoir might account for between 50% and 80% of the cases [73]. Campylobacteriosis cases might also be caused by other risk factors than consumption and handling of poultry meat like as broiler farms could contaminate the environment with further spread to new broiler farms or to humans living in the area and local environmental factors, such as climate [74].

Campylobacter gastroenteritis is a self-limited disease, and antimicrobial therapy is not generally required; however, treatment can decrease the duration and severity of illness if it is initiated early in the course of infection [75]. Infants, elderly people, and immunocompromized individuals are at higher risk of developing more severe campylobacteriosis. In these cases, early antibiotic therapy is especially effective [76]. The primary drugs of choice for treatment of human campylobacteriosis include erythromycin and ciprofloxacin. Resistance to ciprofloxacin and erythromycin in Campylobacter recovered and occurred more frequently among C. coli than C. jejuni [77].

Rapid and appropriate identification of the etiologic agent of infectious gastroenteritis is important, since there are major differences in the treatments required for the different agents. C. jejuni and C. Coli in human stool samples are commonly detected by a bacterial culture test. The test procedure includes culturing of the stool on selective agar medium and the subsequent identification of Campylobacter-suspected colonies. Several another methods, including a commercially available enzyme immunoassay [78], polymerase chain reaction (PCR)-based assays [79], and a DNA hybridization-based assay [80], have already been reported. Although these assays have excellent sensitivity, they require a comparatively complicated and time-consuming procedure. Their simplicity and rapid it appears to be insufficient for routine or emergency use in the microbiology laboratory [81].

**2.3.4. **Yersinia enterocolitica history

Since the causative agent of plague was first identified as a gram-negative bacterium by Alexander Yersin in 1894, our understanding of the epidemiology, genetics and evolution of Yersinia has come a long way [82]. Despite similarities with other Enterobacteriaceae members, slight differences are exist such as slow growth on solid media, colonies of at most 1mm in diameter, and better growth at cooler temperature [83, 84]. Metchock et al., reported yersiniosis most commonly in cooler months [85].
Yersinia spp. is gram-negative rods or coccobacilli with bipolar staining that belong to the Enterobacteriaceae family [86]. Eleven species are known, but only 3 are important human pathogens; Y. enterocolitica is the most common of these, while Y. pseudotuberculosis is less frequent, and Y. pestis is rare [87]. Y. enterocolitica is a zoonotic bacterium causes a variety of gastrointestinal disease in humans (Yersiniosis). Clinical symptoms of yersiniosis first appear after an incubation period of about 5 days and include diarrhea, fever, vomiting, tenesma an abdominal pain. In older children and young adults, abdominal pain in the right lower abdomen can occur, which may be mistaken for appendicitis (pseudoappendicitis). Typically, symptoms disappear within 1-2 weeks after onset.

Sequela such as reactive arthritis or erythematic nodosum sometimes occur. Y. enterocolitica infections are usually sporadic, although outbreaks have been reported. Y. enterocolitica species can be isolated from a variety of domestic and wildlife animals, e.g., pigs, cattle, sheep, goats, dogs, cats, wild boars, and small rodents [88]. Thought to be primarily transmitted to humans via ingestion of contaminated foods, water and milk or ingestion of uncooked meat products, especially pork [88,91]. However, other risk factors, such as pet animal contact, have been reported [92]. The majority of cases of enterocolitis are seen in children aged 1-4 years [93,94]. Moreover, these infections show a modest predilection for males, with male to female ratio of 1.7:1 [95].

2.3.5. Aeromonas hydrophila history

Aeromonas spp. was first isolated from water and diseased animals over 100 years ago. Originally, A. hydrophila was identified as one of four Aeromonas species by Popo (1984) and he placed it in the family Vibrionaceae [100]. The genus Aeromonas currently belonging to the family Aeromonadaceae that includes a group of 21-gram negative bacterial species (one of them is A. hydrophila). A. hydrophila is a pathogenic bacterium, widely distributed in the environment. Although it is clear that members of this genus are primarily aquatic organisms, they can colonize other habitats and cause infections in invertebrates and vertebrates [101,102]. Aeromonas (principally A. hydrophila) currently has the status of a foodborne pathogen of emerging importance [103,104].

Members of the genus Aeromonas are gram negative rods, facultative anaerobic, non-spore-forming, motile by a single polar flagellum, catalase-positive, oxidase positive, neither salt (< 5%) nor acid (min. pH ~ 6.0) tolerant and grows optimally at around 28°C. It has the ability to grow at cold temperatures, reportedly as low as 0.1°C for some strains. It can be metabolize glucose by both the respiratory and fermentative pathways and show resistance to the vibriostatic agent O/129. It can be divided into two groups: the first includes the psychrotrophic Aeromonas, represented by Aeromonas salmonicida and the second, by the mesophilic Aeromonas. It principal reservoir is the aquatic environment such as fresh water lakes and streams and waste water systems [101, 102, 105]. Von Graevenitz and Mensch (1968) reported associations of aeromonads with human disease in a review of 30 cases of Aeromonas infection or colonization, providing evidence for their recognition as human pathogens and suggesting that some aeromonads may be associated with gastrointestinal disease [106].

Gastrointestinal infections of Aeromonas spp. are generally considered waterborne, for this reason, A. hydrophila has been placed on the United State of America (USA) protection Agency Contaminant Candidate List of emerging pathogens in drinking water [109]. In an Egyptian study on 714 patients with diarrhea, they found that 14% were Enterotoxigenic Escherichia coli (ETEC)-associated diarrhea, 1.0% Campylobacter-associated diarrhea and Shigella-associated diarrhea represented 2%. Children with Shigella or Campylobacter-associated diarrhea were reported as watery diarrhea and rarely dysentery [117].

In a study in Jordan investigated the polymicrobial infections in 220 children with diarrhea in a rural population, potential pathogenic agents isolated from 143(65%) children were identified by molecular and
standard microbiological methods. Co-infections with two or more agents were detected in 50(35%) of cases. *Escherichia coli*, *Shigella* spp., *Giardia* and *Entamoeba* histolytica were found to be predominant [118].

Another study in Jordan investigated the enter pathogens associated with cases of gastroenteritis in a rural population in 180 children. Pathogens and potential enter pathogens were identified in 140(77.8%) of the patients, with more than one pathogen being recovered from 67(37.2%) of the patients. Potentially pathogenic parasites were observed in 90(50%) patients; those that were associated significantly with diarrhea were *Giardia lamblia*, *Blastocystis hominis*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Cyclospora cayetanensis*. Pathogenic bacteria were isolated from 72(40%) patients, and, of these, 62.5% were resistant to at least one antibiotic, and 30.6% of these were multidrug resistant. Diarrheagenic *Escherichia coli* strains were found in 14.3% of the samples. The most common enteropathogenic bacteria found were *Shigella* spp., Campylobacter jejuni and *Y. enterocolitica* [111].

A study was conducted to determine the etiology of diarrhea in a hospital setting in Kolkata, India. Active surveillance was conducted for 2 years on two random days per week by enrolling every fifth diarrhea patient admitted to the Infectious Diseases and Beliaghata General Hospital in Kolkata. Most of the patients (76.1%) had acute watery diarrhea in association with vomiting (77.7%) and some dehydration (92%). *Vibrio cholerae* O1, Rotavirus and *Giardia lamblia* were the important causes of diarrhea. Among *Shigella* spp., *S. flexneri* 2a and 3a serotypes were most predominantly isolated. Enteric viruses, Enteropathogenic *Escherichia coli* (EPEC) and Enterohaemorrhagic *Escherichia coli* (EAEC) were common in children <5 year age group. Atypical EPEC was comparatively higher than the typical EPEC, multidrug resistance was common among *V. cholerae* O1 and *Shigella* spp. Including tetracycline and ciprofloxacin. Polymicrobial infections were common in all age groups and 27.9% of the diarrhea patients had no potential pathogen [111]. Other study was carried out to detect the bacterial agents associated with bloody diarrhea in children and to determine their antimicrobial susceptibility patterns between June 2001 and January 2008. 249 children with bloody diarrhea were studied, *Shigella* and *Shiga toxin-producing Escherichia coli* (STEC) were recovered from 48(19.3%) and 3(1.2%) of the total of cases, respectively. In 49 out of 249 children, in whom other enteropathogens were investigated, recovered Campylobacter jejuni from 7 children (14.3%), *Salmonella* spp. from 2(4.1%) and *Aeromonas* spp. from 1(2%) in addition to *Shigella* from 7 children (14.3%). Thirty- four (70%) *Shigella* isolates showed resistance to ampicillin and 13(27%) to trimethoprim-sulfamethoxazole. All *Shigella* isolates were susceptible to nalidixic acid, ciprofloxacin and ceftriaxone. *Salmonella* and STEC isolates were susceptible to all antibiotics assayed. Thus, the use of trimethoprim-sulfamethoxazole or ampicillin would not be appropriate for the empirical treatment of *Shigella* associated diarrhea [111].

In the few cases of acute childhood diarrhea that require antimicrobial therapy, the correct choice of the drug depends on detailed previous knowledge of local strains. In order to establish such parameters in Salvador, researcher reviewed the results of all 260 positive stool cultures of children between 0 and 15 years of age during two years at a pediatric tertiary ca care facility in Salvador, Brazil. Bacterial strains had been presumptively identified by culturing in selective media and by biochemical, testing and their antimicrobial susceptibility patterns were automatically detected.

One study was undertaken to report the current antibiotic resistance in common bacterial enteropathogens isolated in a tertiary care hospital in north India. Stool samples from 119 (88 male, 31 female) patients yielded *Shigella*, *Salmonella*, *Vibrio cholerae* and *Aeromonas*. Fifty two per cent (62/119) of patients were children and 70% were below the age of 5 years old. Twenty-seven patients developed hospital-acquired diarrhea. Among all enteropathogens, *Shigella* spp. was the commonest followed by non-typhoidal *Salmonella* (27), *V. cholerae* O1 El tor serotype Ogawa (19), *Aeromonas* spp. (14), *Salmonella typhi* and *S. Paratyphi* A (2 isolates each).
Resistance to antimicrobial agents was common among all pathogens. Among Shigella an overall resistance of 63.6%, 58.1% and 16.3% was observed for nalidixic acid, trimethoprim-sulfamethoxazole and furazolidone respectively. Seven isolates of Shigella were resistant to ciprofloxacin, while (18.5%) of non-typhoidal salmonella were resistant to ciprofloxacin. V. cholerae were generally susceptible to tetracycline (only 1 isolate out of 13 resistant) and other drugs except nalidixic acid (89.5% resistance) and trimethoprim-sulfamethoxazole (77.8% resistance) [112]. A study was carried out to document the presence of pathogenic A. Hydrophila in diarrheal stool samples in children at Coimbatore, India. Of the 216 samples, (9.7%) were positive for A. hydrophila. Among them 20 isolates were resistant to bacitracin. Most of the isolates showed multiple antibiotic resistances.

In Brazil, the prevalence of enteropathogens associated with diarrheal disease in infants < 5 years old was investigated. Cryptosporidium (85.1%) topped the list of parasite isolates, followed by Entamoeba histolytica (56.4%) and Giardia intestinalis (4.3%). Four samples contained EPEC (4.3%). Salmonella and Shigella, however, were not detected, and only one sample contained Rotavirus (1.1%) [113]. In Sao Paulo, Brazil, the etiologic profile of acute diarrhea in 154 children aging less than 5 years was studied. Intestinal pathogens were detected in 112(72.8%) cases. The association of two or more intestinal pathogens occurred in 47(30.5%) cases. The pathogens identified were, Rotavirus; 32(20.8%), bacteria; 53(34.4%), both; 25 (16.2%), and 2(1.4%) with Giardia intestinalis (in one case associated with Rotavirus and in another one associated with bacteria). Altogether, there were 105 bacterial isolates; 90 were Escherichia coli (EPEC 27, Diffuse adhering E. coli (DAEC) 24, ETEC 21 and EAEC 18), 12 were Shigella spp., 2 were Salmonella spp., and one was Y. enterocolitica spp. Children with mixed infections (viral and bacterial) had increased incidence of severe vomiting, dehydration and hospitalization [114]. The trend in isolation of Vibrio cholerae, Shigella, and Salmonella in neonates with diarrhea in Bangladesh was investigated. The study population included neonates who were admitted with acute diarrhea and other medical complications to the inpatient department of The International Centre for Diarrheal Disease Research (ICDDR) hospital, Dhaka, Bangladesh, in 2001. A single enteric pathogen was detected in 71(29.5%), and multiple pathogens were detected in 12(5%) of the neonates. Enteropathogens identified were as follows: V. cholerae O1 (17.5%), Shigella spp. (9.1%), Salmonella spp. (3.3%), Aeromonas spp. (3.7%), and Hafnia alvei in (0.8%) of the neonates [115].

Researchers studied microorganisms associated with infant diarrhea in a group of 256 children admitted to a public pediatric hospital in Montevideo, Uruguay. Diagnostic procedures were updated to optimize detection of potential pathogens, which were found in 63.8% of cases, and to be able to define their characteristics down to molecular or antigenic type. Mixed infection with two or more agents was detected in more than one-third of positive cases. Escherichia coli, especially (EPEC), were shown to be prevalent. Rotavirus, Cryptosporidium, Campylobacter (mainly Campylobacter jejuni), and Shigella flexneri were also often identified. ETEC, Salmonella, and Giardia lamblia were sporadically recognized. Unusual findings included two EIEC strains, one Shigella dysenteriae, and a non-O:1 Vibrio cholerae culture. EPEC bacteria and S. flexneri (but not Salmonella) showed unusually frequent antimicrobial resistance, especially towards beta-lactam antibiotics, which is the subject of ongoing work [116].

The incidence of enteric pathogens was investigated in 265 Jordanian children suffering from gastroenteritis using PCR and conventional methods. They detected enteropathogens in 66.4% of the examined children. A single enteric pathogen was detected in 50.9% of the children, and multiple pathogens were detected in 15.5%. The prevalence of enteropathogens identified was as follows: Rotavirus (32.5%), EPEC (12.8%), EAEC (10.2%), ETEC (5.7%), Shigella spp. (4.9%), Entamoeba histolytica (4.9%), Salmonella spp. (4.5%), Campylobacter jejuni/coli (1.5%), Cryptosporidium spp. (1.5%), EIEC (1.5%), Giardia intestinalis (0.8%) and Y. enterocolitica (0.4%). No Vibrio cholerae, STEC, microsporidia, adenovirus or small round viruses were detected [117].
In the USA, the regional variation in the incidence of laboratory-confirmed bacterial foodborne illnesses was studied. 12,125 cases were identified. The incidence per 100,000 population was highest for Campylobacter (15.7%), followed by Salmonella (14.4%), and Shigella (7.9%). Lower incidences were reported for E. coli O157 (2.1%), Yersinia (0.4%), Listeria (0.3%) and Vibrio (0.2%). The incidence of Campylobacter and Salmonella among infants proved particularly high, although substantial regional variations were observed [118].

3. Recommendations
1. Improvement of laboratories to increase their ability to isolate all types of enteropathogenic bacteria especially those, which cannot be isolated routinely such as Campylobacter spp., A. hydrophilia and Y. enterocolytica, which require special techniques.
2. Establish a reference laboratory, which is able to identify the serotype of Salmonella, Shigella and other enteropathogenic bacteria to establish the epidemiology of these bacteria.
3. Effective strategies to prevent crowding, improve household ventilation, minimise perennial indoor air pollution, and to improve hygiene.
4. Prevent domestic animals access to the houses especially those capable of transmitting diseases to peoples including poultry and pigeon.
5. Special attention should be paid to improve the water resource situation in the regional level.
6. Combat the health problems created by open sewage pools, and cesspool through the construction of sewage networks especially in Khan Younis to take all the necessary measures to prevent any environmental risks.
7. Monitor the sanitation facilities, treatment plants, and their impacts on the underground water.
8. Conduct studies on the impacts on the public health and raise people's awareness about the risks and prevention, especially with regard to the health impacts on children who live near sanitation facilities.
9. Reduce the emergence and spread of antimicrobial-resistant organisms through reducing the disease burden, spread of infection and education for physician.
10. Further studies is needed in order to provide a broader picture of the burden of acute gastroenteritis, antimicrobial-resistant for enteropathogens, risk factors related to diarrhea in patients, and study in prescribing the antibiotics.

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