

## Microbial Causatives of Diarrhea in Children in Erbil City

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### Abstract

Five hundred samples of stool were collected from patients with diarrhea (infants and children under ten years of age) admitted to the Pediatric and Maternity Hospital in Erbil City from March 2007 to September 2007. The samples were cultured on different culture media and according to the colony morphology, biochemical reactions and by the use of API 20E system, 35 (7%) *E.coli* I, 8 (1.6%) *E.coli* II, 17 (3.4%) *E.coli* III, 22 (4.4%) *E.coli* IV, 8 (1.6%) *Shigella dysenteriae*, 16 (3.2%) *Salmonella arizonae*, 12 (2.4%) *Salmonella typhi* and 6 (1.2%) *Vibrio cholerae*. In addition, cases of *Entamoeba histolytica* 175 (35%), *Giardia lamblia* 102 (20.4%) and *Hymenolepis nana* 2 (2.4%) were identified. No infectious agents were found in 75 (15%) of the samples. 22 (4.4%) of the samples had mixed infections. The sensitivity of the bacterial isolates to different antibiotics was performed. There was a variation in the resistance of the isolates ranging from 2-100% whereas other isolates were sensitive. Most cases of diarrhea were in children less than 3 years of age and the males (64%) had more infection rates than the females (35.8%). Children from urban (77%) areas had higher infection rates than those coming from rural (23%) areas. Children who were bottle fed (31.4%) had higher infection rates than those who were breast fed (17.6%) or those with mixed feeding (16.6%).

### Introduction

Diarrhea is defined either as watery stool or increased frequency, or both when compared with a normal case [1]. Acute diarrheal episodes subside within 72 hours of onset, while diarrhea lasting 14 days or more is considered to be chronic [2]. Diarrhea may be caused by a number of conditions, including bacterial, viral, parasites infection and an intestinal disease [1]. Bacterial infections are very important causes of diarrhea in infants and young children worldwide [3]. The principal microorganisms implicated are *Salmonella* [4], *Shigella* [5], *Vibrio cholerae* [6] and serotypes of *Escherichia coli* including EPEC, STEC, EAEC, EIEC and EHEC [7]. Antibiotic resistance has become a major clinical and public health problem during the lifetime of most people [8]. There are many reasons for this problem, one of which is an over use of antibiotics [9] in addition to the chromosomal changes or the exchange of the genetic material via plasmid and transposons which

help in transmission and spread of drug resistance among pathogenic bacteria.

### Materials and Methods

A total of 500 stool samples were collected in a disposable plastic containers from diarrheal patients (infants and children, below ten years of age) admitted to the Pediatric and Maternity Hospital in Erbil City from March 2007 to September 2007 and the relevant information were recorded from each patient including age (1-10 years), sex, geographical area (rural and urban), clinical symptoms and type of the feeding. Samples were then transferred for direct macroscopical and microscopical examination in the laboratory of Maternity and Children Hospital to be checked for the presence of RBC's, WBC's and parasites and their stages. Then the sample was cultivated on suitable culture media (MacConkey and blood agar) and incubated at 37°C overnight. The growing colonies were cultured on further selective and differential media. The growing bacteria were selected and transferred to nutrient agar slants and incubated at 37°C overnight. The slants were

kept at 4°C until used for microbiological analysis. (API- 20 E Test and Antisera).

To study the effect of different antimicrobials on all isolates of bacteria, both nutrient and Mueller-Hinton agar were used as growth media, after sterilization and cooling at 45°C, final concentration of antibiotics was

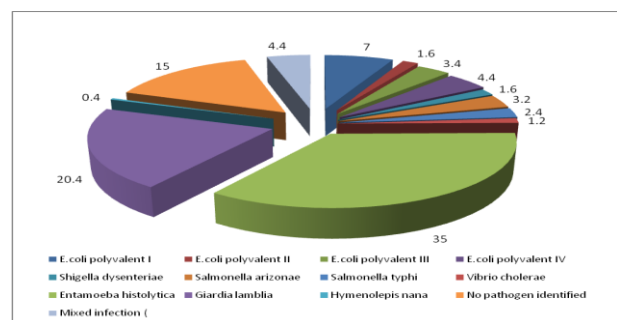
added (Table (1)) to media and poured into sterile Petri dishes. After solidification, the plates were inoculated by streaking method with bacterial isolates then incubated at 37°C for 24 hours. The results were recorded next day [10]. The data were analyzed using the ANOVA test.

**Table (1)**  
**Antibiotics used (11).**

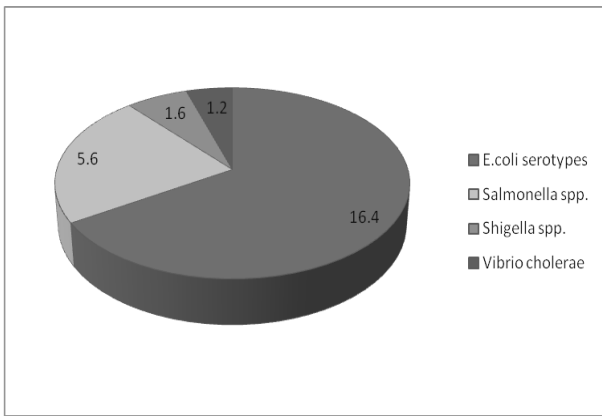
<i>Antibiotic's Names</i>	<i>Symbol</i>	<i>Stock Solution mg / ml</i>	<i>Final (Working) Concentration µg / ml</i>	<i>Solvent</i>
Amikacin	Amk	20	15	D.W.
Amoxicillin	Amc	25	25	D.W.
Ampicillin	Amp	50	50	D.W.
Augmentin (Amoxicillin+Clavulanic acid)	Aug	10	30	Methanol
Cefixime	Cex	20	5	50% Methanol
Cefotaxime	Cef	10	30	D.W.
Cephalexine	Cep	45	30	D.W.
Chloramphenicol	Cm	34	30	Ethanol
Ciprofloxacin	Cip	10	5	D.W. and Ethanol
Doxycyclin	Do	10	30	D.W.
Erythromycin	Ery	10	10	Ethanol
Gentamycin	Gm	10	10	D.W.
Nalidixic acid	Nal	5	30	0.3M NaOH
Nitrfurantion	Nit	30	10	Dimethylformamide
Rifampicin	Rif	34	5	Methanol + 5drops of 10N NaOH / ml
Streptomycin	Sm	10	25	D.W.
Tetracycline	Tc	5	15	50% Ethanol
Tobramycin	To	10	30	D.W.
Trimethoprim-sulfamethoxazole	Tm	20	20	Dimethylformamide

## Results

A total of 500 stool samples were collected from diarrheal patients (infants and children) admitted to the pediatric and maternity Hospital in Erbil City. Regarding the causative agent of diarrhea, the results are presented in Figs.(1,2). The most common isolated enteropathogens were *Entamoeba histolytica*, bacteria and *Giardia lamblia*. The difference between these groups was significant ( $P \leq 0.05$ ).

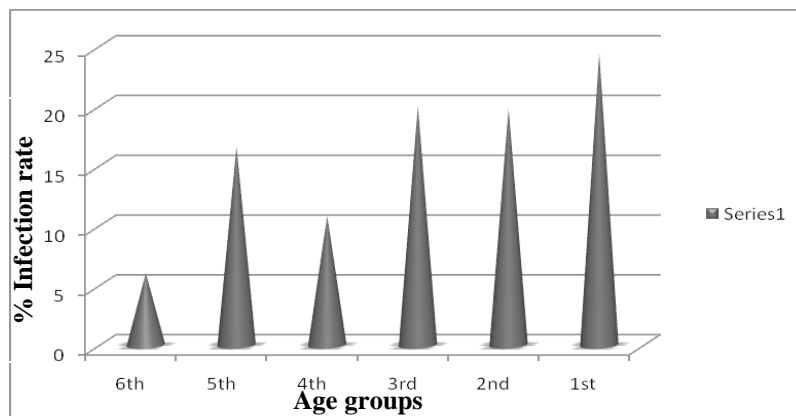


**Fig. (1) Enteropathogens detected in fecal samples from 500 children with diarrhea.**



**Fig.(2) Percentage of bacteria detected in fecal samples from 500 children with diarrhea.**

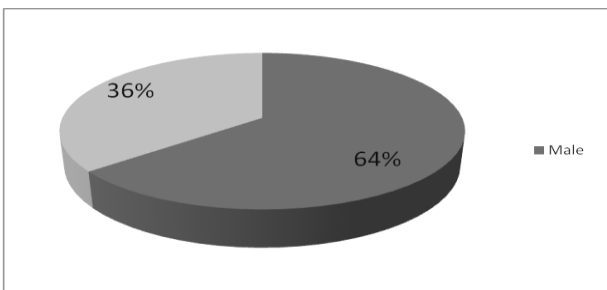
According to the age, patients between 1 month and 10 years were grouped into 7 age groups as shown in Fig.(3), which demonstrates that diarrhea is common in the age group less than 3 years ( $P \leq 0.05$ ).



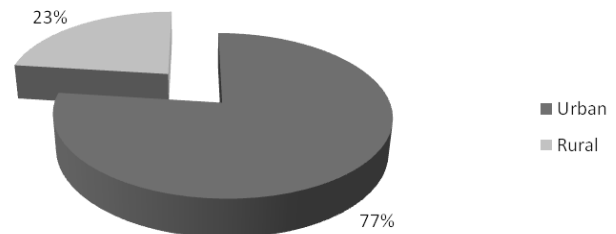
**Fig. (3) Percentage of enteropathogens isolated from stool samples of patients with diarrhea in relation to age groups.**

Concerning the sex, the number of males with diarrhea was 321 (64%) and females were 179 (36%), as shown in Fig.(4), the difference being statistically significant ( $P \leq 0.05$ ).

115 infections (23%), the difference being statistically significant ( $P \leq 0.05$ ).



**Fig.(4) Percentage of enteropathogens isolated from stool samples of patients with diarrhea in relation to sex.**

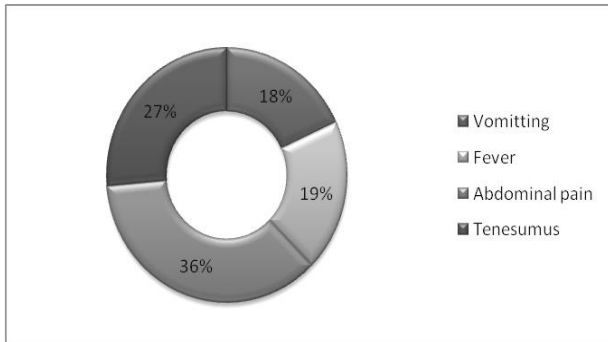


**Fig.(5) Percentage of enteropathogens isolated from stool samples of patients with diarrhea in relation to the geographical region.**

The enteropathogens isolated from patients according to their geographical distribution is shown in Fig.(5), showing the higher infection rate among those in urban areas, 385 infections (77%) in comparison to those in rural areas,

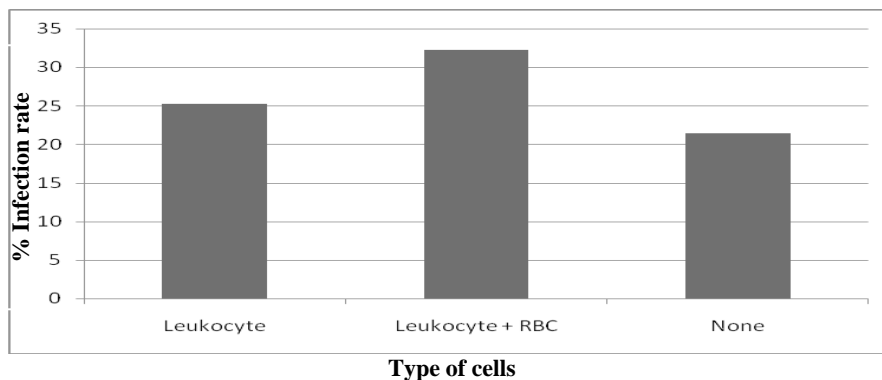
The relation between the clinical symptoms and type of enteropathogens are shown in Fig.(6), which demonstrates that fever, vomiting, abdominal pain and tenesmus are the most common in patients with bacterial

etiology. The difference in the number of these signs and symptoms was found highly significant ( $P \leq 0.01$ ).



**Fig.(6) Enteropathogens isolated from stool samples of patients with diarrhea in relation to clinical signs and symptoms.**

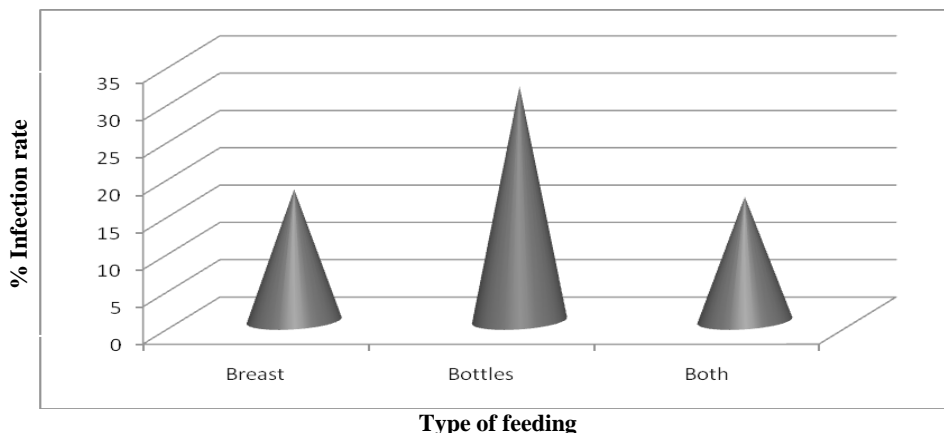
According to the results of stool examination, leukocytes and RBC's were most commonly found in patients with *Entamoeba histolytica* and bacterial etiology as shown in Fig.(7). A significant difference was found in the number of leukocytes and RBC's and the enteropathogens type ( $P \leq 0.01$ ).



**Fig. (7) General stool examination findings of stool samples from patients with diarrhea according to the type of enteropathogens.**

Fig.(8) showed that the highest infection rate (31.4%) was found among children who were bottle fed when compared to the breast

fed group (17.6%) and mixed feeding group (16.6%), the difference being statistically significant ( $P \leq 0.01$ ).



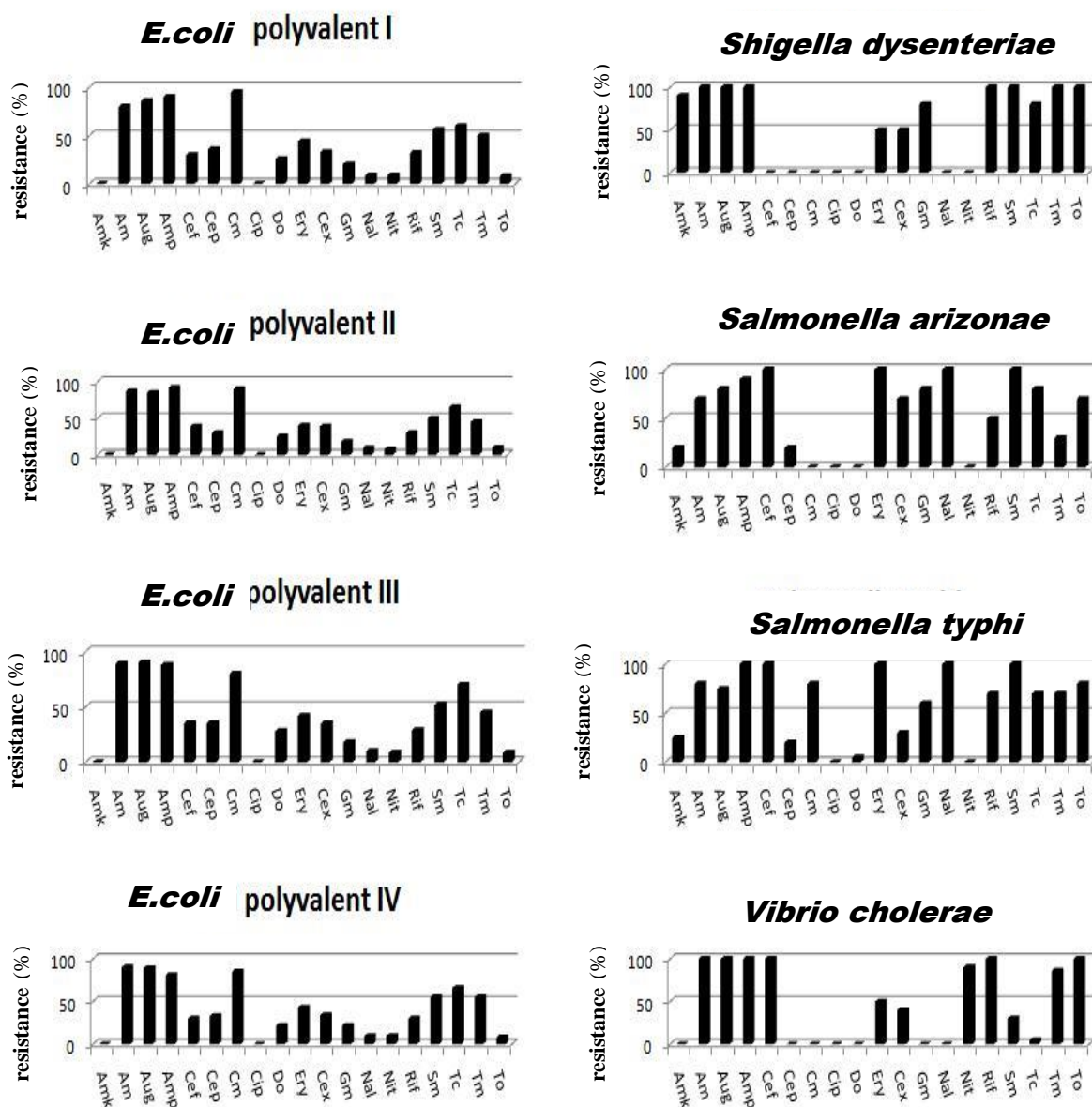
**Fig.(8) Enteropathogens isolated from stool samples of patients with diarrhea in relation to the type of feeding.**

The susceptibilities of 124 isolates were tested against 19 different groups of widely used antimicrobial agents (Amikacin, Amoxicillin, Augmentin, Ampicillin, Cephalexine, Cefixime, Cefotaxime, Chloram-

phenicol, Ciprofloxacin, Doxycyclin, Erythromycin, Gentamycin, Nalidixic acid, Nitrofurantoin, Rifampicin, Streptomycin, Tetracycline, Trimethoprim-Sulfamethoxazole and Tobramycin), and there was variation

in the resistance of the isolates ranging from 2-100% whereas other isolates were

sensitive Fig.(9).



**Fig.(9) Antibiogram groups and percent of resistance to antimicrobials of bacteria isolated from stool samples from patients with diarrhea.**

**Discussion**

Fig.(1) shows that *E.histolytica* (35%), different species of bacteria (24.8%) and *G.lamblia* (20.4%) were the most common enteropathogens isolated from patients. Other enteropathogens that were identified included *Hymenolepis nana* (0.4%) and mixed infections (4.4%) in addition to those patients with no isolated pathogens (15%). Poverty with its associated environmental and educational conditions, social and cultural habits largely determines the incidence of diarrhea and reflects the variations of the infection rate among various groups in

different localities. The high parasitic infection rate in children may be related to fecal-oral transmission of parasites from child to child or food and/ or water borne Giardiasis [12]. An important factor which affects the rate of infection with Giardiasis is the presence of asymptomatic patients which is considered an important source of infection through continuous excreting of *Giardia* cysts with stool [13].

Bacterial pathogens were found in 24.8% of the samples (500), 82 (16.4%) of which were *E.coli* of different pathogenic serotypes, 28 (5.6%) were *Salmonella* spp., 8 (1.6%)

*Shigella* spp. and 6 (1.2%) were *Vibrio cholerae* Fig.(2). All 100 controls were investigated for *E.coli* (serotypes) and none were positive. *E.coli* polyvalent I was isolated in 35 (7%) of the 500 cases, 8 (1.6%) were *E.coli* Polyvalent II, 17 (3.4%) were *E.coli* Polyvalent III and 22 (4.4%) were *E.coli* polyvalent IV. The highest percentage of infection was found in the first, second, and third age groups by different causes (parasites, bacteria and mixed infections), the difference between these groups being significant ( $P \leq 0.05$ ) Fig.(3). The other age groups showed a lower percentage of infection. *Salmonella* spp. was found in a high rate after three years of age. This may be due to contamination of food and milk or climatic factors, socioeconomic status and environmental conditions [14]. *Salmonella typhi* infection rate was high in the age groups 5-9 years. This may be due to the non pasteurized milk used in making ice cream [15] and highest rate of *S.dysenteriae* infection was in age groups 1-4 years. This may be due to the use of contaminated water for bottle feeding in children, low income families and low educational levels for parents [16]. The rate of infection of *S.arizonae* was high in age groups 3-5 years. However, infants are at particular risk. Reptiles are symptom less carriers of this pathogen and are probably infected through contaminated water, food or soil [17]. *Arizonae* is a common gut inhabitant of reptiles, snakes being the most common reservoir for human cases. Because this organism is exceedingly rare, it may infect young infants and immunocompromised individuals. *S.arizonae* can be transmitted by fecal-oral route, through eggs and infected birds can become long-term intestinal carriers. This species is the most sensitive among *Salmonella* and it can still survive for months in water. *Salmonella* antibodies were found to protect children against bacterial infections of the blood caused by these bacteria [18].

The highest percentage of *V.cholerae* infection was shown among age group 6 and above. These results differ from results reported by Sharma *et al.* [19] where the highest incidence was amongst those less than 5 years of age. Cholera differs from other enteric diseases in having a very short

incubation period and rapidly spreading to different countries [20]. All ages were affected but the majority were 6-7 years and above, similar to Al-Abassi *et al.*[20] results where the highest rate was in 15 years or under and different from Sharma *et al.*[19] where high incidence were found in less than 5 years of age. *V.cholerae* can be transmitted from person to person through ingestion of contaminated water, usually from feces or other effluent e.g. effluent from the sewage plant passes into a coastal river [20]. The rate of mixed infections was lower among those less than two years of age than those with no isolated enteropathogens in the same age. The age group of 3 years had the highest rate of mixed infection when compared to the other age groups. All age groups revealed that from some patients, no pathogen was isolated and the age group of four years had the highest level of no isolated pathogen.

Similar results were obtained by Paniagua *et al.* [14]. Diarrhea was found to be at a higher rate in males (64%) than in females (36%) the difference being statistically significant ( $P \leq 0.05$ ) Fig.(4). The sex factor seems to play a role in diarrhoeal infection in children less than 6 years of age. It was found that the incidence in males was higher than in females concerning all pathogens. This may be attributed to the physiological differences between them. The actual reason is not yet known [21]. The mixed infections and those with no isolated enteropathogens were higher in males than females. Diarrheagenic *E.coli* strains transmission is through the fecal-oral route, with contaminated hands, contaminated weaning foods or formula etc. The reservoir of diarrheagenic *E.coli* infection is thought to be symptomatic or asymptomatic children and asymptomatic adult carries including mothers and persons who handle infants. Epidemiological studies in several countries have shown back grounds of asymptomatic carriage. In some studies as many as 17 to 20% of healthy infants younger than 2 years shed *E.coli* of EPEC serotypes in their stools [14].

The prevalence of diarrhoeal children in respect to location or residence Fig.(5) indicated that children living in the center of Erbil City show higher rates of infection

(77%) than in rural areas (23%), the difference being significantly different ( $P \leq 0.05$ ). This may be due to high numbers of inpatient children admitted to Erbil Pediatric and Maternity Hospital from the center zone than the rural. In addition, those living in rural areas might have been treated in their village without referral to one of the city hospitals. This also may be due to sewage contaminated wells from which water was consumed without treatment, or insufficiency of chlorination of well water since the chlorine level sufficient to kill bacteria do not affect the cyst of *E.histolytica* because protozoa cysts are highly resistant to chlorine. These organisms must be removed with coagulation, flocculation, sedimentation and filtration [22]. Moreover, most of the water works in the city center are very old and there are many illegal connections in addition to the use of plastic containers in houses for water storage [23].

Regarding the clinical picture of diarrhea Fig.(6), all types of bacterial infections were frequently associated with tenesmus, fever, abdominal pain and vomiting, except *V.cholerae* (tenesmus was negative). The difference in the number of these signs and symptoms was found to be significant ( $P \leq 0.01$ ), whereas the difference in the signs and symptoms between the bacterial isolates was found to be insignificant ( $P > 0.05$ ). This indicated that all bacterial diarrheas gave similar signs and symptoms.

The association of fecal leukocytes with organisms known to penetrate intestinal mucosa such as *Salmonella*, *Shigella* and some strains of *E.coli* was noted in our study. There was a significant difference ( $P \leq 0.01$ ) in the number of leukocytes and RBC's and the enteropathogens type Fig.(7).

This study has demonstrated a higher incidence of diarrhea caused by EPEC in bottle fed children (31.4%) than in breast fed (17.6%) and mixed fed (16.6%) Fig.(8). Breast feeding has been well documented to prevent and attenuate the severity of diarrhea disease in developing countries and against specific enteric pathogens such as *Shigella* spp., *Campylobacter* spp. and Enterotoxigenic *E.coli* [24].

Most serotypes of *E.coli* were highly resistant to Amc (80-90%), Aug (85-90%) and

Amp (81-92%). These isolates were moderately resistant to Sm (50-56%), Tc (60-70%) and Tm (45-55%). These isolates were sensitive to other antibiotics including Cep (30-36%), Cip (0%), Do (22-28%), Ery (40-44%), Cex (33-39%), Gm (18-22%), Nal (9-10%), Rif (29-32%), to (8-10%) and Cef (30-39%) Fig.(9). This was similar to what was obtained by [25], where all isolates were found to be susceptible to ciprofloxacin and cefotaxime. However, ciprofloxacin and other quinolones are not approved for children because of the risk of damage to immature joints and most parental third-generation cephalosporins (e.g. cefotaxime) are administered only in a hospital setting. These results also agreed with those of Sulaiman [26] who found the isolates to be resistant to Amp (80%), Amc (87.5%) but sensitive to Nal (87.5%), Cef (85%) and Gm (80%). In addition, Salah [27] showed all isolates of *E.coli* to be resistant (97.5%) for Cm and less resistant for Amk (2%). As well as the study reported multidrug-resistance in enteroaggregative *E.coli* with persistent diarrhoea in Kenyan children where the isolates were resistant to Tc and Aug[28]. Another study [29] recorded the highest rates of resistance against Amc, Cm, Tc and Cip (100%) and the most common resistance pattern was for Amc, Gm, Cip, Tc, Cm (38.7%) and the least common resistance patterns was for Nit (3.2%).

The United States food and drug administration emphasizes the spread of drug resistance in the Enterobacteriaceae family from antibiotic-fed animals to human beings. Transmission of the R-plasmid from *E.coli* of poultry to humans occurs very commonly [29]. Antibiotic resistant *E.coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *S.aureus* and *E.coli*, often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. Indeed, *E.coli* is frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E.coli* strains that are pilated to accept and transfer plasmids from and to other bacteria. Thus *E.coli* and other enterobacteria are important reservoirs of transferable antibiotic

resistance [30]. This variation in antibiotic resistance on *E.coli* is due to the genes that are located on conjugant plasmid and by conjugation, transformation or transduction processes, may be transferred to recipient *E.coli*; or the resistance genes may be located on bacterial chromosome and jump to resistance plasmid by transposition process (many composite transposons contain genes for antibiotic resistance and some bear more than one resistance gene). This is found in both Gram-negative and Gram-positive bacteria [31].

*Shigella* seems to be highly resistant to Amp, Amc and Rif. These were similar results to those who detected by Al- Shuwalli [32]. Moreover, *Shigella* was found to be highly sensitive to Cip, Nal and Cef. Hawezy [33] observed that *Shigella* was sensitive to Cef (100%) and Ery (50%) but resistant to Sm, Tm, Cep, Amc and Cip. As comparable to our results, *Shigella* was resistant to all antibiotics except Cep, Cip, Do, Nal, Nit, Cm and Cef, where they were sensitive.

All *Salmonella* were sensitive or intermediately resistant to Tc and amino glycosides, most B-lactam antibiotics, quinolones, co-trimoxazole group antibiotics, Cm, Nit and azithromycin. Apart from slight differences in antibiotic susceptibility between strains of *S.enterica* sub spp. *Enterica* and *S.enterica* sub spp. *Arizonae*, they were susceptible to fosfomycin, whereas typhoid *Salmonella* were intrinsically resistant [34]. *Salmonella* may also harbor temperate phages and plasmids. Plasmids in *Salmonella* may code for antibiotic resistance (resistance plasmids are frequent due to the selective pressure to extensive antibiotic therapy), bacteriocins, and metabolic characteristics such as lactose or sucrose formation or antigenic changes of O antigen [4]. In addition, Charifi and Salehi [35] reported 10.2, 8.3 and 6.6 times increase in resistance of *S.typhi* to Amp, Cm and Tm. This result was in agreement with the results of ours. *Salmonella arizonae* in our study was resistant to Amc, Aug, Amp, Cef, Er, Cex, Nal, Rif (50%), Sm, Tc and To rate of resistance was from 70-100%, except Rif (50%). Gast and Stephens [36] reported resistance of *S.arizonae* to Nal, Sm, Te and Amp which were isolated

from 20% of the rectal samples taken from poultts that had received bacterial strains. This was similar to our results.

Fig.(9) showed that the resistance of *V.cholerae* to Tm and Ery were 86% and 50%, respectively. Similar results were recorded by Keramat *et al.* [37] where there was a 98% and 62% resistance for Tm and Ery, respectively. Sharma *et al.* [19] recorded resistance to Tm of 96% and sensitivity to Cip, Amk, Tc and Do of 92%, 85%, 77% and 67%, respectively. This agrees with our results in this study. In addition, other antibiotics that have been proven effective against *V.cholerae* include Ery, Do, Cm and Fluroquinolones [35]. In *V.cholerae*, several resistance mechanisms such as plasmid encoded resistance, mutation in the quinolones resistance determine regions, integrons, and efflux pumps [38]. Spreading of resistant diarrheal agents in the last few decades has notably increased due to abuse and misuse of antibacterial agents which limits the choice of the therapeutic options for the treatment of infections caused by these bacteria. The wide spread and often indiscriminate use of antibiotics has created drug resistant Gram-negative enteric bacteria that readily acquire multiple resistances through transmission of drug resistant elements. Prolonged antibiotic therapy reduces susceptible strains of the normal gut flora permitting opportunistic coliform strains to colonize and overgrow. The overuse of antibiotics might involve the enhancement of toxin production by the enteric bacteria or an alteration in the normal completing bowel flora leading to an over growth of diarrheagenic *E.coli*. Tm-STX has been reported to enhance toxin production by *E.coli* O 157 strains in vitro [39].

## Conclusions

1. High prevalence of *Entamoeba histolytica* and *Giardia lamblia* that cause diarrhea followed by *E.coli* serotypes, *Salmonella* spp., *Shigella* and *Vibrio cholerae*.
2. Enteropathogenic *E.coli* (EPEC) are considered as one of the major causative agents of children's diarrhea followed by ETEC, STEC and EAaggEC.



3. The isolated bacteria revealed high resistance to most widely used antibiotics, and some isolates were resistant to all tested (nineteen) antibiotics.

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#### الخلاصة

تم جمع خمسمائة نموذجاً من مرضى يعانون من الإسهال (حديثي الولادة و أطفال دون سن العاشرة من العمر) الذين يراجعون مستشفى الأطفال و الولادة في محافظة أربيل من الفترة آذار ٢٠٠٧ الى أيلول ٢٠٠٧. زرعت النماذج على أوساط زرعية مختلفة و إستناداً الى الصفات المزرعية، الشكلية و التفاعلات الكيمياء الحياتية و بإستخدام نظام ال API 20E تم تشخيص ٣٥ (%٧) *E.coli* I ، ٨ (%١,٦) *E.coli* II ، ١٧ (%٣,٤) *E.coli* III ، ٢٢ (%٤,٤) *E.coli* IV ، ١٦ (%١,٦) *Shigella dysenteriae* ، ١٢ (%٣,٢) *Salmonella arizonae* ، ٦ (%١,٢) *Salmonella typhi* و عزلت *Vibrio cholerae*. كما تم تشخيص طفيلي *Entamoeba histolytica* ١٧٥ (%٣٥) و *Giardia lamblia* ١٠٢ (%٢٠,٤) و ٢ (%٢,٤)

*Hymenolepis nana* من النماذج. لم يتم تشخيص أي مسبب مرضي نسبة الى الطرائق المستخدمة للتشخيص في هذه الدراسة في ٧٥ (١٥%) نموذج. وجد أن ٢٢ (٤,٤%) من النماذج كان فيها مسببات مختلطة. تم إختبار حساسية العزلات للمضادات الحيوية. و أظهرت العزلات تباينا من حيث مقاومتها للمضادات الحيوية تتراوح من ٢ الى ١٠٠% بينما أظهرت عزلات أخرى حساسية لها. تركزت غالبية حالات الإصابة بالإسهال لدى الفئة العمرية أقل من ثلاث سنوات و لوحظ أن إرتفاع حالات الإسهال لدى الذكور و بنسبة ٦٤% أعلى مما عليه لدى الإناث و بنسبة ٣٥,٨%. كانت نسبة الإصابة في المدينة (٧٧%) أعلى مما هي عليه في الأرياف (٢٣%). و لوحظ أن نسبة الإسهال بين الأطفال اللذين يتناولون تغذية صناعية (٣١,٤%) أعلى مما هي عليه في الأطفال اللذين يتناولون تغذية مختلطة (١٦,٦) و طبيعية (١٧,٦). وجد أن الأعراض الأكثر شيوعا عند مرضى الإسهال هي الحمى، آلام البطن و التقيؤ و الأختلاجات ووجود كريات الدم البيضاء و الحمراء في النماذج المفحوصة.