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EMERGING FLUOROQUINOLONES RESISTANCE IN DIFFERENT TYPES OF BACTRIA ISOLATED FROM DIARRHEAL CHILDREN IN THI-QAR PROVINCE, IRAQ DURING 2017-2018

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Abstract

Diarrhea is defined by the World Health Organization as having three or more loose or liquid stools per day, or as having more stools than is normal for that person. This study was carried out to Emerging fluoroquinolones resistance in Bacteria isolated from diarrheal children in Thi-Qar province ; Total of 150 sample were collected then subjected to diagnosis, According the age; the age groups of (1 - 5) years were the highest percentage of diarrheal cases (64.1%) and the diagnosis appeared that (8) types of bacteria caused diarrhea which were (7) isolates of *E. coli,* (7) isolates of *Salmonella*, (6) isolates of *Klebseilla*, (5) isolates of *Aeromonas* spp., (5) isolates of *Protues*, (4) isolates of *Acintobacter*, (3) isolates of *Vibrio cholerae*, (2) isolates of *Shigella* from diarrhea children in Thi-Qar province of people with diarrhea have been diagnosis in the laboratory of biology department of science collage in Thi-Qar University. Antimicrobial susceptibility test appear that *E. coli* resistance to ciprofloxacin (14%), nalidixic acid (100%). while sensitive to ciprofloxacin (86%), Nitrofurantion (100%). While *Salmonella* spp.: resistance to nalidixic acid was (14%) sensitive to ciprofloxacin (100%), nalidixic acid (85%), Nitrofurantion (100%) and *Klebsiella* spp.: sensitive to ciprofloxacin (100%), nalidixic acid (100%), Nitrofurantion (100%). However *Aeromonas* spp. : resistance to ciprofloxacin (80%), nalidixic acid (60%) while sensitive to ciprofloxacin (20%), nalidixic acid (40%), Nitrofurantion (100%) and *Proteus* spp. : resistance to ciprofloxacin (20%), Nitrofurantion (20%) while sensitive to ciprofloxacin (80%), nalidixic acid (100%), Nitrofurantion (80%) while *Acintobacter* spp.: sensitive to ciprofloxacin (100%), nalidixic acid (100%), nitrofurantion(100%).

Key words : fluoroquinolones resistance, diarrheal children.

Introduction

Diarrhea is defined by the World Health Organization, which has three or more loose or fluid stools per day or has more stool than normal, The most common cause is the infection of the intestine due to a virus, bacterium or parasite - a disease also known as gastroenteritis (WHO, 2003). These infections often come from food or water that is infected through stool (Tedros 2014). Diarrhea is usually classified according to the timing. If symptoms suddenly appear in less than two weeks, is called acute diarrhea (Feakin, 2013). In the United States, this type of diarrhea is one of the most severe acute diarrhea, usually caused by bacteria, viruses or parasites, Acute diarrhea is one of the major causes of death in children under the age of five due to common illnesses worldwide (Tedros 2014). Persistent diarrhea is when the disease lasts two to four weeks. This can be from a number of conditions, such as irritable bowel syndrome (IBS), Crohn's disease, ulcerative colitis, celiac disease, diverticular disease, and colon cancer. You can also get this type of diarrhea after gastric surgery (for example, gastrectomy) (Powell, 2005). If the condition lasts longer than four weeks, chronic diarrhea is considered. Although often due to digestive disorders, other types of diarrhea with regard to pathophysiology, the second category of diarrhea involves four mechanisms that occur, but note that their integrity

may exist, Discharge Diarrhea When the electrolyte absorption is affected, the body releases water into the small intestine and causes intestinal deficiency (WHO, 2003). Dysentery: If blood is visible in the stool, it is also known as Dissonne. Blood is the trace of invasion of the intestinal tissue. Diastrophe is one of the symptoms, including *Shigella*, *Entamoeba histolytica* and *Salmonella* (Moon, 2015). The are many cause of diarrhea, gastroenteritis is one of the most common causes of bacterial infection that causes diarrhea, but its viral infection is low or higher among children. Common bacteria cause diarrhea of *Salmonella*, *Shigella*, *Entamoeba histolytica*. Polycystic ovary syndrome is one of the major causes of infectious diarrhea that results in death. (Prober, 2012). Poverty is a good indicator of the rate of infectious diarrhea in a population, Water is one of the most common causes of infectious diarrhea, a lack of clean water. Inappropriate faecal excretion often results in groundwater contamination (Brown and Ensink, 2013). Nutrition is important for health and performance, including preventing infectious diarrhea. This is especially true for young children who do not have complete immunity (Black and Sazawal, 2001). Antibiotics used to treatment the diarrhea are ciprofloxacin were used to treat various types of bacterial infections, and are also used to treat people who are prone to detoxification or cancer. Ciprofloxacin and other new antimicrobial agents of quinolone have higher potency than spontaneous bacteria than old analogues such as nalidixic acid. New and published observations on the mechanisms of action and resistance to ciprofloxacin in Escherichia coli have been discussed and discussed. The genetic and biochemical studies of the DNA subclass identified the essential bacterial enzyme genes as the target of ciprofloxacin and other quinolones. For a series of quinolones, inhibition of pure gyrase DNA is associated with antibacterial activity. Antibacterial activity of ciprofloxacin and nalidixic acid, compared with some other quinolones, is somewhat less affected by rifampin and sore throat, indicating that there is a place of action in addition to DNA (Jawetz, 2001). In short, a subunit of girase DNA targets ciprofloxacin and other quinolones. The resistance of Ciprofloxacin appears to occur by mutation in this target and by changing the way the drug penetrates through the outer membrane of the cell (Wolfson and Hooper, 1985). The aims of this study are determination the bacteria caused diarrheal children in Thi Qar province then determination the bacterial resistance against different types of quinolone antibiotics.

Materials and Methods

1. Children samples collection

150 fecal samples were collected from children suffering from diarrhea, from both sexes and age from 1 to 13 years 86. Fecal samples were put immediately in transport media.

2. Bacterial cultures

Transport media with stool spread XLD, TCBS, on MacConkey agar plates and Blood agar then incubate at 37°C overnight (18-24 hours) and read the plates.

3. Biochemical tests

The important biochemical tests were achieved according to Winn *et al.,* 2007[15]. The tests are (Kligler iron (KI), Oxidase test, Urease test, Indole test, Citrate utilization test).

4. Api-20E system (Analytical profile index for Enterobacteriaceae test)

Api-20E system is used clinically for the rapid identification of the isolates this test done according to (Leboffe and Piercr, 2005).

5. Antimicrobrial susceptibility test

The antimicrobial susceptibility test to fluoroquinolones antibiotic has been done by disc diffusion method according to Bauer *et al*., (1966)

Results

1. Percentage of diarrheal children according the age

150 Patients (children 1day-13years) suffering from diarrhea, from both sexes were include in this study. According the age; the age groups of (1 - 5) years were the highest percentage of diarrheal cases (64.1%) as in table 1 and fig. 1.

2. Diarrheal bacteria diagnosis

The results of isolation of 39 types of diarrheal bacteria which were diagnosis (7 isolates of *E. coli,7 isolate*s of *Salmonella*, 6 isolates of *Klebsiella*, 5 isolates of *Aeromonas* spp., 5 isolates of *Protues*, 4 isolates of *Acinetobacter*, 3 isolates of *Vibrio cholera*e, 2 isolates of *Shigella* from diarrhea children in Thi-Qar province of people with diarrhea have been diagnosis in the laboratory of biology department of science collage in Thi-Qar university.

3. Emerging fluoroquinolones resistance in isolates

The antimicrobial susceptibility test to fluoroquinolones antibiotic appeared bacterial resistance as the following:

1. *E. coli*: resistance to ciprofloxacin (14%), nalidixic acid (100%). while sensitive to ciprofloxacin (86%), Nitrofurantion (100%).

Table 1: Percentage of diarrheal children according the age.

Age of patients	1 day-1 year	1 year 2 year	2 year-3 year	3 year-4 year	4 year-5 year	5 year-6 year	6 year-7 year	7 year-8 year	8 year-9 year	9 year-10 year	10 year-11 year	11 year-12 year	12 year-13 year	Total
No. of diarrheacases	41	14	15	13	15	6	10	9	6	2	5	5	11	150
Percentage %	27	8.4	10	8.7	10	4.4	6.4	6	4	1	3.4	3.7	7	100

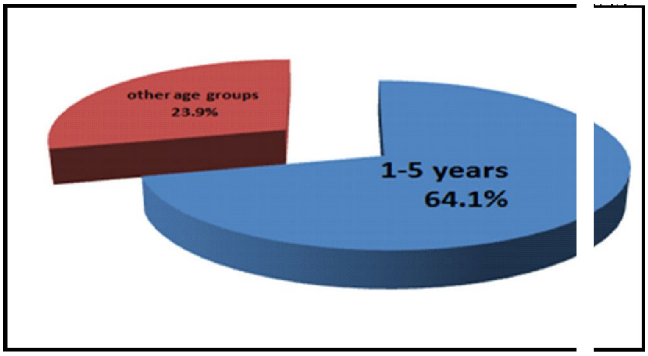

Fig. 1: Distribution of diarrheal children according the age.

2. *Salmonella* spp.: resistance to nalidixic acid (14%).While sensitive to ciprofloxacin (100%), nalidixic acid (85%), Nitrofurantion (100%).
3. *Klebsiella* spp.: sensitive to ciprofloxacin (100%), nalidixic acid (100%), Nitrofurantion (100%). as in Table 4.
4. *Aeromonas* spp. : resistance to ciprofloxacin (80%), nalidixic acid (60%) while sensitive to ciprofloxacin (20%), nalidixic acid (40%), Nitrofurantion (100%).
5. *Proteus* spp. : resistance to ciprofloxacin (20%), Nitrofurantion (20%) while sensitive to ciprofloxacin (80%), nalidixic acid (100%), Nitrofurantion (80%).
6. *Acinetobactor* spp.: sensitive to ciprofloxacin (100%), nalidixic acid (100%), Nitrofurantion (100%).
7. *Vibro cholerae* : resistance to ciprofloxacin (100%), nalidixic acid (100%), Nitrofurantion (33%). while sensitive to Nitrofurantion (67%).
8. *Shigella* spp.: resistance to nalidixic acid (100%). while sensitive to ciprofloxacin (100%), Nitrofurantion (100%).

4. Persantage of Antimicrobial susceptibility test

The antimicrobial susceptibility test for 39 isolates showed different percent to resistance as in table 2 and fig. 2.

Discussion

Diarrhea: A common condition that involves unusually frequent and liquid bowel movements. The opposite of constipation, There are many infectious and noninfectious causes of diarrhea (WH0, 2003). A number of diseases and conditions can cause diarrhea, including

Table 2: Percentage of Fluoroquinolones resistance according to CLSI, 2010 (n=39).

NIT	NA	CIP	Types of bacteria
100%	0%	86%	*E. coli*
100%	86%	100%	*Salmonella* spp.
100%	100%	100%	*Klebsiella* spp.
100%	40%	20%	*Aeromonas* spp.
80%	‘100%	80%	*Proteus* spp.
100%	100%	100%	*Acinetobacter* spp.
67%	0%	0%	*Vibrio cholera*
100%	0%	100%	*Shigella* spp.

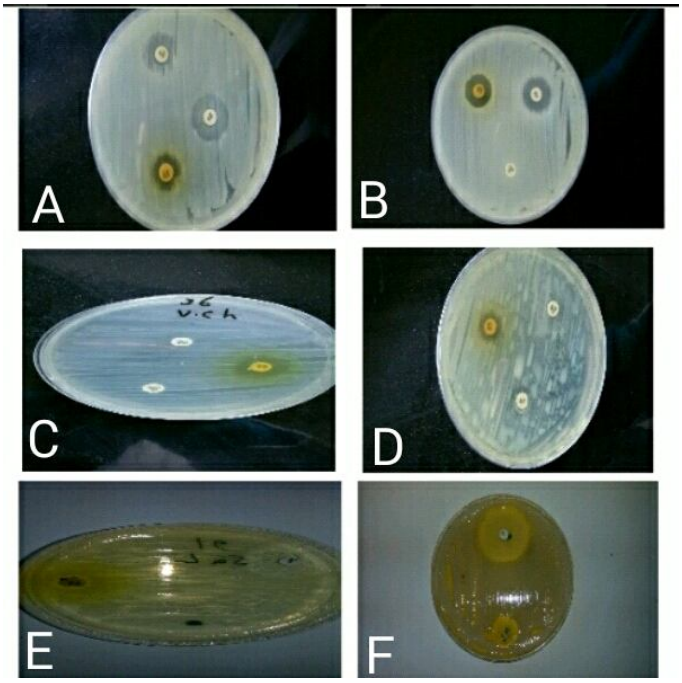

Fig. 2: Antimicrobial susceptibility test for A- *Salmonella* spp., B- *Shigella*, C-*Vibrio cholera*, D-*Aeromonas* spp., E-*Klebsiella* spp., F-*E. coli*.

Viruses. Viruses that can cause diarrhea include Norwalk virus, cytomegalovirus and viral hepatitis. Rotavirus is a common cause of acute childhood diarrhea. Bacteria and parasites. Contaminated food or water can transmit bacteria and parasites to your body. Parasites such as *Giardia lamblia* and *Cryptosporidium* can cause diarrhea. Common bacterial causes of diarrhea include *Campylobacter*, *Salmonella*, *Shigella* and *Escherichia coli*. When traveling in developing countries, diarrhea

caused by bacteria and parasites is often called traveler's diarrhea. *Clostridium difficile* infection can occur, especially after a course of antibiotics). Jawetz, *et al.*, 2007). Total of 39 sample which were collected 7 isolates of *E. coli, 7* isolates of *Salmonella*, 6 isolates of *Klebsiella*, 5 isolates of *Aeromonas* spp., 5 isolates of *Protues*, 4 isolates of *Acinetobacter*, 3 isolates of *Vibrio cholera*, 2 isolates of *Shigella* from diarrhea children in Thi-Qar province of people with diarrhea. 39 Patients (1-13years) suffering from diarrhea, from both sexes were include in this study. According the age groups of 1-6 years were the highest percentage of diarrheal cases (62%) and 6-13 years were (38%).

There was a study in India on diarrheal bacteria for the infected children and their results were almost agree with our results and their results were confirmed Out of 280 children frequency of diarrhoeagenic bacteria isolated from the samples showed that *Escherichia coli* was recorded as the predominant bacteria with 44.2% of prevalence followed by *Shigella, Salmonella, Klebsiella* and *Campylobacter* with 28.2%, 13.6%, 7.8% and 6.1% respectively (Vyas *et al.*, 2014). Similarly, there was a study of the detection of diarrheal bacteria for infected children in Sudan and were almost agree with our results as well and their results Of the 437 samples analyzed, 211 (48 %) tested positive for diarrheagenic *Escherichia coli*, 96 (22 %) for rotavirus A, 36 (8 %) for *Shigella* spp., 17 (4 %) for *Salmonella* spp., 8 (2 %) for *Campylobacter* spp., 47 (11 %) for *Giardia intestinalis* and 22 (5%)

(Amir, *et al*., 2015). Antimicrobial susceptibility test was sen sensitive to ciprofloxacin (86%), nalidixic acid and Nitrofurantion (100%). while *Salmonella* spp. was sensitive to nalidixic acid (86%) ciprofloxacin and Nitrofurantion(100%),on other *Klebsiella* spp. and *Acintobacter* spp. were sensitive to ciprofloxacin and nalidixic acid and Nitrofurantion (100%).our study show *Vibro cholerae* sensitive to ciprofloxacin and nalidixic acid (0%), Nitrofurantion (67%).while *Aeromonas* spp. sensitive to ciprofloxacin and nalidixic acid(20% and 40%) respectively, Nitrofurantion (100%). Also *Shigella* spp. sensitive to nalidixic acid(0%), ciprofloxacin and Nitrofurantion (100%). Our study show *Proteus* spp. sensitive to ciprofloxacin and Nitrofurantion (80%), nalidixic acid (100%). There has been research in Africa to study Fluoroquinolones resistance to bacteria and their results have been almost agree with our results and their results have been confirmed *E. coli (9/19) V. cholera* (0/9)*, Salmonella* (19/89) (Marie *et al*., 2016)

The increased use of fluoroquinolones has led to increasing resistance to these antimicrobials, with rates of resistance that vary by both organism and geographic region. Resistance to fluoroquinolones typically arises as a result of alterations in the target enzymes (DNA gyrase and topoisomerase IV) and of changes in drug entry and efflux. Mutations are selected first in the more susceptible target: DNA gyrase, in gram-negative bacteria, or topoisomerase IV, in gram-positive bacteria. Additional mutations in the next most susceptible target, as well as in genes controlling drug accumulation, augment resistance further, so that the most-resistant isolates have mutations in several genes. Resistance to quinolones can also be mediated by plasmids that produce the Qnr protein, which protects the quinolone targets from inhibition. Qnr plasmids have been found in the United States, Europe, and East Asia. Although Qnr by itself produces only low-level resistance, its presence facilitates the selection of higher-level resistance mutations, thus contributing to the alarming increase in resistance to quinolones (Hanan, 2006)

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