THE UNIVERSITY OF ALQADISIYA

COLLEGE OF PHARMACY

2016-2017

The difference in Effectiveness of ceftriaxone by three different manufacturers on *Escherichia coli* which Detection in Diarrhea among Children Under two Years Old

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيم

((قالَ رَبِّ اشْرَحْ لي صَدْري(25) وَيَسِرْ لي
أمْري(26) وَاحْلُلْ عُقْدَةً مِنْ لِساني(27) يَفْقَهوا
قَوْلي(28))

صَدق الله العَلي الْعَظِيم

سورة طه(25-28)

DEDICATION

To

The sake of Allah, my Creator and my Master

My great teacher Hams (May Allah bless and grant her).

My homeland lraq

My great parents, who never stop giving of themselves in countless ways

My beloved brothers and sisters

To all my family, the symbol of love and giving

My friends who encourage and support me,

All the people in my life who touch my heart,

I dedicate this research.

Ghufran & Noor

ACKNOWLEGEMENT

Firstly, all thanks to God for helping our in performing this work. We would like to thank Dr. Hams Hussein (supervisor) for her encouragement and advice on how to develop this thesis whose experience, constructive criticism, generous support and encouragement gave me energy, strength and self-confidence to finish this study. Also we would like to think her, for her idea and recommendation for title of this study. The director and all staff especially biologist Anmar Hammed of the microbiology laboratory at teaching hospital of AL-Diwaniyah for their participation in this study. All patients, who decided to participate and answered the questionnaire in this study. Millions of thanks to our parents their patience and encouragement during the whole time of this study.

ABSTRACT

The incidence of infectious diarrhea and the prevalence of a given causal agent are strictly associated with socioeconomic factors such as nutrition, sanitation and habitat of the population. Our study showed that 38(76%) of samples were give positive results for *Escherichia* coli , 12(24%)of samples may be another cause of children diarrhea. All *Escherichia coli* isolates (100%) sensitive to Ciprofloxacin, Gentamycin, and Norfloxocin. However, 16.6% of *E.coli* isolates were sensitive for Carbenicillin and Amikacin. But 33.3% of *Escherichia coli* isolates were sensitive to Amoxacillin-clavulanic and Aztreonam.

The results show that there is no significant difference in antibacterial activity of ceftriaxone from three origins against *Escherichia coli* which isolated , from the (LDP) company have inhibition zone about (35mm) at (100%) antibiotic concentration, (32mm) at (75%) ,(30mm) at (50%),(25mm) at (25%) , the antibacterial activity against *E.coli* for ceftriaxone from the Ranbaxy company show(30mm) at (100%) antibiotic concentration, (25mm) at (75%),(22mm) at (50%),(20mm) at (25%) , While the antibacterial activity against *E.coli* for ceftriaxone from the mn pharmaceuticals company show(35mm) at (100%) antibiotic concentration, (30mm) at (75%), (28mm) at (50%), (25mm) at (25%). The study show that all of these companies have the same MIC and MBC.

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List of abbreviations and acronyms

APIanalytical profile index
CDC ……..Center for Disease Control
DAEC ……..diffusely adherent *Escherichia* coli
DNA …….Deoxyribonucleic Acid
E.coli……..*Escherichia Coli*
EAEC ……..Enteroaggregative *Escherichia* coli
EIEC …….Entero-invasive *Escherichia coli*
EHEC ……Enterohemorrhagic Escherichia coli
EPEC…… *Escherichia.Enteropathogenic Escherichia coli*
ETEC ……Enterotoxigenic *Escherichia* coli
H2S……... Hydrogen sulfide
HUS……Haemolyticus-uramic syndrome
MBC …….minimum bactericidal concentration
MIC ……..Minimum Inhibitory Concentration
MHA…….MullerHelton agar
MOH…….Ministry of Health
ORS……..Oral Rehydration Salt
STEC ……. shigalike toxin –producing Escherichia coli
UNICEF ….United Nation International Children and Emergency Fund
WHO ……..World Health Organization

Chapter one

(introduction and literature review)

1.1 Background to problem

Diarrhea is a leading killer of children, accounting for 9 per cent of all deaths among children under age 5 worldwide in 2015. This translates into over 1,400 young children dying each day, or about 530,000 children a year, despite the availability of simple effective treatment. From 2000 to 2015, the total annual number of deaths from diarrhea among children under 5 decreased by more than 50 per cent – from over 1.2 million to half a million. Many more children could be saved through basic interventions to improve drinking water, sanitation and hygiene (WASH) for diarrhea prevention, and the widespread use of a simple solution of oral rehydration salts (ORS) and zinc supplementation during episodes of diarrhea .[1]

Update in 2016.About 40% of cases of acute diarrheal illness in the first 5 years of life are caused by rotaviruses, while a further 30% are caused by other viruses, mainly nor viruses and adenoviruses (8). In about 20% of affected children, a bacterial pathogen can be identified in the stool (Campylobacter jejuni, Yersinia, salmonella, shigella, pathogenic *Escherichia* coli, or clostridium difficile). Parasites are the cause in fewer than 5% (lamblia, cryptosporidia, Entamoeba histolytica, andothers.[2]

1.2 Objectives

To determine effectiveness of ceftriaxone from three different company (LDP ,Ranbaxy ,mn pharmaceutical) on *Escherichia coli* which isolated from diarrhea of childrens under two years old, and To determine if there is difference in biological activity of ceftriaxone among these manufactories.

1.3 Literature review

1.3.1definition of terms

Diarrhea is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual. It is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and

parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene. Severe diarrhea leads to fluid loss, and may be life-threatening, particularly in young children and people who are malnourished or have impaired immunity.[3]

Persistent diarrhea occurs when the duration of symptoms exceeds seven days and chronic diarrhea when it lasts more than 14 days.[4]

Prevalence of a disease: Is the total number of existing cases respective to the entire population. In community statistic, it is usually represented as the percentage of the population having a particular disease at any given time.[5]

Antibiotic resistance: Is the natural ability of microorganisms to withstand the effect of drugs that are lethal to most members of its species or is the ability of a bacterium or other microorganism to survive and reproduce in the presence of antibiotic dose that were previously thought effective against them. [6]

Susceptibility to antibiotics: is the quality or state of being susceptible or the state of being predisposed or lacking the ability to resist something.[7]

The antimicrobial susceptibility testing is to predict the in vivo success or failure of antibiotic therapy. Test is performed in vitro, and measures the growth response of an isolated organism to particular drug. This helps to provide clinical information for selecting appropriate antibiotic for your patient. The data from the test will be interpreted as susceptible, resistance or intermediate.

1.3.2 Types of diarrhea

Acute watery diarrhea: This term refers to diarrhea characterized by abrupt onset of frequent, watery, loose stools without visible blood, lasting less than two weeks. It is accompanied by flatulence, malaise and abdominal pain, nausea, vomiting, fever also may be present. The common causes of acute watery diarrhea are viral, bacterial and parasitic infections. In general the pathogenic bacteria which are most important includes; Shigellae, Pathogenic *Escherichia* coli, Vibrio spp, Camplobacter jejuni, and Salmonella spp.[8]

Dysentery: May be defined as diarrhea containing blood and mucus in feaces. The illness also includes abdominal cramps, fever, and rectal pain. The most important cause of bloody diarrhea is shigella. The Shigella can

cause septicemia (blood poisoning), rectal prolapsed and haemolyticus-uramic syndrome (HUS) which affect the kidney and blood clotting system.[9]

Persistent diarrhea: Is defined as diarrhea episodes of presumed infectious etiology that have an unusually long duration and last at least 14 days. About 10 percent of diarrhea in children from developing countries become persistence, especially among those less than three years and more so among infants. This diarrhea cause substantial weight loss in most patients and it may be responsible for about one third to half of all diarrhea related deaths. The pathogens responsible for persistent diarrhea are Entropathogenic *Escherichia* coli (EPEC), EnteroAggregassive Escherichia coli (EAggC) and Cryptosporidium.[10]

Chronic diarrhea: This term refers to diarrhea which is recurrent or long lasting due to mainly noninfectious causes. This may be caused by gastrointestinal diseases, or may be secondary to systematic disease. In addition chronic diarrhea could because by enteritis, food intolerance, medication and irritable bowel syndrome etc. [11]

1.3.3 the main causative agents of diarrhea

The most common cause of acute diarrhea is a viral infection. Other causes include bacterial infections, side effects of antibiotics, and infections not related to the gastrointestinal (GI) system. In addition, there are many less common causes of diarrhea.

Viral, bacterial, and parasitic infections are all contagious, and parents/caretakers can assist in preventing spread of the infection. Children are considered contagious for as long as they have diarrhea. However, depending on the microorganism, some children can spread diarrhea even before they develop symptoms, and a minority will continue to spread diarrhea pathogens for weeks, months, or years after their symptoms resolve. Microorganisms causing diarrhea are spread from hand to mouth; hand washing and separating food-handling and feces disposal (eg, not changing diapers in the kitchen) are very important to preventing infection in family and other contacts.

Viral infection — Viral infection is the leading cause of diarrhea in children and is seen most commonly in the winter months in temperate climates. Symptoms of

viral infection can include fever (temperature higher than 38ºC or 100.4ºF), watery diarrhea, vomiting, abdominal cramps, lack of appetite, headache, and muscle aches.Viral infection usually begins 12 hours to 5 days after exposure, and resolves within three to seven days. No specific treatment is available for viral causes of diarrhea. Children with diarrhea from viral infections are best treated with supportive measures (oral rehydration solution; age-appropriate diet, limiting foods high in fat and simple sugars; and rest).

Rotavirus is one of the most common causes of severe diarrhea. Also other virus may be important causes of diarrheal diseases in human, these include; Norwalk virus, Norwalk-like viruses, adenoviruses, calici viruses and astroviruses .[12]

Bacterial infection — Bacterial infection is sometimes hard to distinguish from viral infection. Bacterial infections are more common in locations where there is unsafe drinking water and poor handling of sewage. Persistent high fever (higher than 40ºC or 104ºF) and diarrhea that is bloody or contains mucus are more common with bacterial diarrhea. Most children with bacterial infection do not require antibiotics and will improve with time and supportive measures, however, treatment may be necessary in certain situations.

Parasitic infection — Parasitic infections are more common in locations where there is unsafe drinking water and poor handling of sewage. Infection with a parasite is uncommon in developed countries but may be seen in children who have recently ingested contaminated water or who have traveled to or lived indeveloping countries. Diarrhea from parasitic infections may last for weeks to months.

Antibiotic-associated diarrhea — A number of antibiotics can cause diarrhea in both children and adults. The diarrhea is usually mild and typically does not cause dehydration or weight loss. In most cases, antibiotics should not be stopped and the child's diet does not need to be changed. The diarrhea usually resolves one to two days after antibiotics are finished. Contact a healthcare provider if a child on antibiotics has diarrhea that is severe , contains blood, or does not resolve after the antibiotic is stopped.[13]

1.3.4 Transmission Routes of Causative Agents (Pathogens)

Infectious diarrhea is acquired by fecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with fecal matter. With regard to water–borne diarrhea, transmission patterns occur when domestic water storage facilities or/and water sources for human consumption are contaminated.[14]

There are four transmission routes that the major infectious agents use to reach human host, namely human to human via the environment, human to human multiplying in the environment, human to animal to human via the environment, and animal to human via the environment. In situation where feacal contamination of the domestic environment is high, the majority of cases of endemic disease probably occur either by human-to-human transmission, or from the human-to-human transmission of the pathogenic agent, which have multiplied in the environment.[15]

In study area the common routes of infection of theses enteric bacteria to under five years children are through poor person and environmental hygienic practices involves poor food handling, lack or poor knowledge of hands washing by children and parents as well poor waste disposal of children feace.

1.3.5 Common Enteric Isolated Bacterial Pathogens Associated with Diarrhea

* Escherichia coli

Escherichia coli are the most prevalent infecting organism in the family of gram-negative bacteria and a facultative anaerobe known as enterobacteriaceae.[16]

Escherichia coli bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich.[17]

Dr. Escherich also showed that certain strains of the bacterium were responsible for infant diarrhoea and gastroenteritis, an important public health discovery. Although *Escherichia* coli bacteria were initially called Bacterium

coli, the name was later changed to Escherichia coli to honor its discoverer . *Escherichia* coli is often referred to as the best or most-studied free-living organism .[18] More than 700 serotypes of Escherichia coli have been identified . The "O" and "H" antigens on the bacteria and their flagella distinguish the different serotypes.[19]

It is important to remember that most kinds of *Escherichia* coli bacteria do not cause disease in humans . Indeed, some *Escherichia* coli are beneficial, while some cause infections other than gastrointestinal infections, such as urinary tract infections.

The strains of *Escherichia* coli that cause gastroenteritis are divided into the following 6 groups: enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC) or Shigalike toxin-producing E. coli (STEC), enteroaggregative (EAEC), and diffusely adherent *Escherichia* coli (DAEC). The clinical spectrum of diarrheal disease caused by these strains is dependent upon the characteristics of the secreted enterotoxin and plasmid-mediated virulence factors that allow for attachment and invasion of intestinal epithelium.[20]

Enterotoxigenic *Escherichia* coli (ETEC) are a common cause of "traveler's diarrhea" and a very important cause of diarrhea in children less than 5 years of age in developing countries. ETEC produces heat-labile and heat-stable enterotoxins which affect the small intestines and cause a secretory diarrhea.

Shiga toxin-producing *Escherichia* coli (STEC) are named for the cytotoxic toxins they produce. There are at least two antigenic forms of the toxin referred to as Shiga-like toxin 1 and Shiga like toxin 2. STEC has been associated with mild non-bloody diarrhea, hemorrhagic colitis, a severe form of diarrhea, and with hemolytic uremic syndrome, a disease resulting in acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. Shiga-like toxin 1 is identical to the Shiga toxin of Shigella dysenteriae type 1, and Shiga-like toxin 2 also has many properties that are similar to the Shiga toxin; however, the two toxins are antigenically and genetically distinct. EHEC produces cytotoxic Shiga Toxins (Stx-1 and Stx-2) that destroy intestinal villi and cause dysentery.[21]

Enteropathogenic *Escherichia* coli (EPEC) are an important cause of diarrhea in infants, especially in developing countries. EPEC causes diarrhea by destroying microvilli in the small intestines.

Enteroinvasive *Escherichia* coli (EIEC) produce a disease very similar to shigellosis. The disease occurs most commonly in children in developing countries and in travelers to these countries. EIEC causes bloody diarrhea by causing destruction of epithelial cells in the large intestines.[22]

Enteroaggregative *Escherichia* coli (EAEC) causes acute and chronic diarrhea (>14 days in duration) in persons in developing countries. These organisms also are the cause of foodborne illnesses in industrialized countries and have been associated with traveler's diarrhea and persistent diarrhea in patients with HIV.[23]

1.3.6Antibiotic treatment

Medications such as antibiotics agents are generally not necessary and could be harmful for infants or children with diarrhea. Rarely, antibiotics may be used in cases of bacterial infection when a specific cause of the diarrhea has been found or is strongly suspected, particularly after recent travel. Inappropriate use of antibiotics will not improve diarrhea. Furthermore, antibiotics can cause side effects and lead to development of antibiotic resistance.[24],[25]

Antimicrobial therapy of acute diarrhea varies depending on the etiologic agent. Since viral agents are the predominant cause of acute diarrhea, antimicrobial agents play only a limited role in case management. Certain diarrheal diseases, however, require appropriate drugs in addition to fluid and nutritional therapy. Identification of patients requiring antimicrobial therapy relies on clinical, epidemiologic, and laboratory evidence. For instance, bloody diarrhea or the presence of white blood cells on methylene blue stain of the stool specimen suggests a bacterial agent causing invasive mucosal damage and indicates that stool cultures should be performed to identify the organism. Other clinical clues suggesting a cause of infectious diarrhea amenable to antimicrobial therapy include a history of recent antibiotic use (in which case Clostridium difficile should be suspected), exposure to children in day care centers where Giardia or Shigella is prevalent, recent foreign travel, and immunodeficiency, in which infectious causes of diarrhea should be diligently evaluated. Conversely, watery diarrhea and

vomiting in a child less than 2 years of age most likely represent viral gastroenteritis and therefore do not require antimicrobial therapy.[26]

1.3.7 Ceftriaxone

Ceftriaxone is a broad-spectrum bactericidal cephalosporin antibiotic. Ceftriaxone is active *in vitro* against a wide range of Gram-positive and Gram-negative organisms, which include β-lactamase producing strains. Ceftriaxone belong to Third-Generation Cephalosporin.

Cephalosporin's exert bactericidal activity by interfering with bacterial cell wall synthesis and inhibiting cross-linking of the peptidoglycan. The cephalosporin's are also thought to play a role in the activation of bacterial cell autolysins which may contribute to bacterial cell lysis.

Chapter Two

(Materials and methods)

2.1 study area

The study was done in teaching hospital and AL-Hussein hospital for children of Al-Diwaniyah city with collaboration and support of Ministry of health (MOH). The period of study was extend from 1/8/ 2016 to 15/1/2017.

2.2 study population

This is a controlled clinical trial on 50 children. Study population was included children less than two years of age who attend hospital with diarrhea.

2.3 sample collection

Overall population of children less than two years age from center of Ad-Diwaniyah . The stool samples were collected from diarrhea patients in AL-Hussein hospital for children and the teaching hospital.

2.4 diagnosis

The diagnosis was based on the presence of clinical symptoms and signs . Initial tests performed is stool culture. The culture state that the causative organism is *Escherichia* coli .

2.5 isolation of *Escherichia* coli

Isolation and Identification of *Escherichia* Coli 50 stool samples from children with diarrhea (aged under 2 years) were cultured on MacConkey agar and Eosin Methylene Blue (EMB). All enteric bacteria isolated were identified on the basis of colonial characteristics, Gram stain and biochemical tests, IMViC, Urea, Kligler Iron Agar [27]

2.5.1 Detection of Hemolysin Production

The Escherichia coli isolates tested for blood hemolysis were streaked on blood agar plates containing 5% (v/v) human blood and incubated aerobically at 37°C for 24 hours. The clear zones around the growth colonies indicate a positive reaction [28].

2.5.2 API 20 E test :

API (Analytical Profile Index) 20E presented is a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae.

In API 20E for identification of members of the family Enterobacteriaceae, the plastic strip holds twenty mini-test chambers containing dehydrated media having chemically-defined compositions for each test. As shown in Fig.1

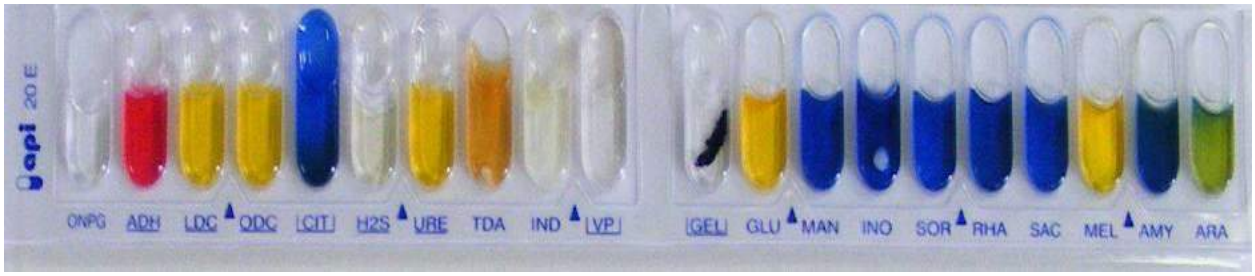

Fig.1 API 20 E Biochemical Test strip

Setting up an API20E Biochemical Test Strip

- Pick up a single isolated colony (from a pure culture) and make a suspension of it in sterile distilled water.
- Take the API20E Biochemical Test Strip which contains dehydrated bacterial media/bio-chemical reagents in 20 separate compartments. API20E Biochemical Test Strip is commercially available. (Bacteria will react with them and will give different colors which will help to identify bacteria to the species level).
- Take a Pasteur pipette and fill up (up to the brim) these compartments with the bacterial suspension.
- Add sterile oil into the ADH, LDC, ODC, H2S and URE compartments.

- Put some drops of water in the tray and put the API Test strip and close the tray.
- Mark the tray with identification number (Patient ID or Organism ID), date and your initials.
- Incubate the tray at 37oC for 18 to 24 hours.

2.6 Antibiotic Sensitivity Test

2.6.1 preparation of Muller Helton Agar.

1. Suspend 38 g of the powder in 1 L of purified water. Mix thoroughly.

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.OPTIONAL: Cool medium to 45-50°C .

4. Pour cooled Mueller Hinton agar in the dishes on a level, horizontal surface to give a uniform depth of about 4 mm (60-70 mL of medium for 150 mm plates and 25-30 mL for 100 mm plates) and cool to room temperature.

5. Check prepared medium to ensure the final pH is 7.3 ± 0.1 at 25°C.

2.6.2Kirby-Bauer disk diffusion test

Antibiotic susceptibility test of *Escherichia* coli isolates was determined by the standard Kirby-Bauer disk diffusion method [29]

These antibiotics with their respective disk concentrations are

Amoxicillin-clavulanic acid (30µg), Amikacin (10µg), Aztreonam (30µg), Carbenicillin (25µg), Cephalothin (30µg), Ciprofloxacin (10µg), Gentamycin (10µg), Norfloxacin (10µg) (Bioanalyse)., Bacterial cultures suspension equivalent of 0.5 tube McFarland turbidity standards were spread on Muller-Hinton agar plates using sterile swabs and incubated aerobically at 370C for 24 hours, then inhibition zones diameter around antibiotic disks were measured. Results were

expressed susceptible or resistant according to the criteria recommended by the CLSI [30]

Fig.2 Kirby-Bauer disk diffusion test

2.7 Antibiotics dilution method

2.7.1 Antibiotics dilutions

A:mean 100% antibiotic (4ml antibiotic).

B:mean 75% antibiotic (3ml antibiotic +1ml DW).

C: mean 50% antibiotic (2ml antibiotic +2mlDW).

D: mean 25% antibiotic (1ml antibiotic+3ml DW)

E: mean 100% DW.

Fig.3(LDP ,mn pharmaceutical andRanbaxymanufacturers of ceftriaxone)

2.7.2 Preliminary screening of prepared Antibiotics dilutions:

Evaluated the susceptibility prepared dilutions on the inhibition of bacterial growth (bacteria *Escherichia* coli) according to the method [31] as the method is used Agar – well diffusion method , and included the preparation of Muller- Hinton agar , as instructed by the company processed, pour in Petri dishes, then vaccinated agar with (0.1) ml of airborne bacterial with concentration (1×106) bacteria cell \ ml, publish shake the publisher glass, left the dishes for a period of (15 - 30 minutes), (3-4) diameter hole (8 ml) \ hole ,and the added (100) Microliters' of concentration of (25,50,75,100) into each hole using, dishes were incubated at 37 ° C was measured inhibition zone diameter.

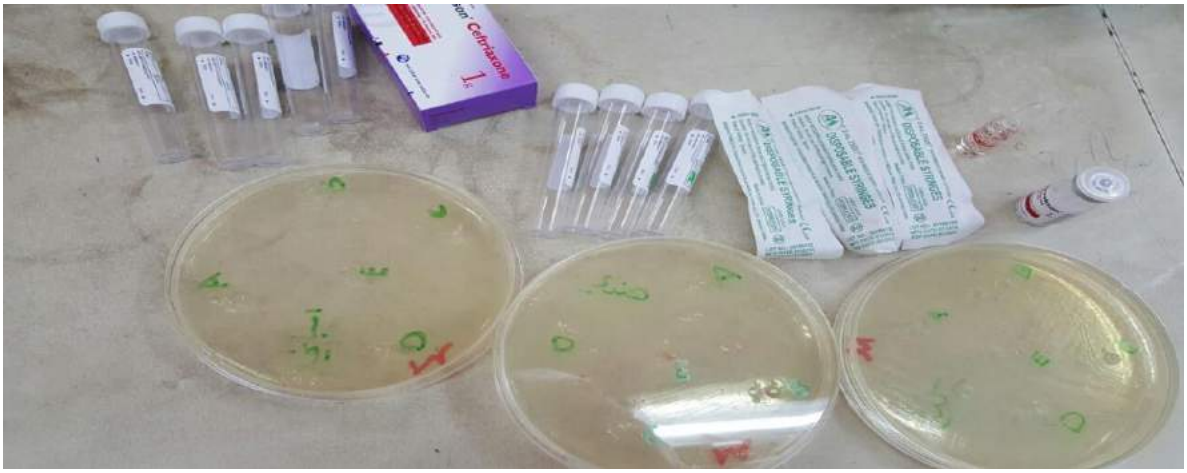

Fig .4 (holes on MHA)

2.7.3 Measuring inhibition zone

Following removal of the plate from the incubator:

- Hold the plate a few inches above a black nonreflecting surface.
- Measure to the nearest millimeter with a ruler or calipers.

This procedure applied on ceftriaxone from three companies : (LDP,Ranbaxy,mnPHARMACEUTICALS).

Antibiotic	R	I	S
Ceftriaxone	≤19	20—22	≥23

[32]

R stands for resistance which is the ability of a bacterium or other microorganism to survive and reproduce in the presence of antibiotic dose that were previously thought effective against them .[33]

S stands for susceptibility which is the quality or state of being susceptible or the state of being predisposed or lacking the ability to resist something. [34]

I stands for intermediate: Include isolated with antimicrobial minimum inhibitory concentration (MICs) that approach usually attainable blood tissue level and for which response rates may be lower than for susceptible isolates .[35]

2.8 Minimal Bactericidal Concentration (MBC) and Minimal Inhibitory Concentration (MIC)

2.8.1preparation of broth media

Suspend 8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete

dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2-8°C. The color is amber, slightly opalescent.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

Fig .5 (broth preparation)

2.8.2: MIC and MBC test procedure:

1. Prepare 0.5 McFarland suspension of bacteria were prepared by using standard turbidity tube from API 20E standard McFarland tubes ,and in contrast with this turbidity we was inoculated the CFU in the broth medium.
2. then we distributed the suspension in all over (30) tubes (each tube contain 9 ml).

***preparation of ceftriaxone concentrations :

(1)gm were added to (10)ml of DW. BY which we obtain(1/10) of this antibiotic that considered stock solution .

(1)ml of stock solution were added to (9)ml of DW.to obtain (1/100) concentration of this antibiotic.

This procedure were repeated until reaching (10^10) dilution of the antibiotic conc. To each origin of synthetic company by which we to get (10)dilutions of each origin of antibiotic.

3. adding of antibiotic :

From each conc. Of antibiotic we added (1)ml to each (9)ml of broth that inoculated by the McFarland (0.5) standard of *Escherichia* coli 1 (API 20 E number 5144132){commercial identification system of Enterobacteracaea}.All tubes were inoculated at (35°C) for (24) hour.

**examination :

The turbidity of bacteria in the tube were degraded from (1/10) clear to 10^5 very turbid , All turbidity of each origin of antibiotic ceftriaxone were cultured on blood agar by separation way by adding (50)mcg to the surface of agar and separated by using of glass separator to distribute the bacteria in all over the surface of agar . the reincubating.

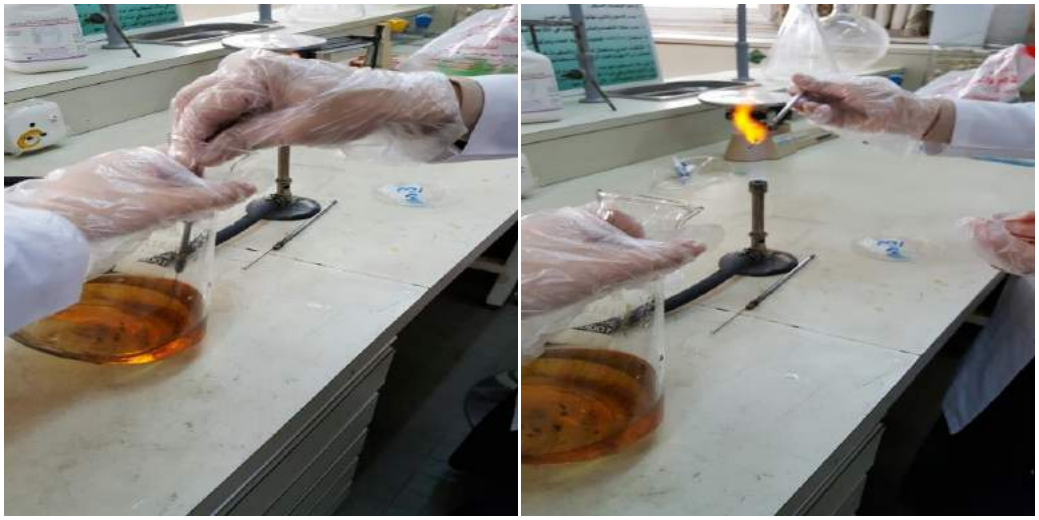

Fig .6 (preparation of bacterial suspension)

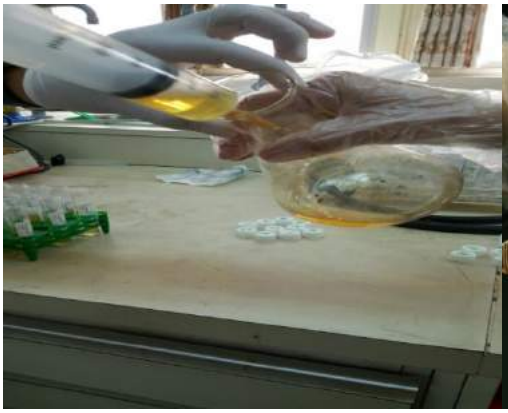

Fig .7 (filling test tubes with broth Media Containing *Escherichia* coli)

Fig .8 (adding of antibiotic to broth media containing *Escherichia* coli)

Data Analysis:

All received data were ranged, tabled and analyzed by using a computerized Microsoft Office Excel (2013) and IBM/SPSS programs. Chi-square test were used at a level of $P \leq 0.05$ to detect the variations in an inhibition zone between the different Ceftriaxone concentrations with manufacturer companies (Table 1), or between MIC and MBC with different manufacturer companies (Table 2)

Chapter Three

(The results)

THE RESULTS

"The incidence of infectious diarrhea and the prevalence of a given causal agent are strictly associated with socioeconomic factors such as nutrition, sanitation and habitat of the population" (32). Our study showed that 38(76%) of samples were give positive results for *Escherichia* coli , 12(24%)of samples may be another cause of children diarrhea (Table 1).

Table 1: The microbial spectrum in stool specimens from children diarrhea

Escherichia. Coli isolates	Klebsiella spp.	Total sample
38(76%)	12(24%)	50(100%)

3.1 API 20

API20 test gives this show in figure (9)

Fig. 9: Numbering in API 20E Test Strip

Numbering in API 20E Test Strip

The profile for this combination of reactions is therefore 5144132

By using API catalog , the organism Identified as Escherichia coli type 1

3.2 sensitivity test

3.2.1 Disk diffusion test

All *Escherichia* coli isolates 100% sensitive to Ciprofloxacin, Gentamycin, and Norfloxocin. However, 16.6% of *Escherichia* coli isolates were sensitive for Carbenicillin and Amikacin. But 33.3% of *Escherichia* coli isolates were sensitive to Amoxacillin-clavulanic and Aztreonam. Figure (1). This is similar to the study of [36] in which gram negative bacteria were multi drug resistant to Ampicillin, Amoxicillin, Ceftizoxime, Cefepime, Tetracycline. Many studies have shown that active efflux pump or produced beta lactamase enzyme, can be a mechanism of resistance for almost all antibiotics like penicillin's, cephalosporin and carbapenems.and this agree also with the study of [37]

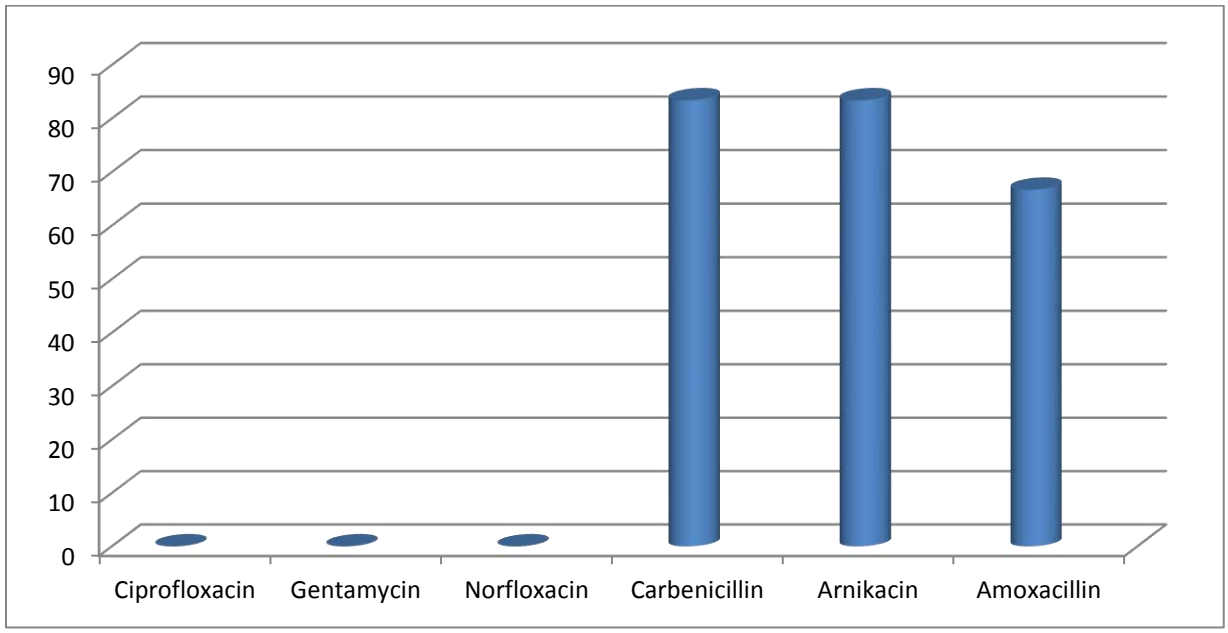

Fig.10 : Percentage of *Escherichia coli* isolates resistance for antimicrobial agents

3.2.2 dilution method

The results show that antibacterial activity against *Escherichia* coli for ceftriaxone from the (LDP) company have inhibition zone about (35mm) at (100%) antibiotic concentration, (32mm) at (75%) ,(30mm) at (50%),(25mm) at (25%) , the antibacterial activity against *Escherichia* coli for ceftriaxone from the Ranbaxy company show(30mm) at (100%) antibiotic concentration, (25mm) at (75%),(22mm) at (50%),(20mm) at (25%) , While the antibacterial activity against *Escherichia* coli for ceftriaxone from the mn pharmaceuticals company show(35mm) at (100%) antibiotic concentration, (30mm) at (75%), (28mm) at (50%), (25mm) at (25%) , as show in the table (2)

Table (2):Variation in Ceftriaxone Inhibition Zone with Different Concentration

Concentration	Inhibition zone of ceftriaxone /mm		
	LDP	Ranbaxy	mn - pharmaceutical
A (100%)	**35** [a]	**30** [b]	**35** [a]
B (75%)	**32** [a]	**25** [b]	**30** [a]
C (50%)	**30** [a]	**22** [b]	**28** [a]
D (25%)	**25** [a]	**20** [b]	**25** [a]

Different small letters, horizontally, referred to significant differences at a level of $P \leq 0.05$

LDP company at all antibiotic concentration (100% ,75% ,50%, 25%) *Escherichia* coli show susceptibility to ceftriaxone.

mn pharmaceutical company this company show the same result as LDP at all antibiotic concentration (100% ,75% ,50%, 25%) *Escherichia* coli show susceptibility to ceftriaxone.

Ranbaxy company the result state at ceftriaxone concentration (100%, 75%) , *Escherichia* coli show susceptibility ,while at ceftriaxone concentrations (50% , 25%) , *Escherichia* coli is intermediate . Figure (2)

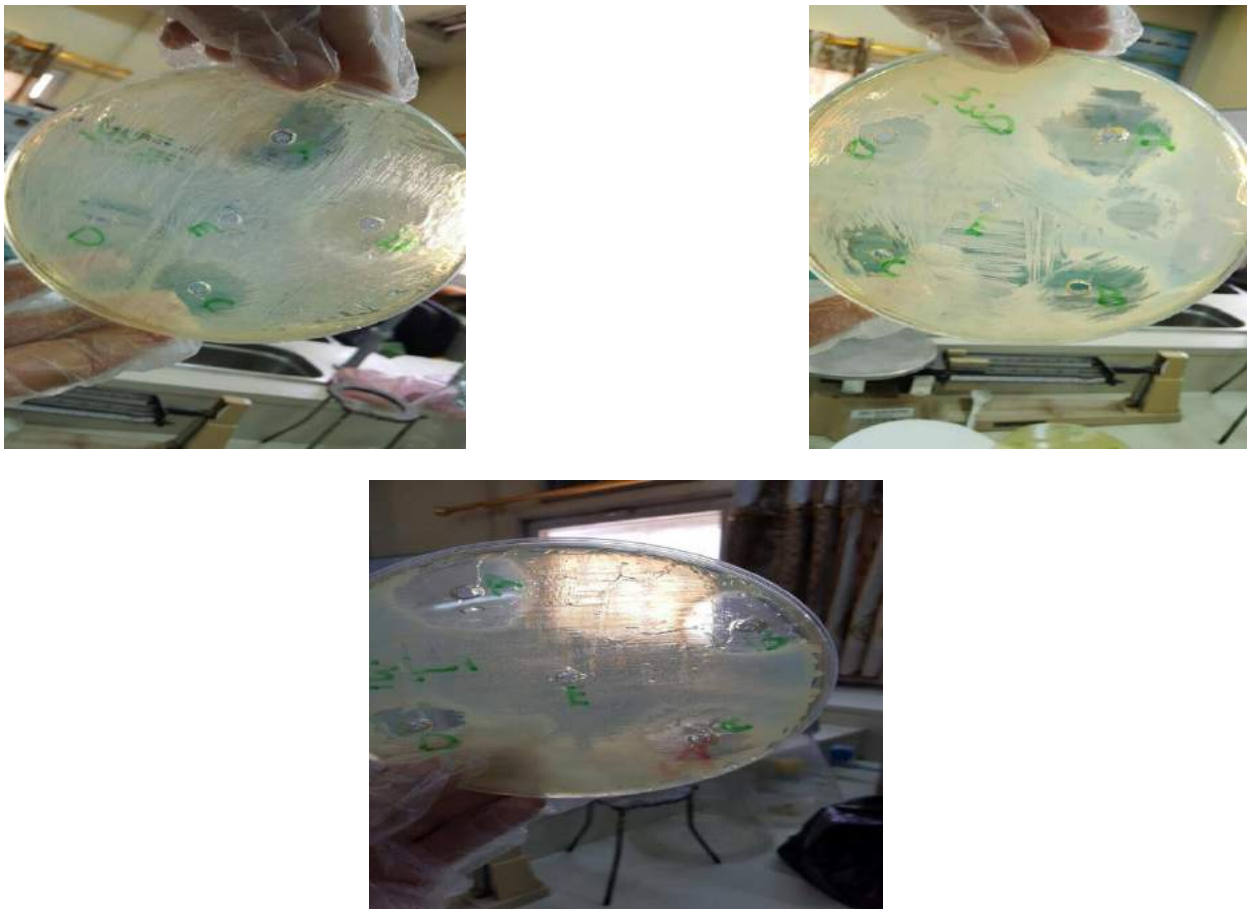

Fig.11 :Agar Wall Diffusion method with different concentrations of ceftriaxone (3 companies)

3.3 MIC and MBC test:

The dilution of (1/100000) the lowest concentration of antibiotic gave us high bacterial growth while the concentration (1/10) was clear that represent MBC and (1/100) gave us growth of (3) colonies on agar that represent MIC.

The study show that all of these companies have the same MIC and MBC , as a show in table(3), Figure(4)

Table (3):Variation in Ceftriaxone Inhibition Zone with MIC and MBC

Company	LDP	Ranbaxy	mn - pharmaceutical
Minimal Inhibitory Concentration (**MIC**)	**(0.01)**[b]	**(0.01)**[b]	**(0.01)**[b]
Minimal Bactericidal Concentration (**MBC**)	**(0.1)**[b]	**(0.1)**[b]	**(0.1)**[b]

Different small letters, horizontally, referred to significant differences at a level of P≤ 0.05

Fig.12 :MIC and MBC test tubes

Note: The susceptibility of most pathogenic bacteria is usually determined by the diffusion method. But, some clinically important bacteria grow slowly, need special media and atmosphere, and it is not possible to use the diffusion method. In these cases the best method is the Minimum Inhibitory Concentration, and the ability to produce _-lactamase.

Chapter Four

Discussion

4.1 Isolation of *Escherichia*.coli from stool of children. Why?

Diarrhea remains the second leading cause of death in children younger than 5 years globally, accounting for 1.3 million deaths annually .Entropathogenic *Escherichia coli(EPEC)*, one of the diarrheagenic *Escherichia coli* pathotypes, are among the most important pathogens infecting children worldwide because of their high prevalence in both the community and hospital setting, and because they are one of the main causes of persistent diarrhea .[38]

This finding is in agreement with the report from Saudi Arabia which reported high prevalence of enteric bacteria associated with diarrhoea at 1-2 years. agreement with the results of (CSB, 2003) that reported the highest prevalence at ages of 12-23 months at about 14.8% and the lowest prevalence at 6-11 months. The present study also concurs with studies in study in Bangladesh (Piechulek *et al*., 2003), Vietnam (Takanesh *et al*., 2009). Also a study from Kenya by Onyango *et al*., (2010) reported prevalence of 16.7% at the ages of 13-24 months. Protection against diarrhoea at the youngest age group may be conferred by several mechanisms such as maternal antibodies against enteric pathogens and current breastfeeding. Transmission of Enterotoxigenic *Escherichia coli* (ETEC) is known to be specifically associated with contaminated weaning foods among younger children in developing countries (Rao *et al*., 2003, Blak et al 1982), and was considered to be responsible for the diarrhoea-induced weight faltering (Motarjeni et al., 1993) . This finding may be explained by the fact that weaning foods for young children prepared under in hygienic conditions are frequently contaminated with pathogens and are an important risk factor of diarrhoea transmission (Motarjeni *et al*., 1993). The increased risk of having diarrhoea in children with age less than five years whose mothers had poor food hygiene practices in our study was observed. Direct association between food hygiene practices and diarrhoea in children have been suggested in several epidemiological studies in developing countries, such as Vietnam (Takanashi *et al*., 2009).

4.2 Choice of ceftriaxone .why?

ceftriaxone is third generation cephalosporins with greater activity than second generation cephalosporins against certain gram negative bacteria. Ceftriaxone has longer half life and therefore needs to be given only once daily . It works by interfering with the formation of the bacteria's cell wall so that the wall ruptures, resulting in the death of the bacteria.[39] Ceftriaxone may be administered both intravenously and intramuscularly. Besides the need for parenteral administration and the high cost, the major drawback of the widespread use of ceftriaxone for the treatment of acute infectious diarrhea is the immediate danger of increasing microbial resistance to this useful drug. For all of these reasons, this drug should be reserved for very severe cases.

4.3 Components of antibiotic from three companies

LDP ceftriaxone

(vial LDP contain ceftriaxone sodium equivalent to 1gm ceftriaxone)

mn pharmaceuticals ceftriaxone

(each vial contains ceftriaxone sodium equivalent to 1gm ceftriaxone)

RANBAXY ceftriaxone

(each vial contain ceftriaxone sodium equivalent to 1gm ceftriaxone)

Action of ceftriaxone from these companies (LDP, mn pharmaceuticals , Ranbaxy) are similar.

4.4 size of inhibition zones

4.4.1 LDP ceftriaxone:

At high concentration (100%) ,large inhibition zone (35mm) is present . when the antibiotic concentrations decrease (75%, 50%, 25%, 0%) ,the size of inhibition zones are decreased (32,30,25,0mm). At all concentration (100%,75%,50%,25%) except (0%) ,bacteria show susceptibility to ceftriaxone

4.4.2 mn pharmaceutical ceftriaxone:

At high concentration (100%) ,large inhibition zone (35mm) is present . when the antibiotic concentrations decrease (75%, 50%, 25%, 0%) ,the size of inhibition zones are decreased (30,28,25,0mm).At all concentration (100%,75%,50%,25%) except (0%) ,bacteria show susceptibility to ceftriaxone.

4.4.3 Ranbaxy ceftriaxone:

At high concentration (100%) ,large inhibition zone (30mm) is present . when the antibiotic concentrations decrease (75%, 50%, 25%, 0%) ,the size of inhibition zones are decreased (25,22,20,0mm).At (100%,75%) concentrations , bacteria show susceptibility to ceftriaxone. While (50%,25%) concentration is intermediate .

Bacterial responses to antibiotics are concentration-dependent. At high concentrations, antibiotics exhibit antimicrobial activities on susceptible cells, while sub inhibitory concentrations induce diverse biological responses in bacteria. At non-lethal concentrations, bacteria may sense antibiotics as extracellular chemicals to trigger different cellular responses, which may include an altered antibiotic resistance/tolerance profile. In natural settings, microbes are typically in polymicrobial communities and antibiotic-mediated interactions between species may play a significant role in bacterial community structure and function.[40]

The MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents.[41]

the MICs of ceftriaxone were determined by broth dilution method.MBC determined where no visible growth found. This study showed that MIC & MBC values of ceftriaxone found highest against *Escherichia* coli .

conclusion

In this study, we determined the inhibition activity of the antibiotic from the three different Origins on bacteria ***E.coli*** which isolated from children's diarrhea under 2 year,. The results of the study showed no significant differences among these Origins

Recommendations

Its necessary to perform the additional studies of this subject of the research to determine the pharmacokinetics of this antibiotic, and the necessity also to perform histological studies to detect the existence of differences in the mechanism of action on tissues.

Chapter Five

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