Effect of Antibiotics Combination on *Escherichia coli* Contaminated Pacifier used by children suffering from diarrhea

Mohammed Oudah Hamad*

***Department of Ecology, Faculty of Science , University of Kufa**

mohammeda.alkhafaji@uokufa.edu.iq.com

Abstract

A total of eighty nine clinical samples were collected from pacifier of children patients with age1-48 months from both gender complaining from diarrhea in Hilla city. 19 isolates of *E.coli* were isolated and identified according to their morphological properties(cultural and microscopical) and biochemical tests and percentage (21.3%).Some clinical samples were showed *Entamoeba histolytica* ,*Giardia lamblia* and *Trichomonas hominis* and Monilia. The susceptibility of the all current isolates were carried out against 17 antibiotics, *E.coli* isolates were very sensitive to meropenem and impenem ,and sensitive to sefitrixon ,amikacin, and ciprofloxacin ,while its resistance to others antibiotics were different. The minimum inhibitory concentrations(MIC) of some present isolates were higher.The combination of β - lactam antibiotics (amoxicillin and cefotaxim)with quinolones(ciprofloxacin)from side and aminoglycoside (amikacin) from other side , demonstrated effective synergism of these mixtures anti multidrug resistance *E.coli* isolates.

Keyword: *E. coli*, Diarrhea ،Dummy Sucking, Children, Combination of Antibiotics.

Introduction

Diarrhea sickness is a major public health problem for the world, causing 2 million deaths every year, especially for infants up to 5 years,The main and more common cause of diarrhea in infants is EPEC ,bacteria have been previously considered to be the common pathogens of humans, animals and plants leading to the diseases of living organisms all over the worldwide, especially the poor countries, including the gram-negative bacteria, especially the enterobacteriaceae, such as *E. coli*, which are divided into a non-harmful to gastro intestinal tract and opportunistic pathogen, causing intestinal diseases and others its strains capable of causing the disease outside the bowel, which is one of the main and common causes of intestinal infections and diarrhea, especially in children in the first months of birth, people who use antibiotics in long periods,as well as the elderly, diabetics and immune impairment (Wong). Diarrheagenic *E. coli* or Pathogenic *E.coli* is classified according to its genetic and phenotypic traits to six pathotypes: Enteropathogenic *E.coli* (EPEC), Enterotoxigenic *E.coli* (ETEC), Enterohemorrhagic *E.coli* EHEC, Enteroinvasive *E.coli* (EIEC), Enteroaggregative *E.coli* (EAEC), Enteradhesive *E.coli* (EAEC) are an antibiotic-resistant opportunistic pathogen, and it resist for many sterilizers and disinfectants used in sterilization and disinfection of hospitals, therefore *E.coli* one causes of hospital infection (Barati et al., 2012) The (EPEC) strain is the primary pathogen for diarrhea in infants in developing countries (Torres et al., 2005), with EPEC infection adhering complete to the intestinal epithelial tissue and vandalizing of microvillus by means of a mechanism of complex signals that eventually lead to diarrhea. These effects are called adhesion and effacing (A / E) lesion, which are genetically encoded through chromosome by the locus of enterocyte effacement, (EPEC) is classified as typical and atypical, depending on the difference in the virulence factors and evolutionary pathways of their strains (Bugarel et al., 2011). The typical strain has the ability to form an appearance pattern called localized adherent (LA) for fine colonies,encoded by plasmid, this plasmid contains important resistant antibiotics genes, as well as containing operon composed of several common genes for enciphering in the bundles forming pilli, a localized factor of adhesion. This Plasmid encoded for the adhesion factor is found in all typical strain while the non-typical strains lack or are different from the first. Most pathogenic

genes are grouped together in large groups called pathogenicity associated islands, the pathogens of *E. coli* that cause diarrhea in children, who use pacifier as a result of recent contamination. The most common cases of infection due to drinking water and contaminated food, causing many diseases of the human, including inflammation of the urinary tract, inflammation of the wounds, ear infection, vaginal infections in women, and other infections, and is considered inflammatory gastrointestinal tract of common and acquired-common diseases, especially in infants and recurrence and come in second place after infections of the respiratory tract. These bacteria have multiple mechanisms to resist antibiotics. It is one of the most harmful pathogens for children when they become infected. Therefore, it is necessary to investigate the pharmacological sensitivity of the disease isolates to know the suitable effective drug, as well as to limited the spread of resistance in hospitals or society. Resistant bacteria such as *E. coli*, as the study of Burgess, 2005), From the suggests that reducing the risk of bacterial resistance is by mixing some antibiotics with each other, thus increasing their effectiveness through synergy ,The infection of the pathogenic bacteria, especially in children who suffering from diarrhea and resistance to many antibiotics, to the spread of diseases and deaths in newborns to weaken their immunity and contribute to the increase in the cost of primary health care globally, and despite the availability of modern antibiotics, but the emergence of resistance and development in many microorganisms become a common problem in the economically poor countries and suffers from it by the patient and the doctors. These bacteria are characterized by their multiple resistance to antibiotics, because of the transmission of resistance genes between the many genus and the convergent species, resistance genes and pathogen genes on transient genetic factors and thus can be transmitted to the cell received (Abubaker) for the danger of *E. coli* bacteria in causing health problems such as diarrhea in children using dummy sucking, so the current study aimed to isolate and identification the causes of diarrhea for newborn children and to know the resistance of this bacteria against antibiotics and the effect of mixing some antibiotics on it. The dummy is a tool used by children to stimulate the behavior of special sucking lest cry remain calm and quiet away from the bad habit Sucking his fingers, especially in the first months of his life, the components of a wide flat plastic base prevents swallowing flexible part of the dummy by the child and simply can become a pest in the case of the poor use of it and lack of care for cleanliness, leading to pollution in many of the microorganisms caused by many infections such as inflammation of the mouth, middle ear, teeth, respiratory tract and inflammation of the intestine causing diarrhea leading to deterioration of children's health and drought and loss of appetite and pain Tons accompanied by stingers during defecation and crying(Canadian).

Materials and Methods

1.Collection of specimens

A total of 89 clinical samples were taken by disposable swabs cotton, from the outer part of the pacifier nipple used by children at age 1-48 months for both gender and suffering from diarrhea, it were placed with 5 ml test tubes of physiological saline solution or brain heart infusion broth, and transport to bacteriological laboratory to culture in blood agar , MacConkey agar , EMB agar and incubated for 24 hours at 37°C under aerobic conditions, examined to isolate the agent causing the diarrhea and identification by subsequent examinations.

2.Bacterial isolates

E.coli isolates were isolated from the cotton clinical swabs from the outside of the pacifier nipple after culturing in blood agar base, MacConkey agar (Hymedia) and EMB with a streaking technique, identified based on its characteristics on the culture and under microscopic chemical tests .

3.Antibiotic susceptibility test

Antibiotics sensitivity was tested against17antibiotics, prepared by Bioanalyse-Turky-ready disks and the lowest inhibitory concentration of antibiotics with macro-dilution method done

. 4.Antibiotics Combination

The half-chess board technique referred to by Garrod was used. Concentrations of 10 µg / ml for amoxicillin, cephalaxin, tetracycline and trimethiprim and 5 µg / ml for ciprofloxacin, amikacin and cephotaxim were used.

Results & Discussion

19 bacterial isolates of *E. coli* were isolated from pacifier used by male and female children with diarrhea and numbers and percentages shown in Table 1. Isolation and identification depending on its culture, microscopic and biochemical characteristics Table 2, 3.

Table (1). Numbers and percentages of *E.coli* isolates according to age and gender.

and age(month) Gender	No. Samples	Isolates	(%)
Males Children (1-48 months)	58	13	22.4
Females Children(1-48 months)	31	6	19.4
Total	89	19	21.4

The current study showed that the isolation rate of *E.coli* from pacifier used by children with diarrhea for both gender and age 1-48months were (4.21%), and 11(57.9%) isolates from it, isolated from children in rural areas, while 8 (42.1%)isolates from the urban of Hilla city.The present bacterial isolates were distributed among males and females, the percentage of males in the rural areas was7(53.8%), while 6(46.2%) isolates belonged to males belonging to the residents of the urban areas. Four (66.7%) isolates belonged to females belonging to rural residents and two (33.3%) isolates were from urban , *E. coli* has clinical significance in the incidence of diarrhea and its associated complications compared to the intestinal family and other bacterial species (Sahuquillo-Arce) . (Seidman) could to isolated *E.coli* from children of male and female in rular. Al-Dawmy (2013) refers to isolations 122 of *E. coli* were isolated from 230 diarrhea cases for children aged 1-10 years ,as well as showed *E.coli* prevalent on other bacterial species, The present results were that the occurrence of diarrhea due to intestinal infection and associated fever in the children under study was the result of pacifier contamination by *E. coli*, as well as many types of bacteria including normal flora in the body, and diarrhea may result due to lack of personal hygiene of mother and child, of the microorganisms of the external environment through the infancy of children as well as the use of one infusion of more than one child or between an infected child and a healthy child, as well as the natural flora of the body and its transfer from the hands of mothers and members of the family of children to pacifier .They are the child's body while changing his diaper contaminated with feces and the lack of care by the mother, clean the child and not change his diaper at the appropriate time leads to the transmission of bacteria from its natural places to the pacifier and child hand and then to his mouth through it and thus get contamination and diarrhea, as well as direct kissing of the child in his mouth by family members ,and this were negative effect on children by bacterial infections. Contaminated pacifier had role in the infection of children in various diseases, including diarrhea , oral diseases , respiratory infections and the middle ear by fungi and bacteria especially *Candida albicans* and *E,coli*. Al-Hashemi and others (2010) showed that the risk of pacifier lies in the possibility of contamination with various microbes, especially bacteria that coexist naturally in humans, and use it without cleanliness and poor hygiene causes acute inflammation of the intestines and middle ear.

Table2.Morphological and biochemical tests of *E. coli* isolates isolated from pacifier of children

Type of Test	*E.coli*
Gram Stain	(-ve)
Production of catalase	(-ve)
Growth in EMB	Metallic Sheen
Production of oxidase	(-ve)
Blood lyses	γ
Motility test	(+ve)
Citrate utilization	(-ve)
Indol test	(+ve)
Proskauer test	(-ve)
Methyl red test	(+ve)
Urea lyses	(-ve)
Gelatin Liquefaction	(-ve)
Kligler iron test	Acidic/Acidic
H_2S Production	(-ve)
Gas production/ Sugar fermentation	(+ve)/(+ve)
Lactose fermentation	(+ve)
Maltose fermentaion	(+ve)
Sucrose fermentaion	(+ve)
Trehalose fermentaion	(+ve)
Xzylose fermentaion	(+ve)
Manose fermentaion	(+ve)
Manitol fermentation	(+ve)
Phenyl aniline deaminase	(+ve)
Decarpoxyl from arginine	(+ve)

No haemolyses **γ: Symbols: +ve:Positive, -ve:Negative,**

Tomasi and others pointed to the isolation of *E.coli* high percentage of children pacifier users of the infestation leading to increased contamination in their bodies causing inflammation of the bowels and diarrhea. Ifeanyi and others found that 92.3% of 400 samples were taken from children with diarrhea and age1-60 months were due to *E.coli.* The current isolates which was isolated in these numbers and proportions from children with ages 1 - 48 months, due to their weak immunity and immune system incomplete and the absence of health awareness in the mother is the sterilization of the infestation and fight flies carrier of various bacteria, which often stable on the dummy or mouth of the child, making them more susceptible to infection, including inflammation of the intestines and diarrhea. Several studies refers to the presence of antibodies and immune cells within the body, such as gastric acid as a protective device, protects the child or reduces bacterial infection, as it has a strong antibacterial effect.

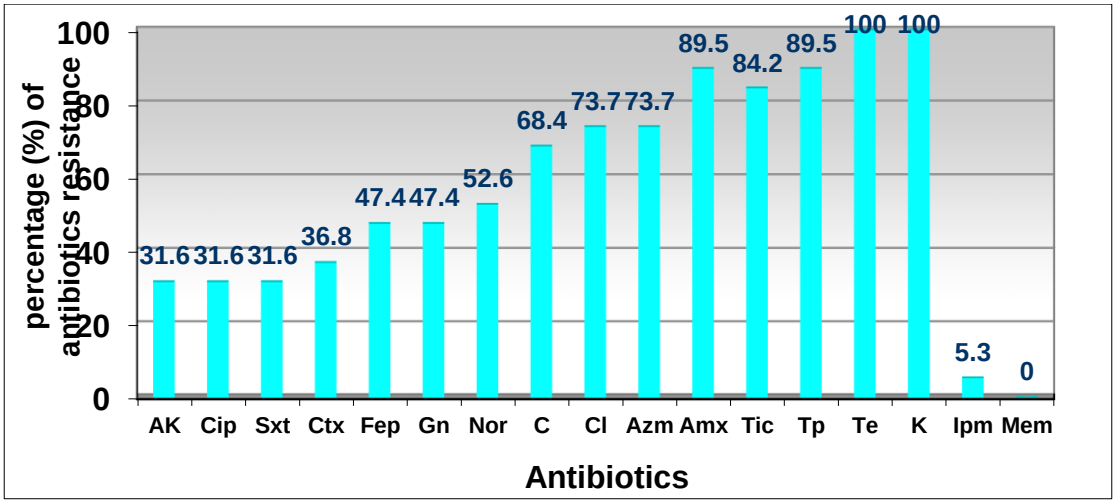
Table 3. The antibiotics sensitivity of *E.coli* isolates isolated from children's Dummy sucking.

Isolates / *E.coli*	Meropenem	Impenem	Kanamycin	Norfloxacin	Trimethprim	Cifipiem	Ticarcillin	Amoxcillin	Aztronam	Cloramphenicol	Cefalexin	Gentamycin	Sifitrixon	Ciprofloxacin	Tetracycline	Amikacin	Cefotaxime	No. of isolates resistance
1	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	15
2	S	S	R	R	S	S	R	R	R	S	R	S	S	I	R	S	S	7
3	S	S	R	R	R	S	R	R	S	R	S	S	R	I	R	R	S	10
4	S	S	R	S	R	S	R	R	R	R	R	S	R	S	R	R	S	10
5	S	S	R	R	R	S	R	S	R	R	R	R	I	S	R	S	S	9
6	S	S	R	S	R	R	S	R	S	S	R	S	S	S	R	S	R	7
7	S	S	R	S	R	S	R	I	R	R	R	R	R	S	R	R	R	11
8	S	S	R	S	R	S	R	R	S	R	R	R	S	R	R	I	S	9
9	S	S	R	S	R	R	R	R	R	S	R	S	R	S	R	S	S	9
10	S	S	R	R	R	R	R	R	R	S	R	R	I	R	R	S	R	12
11	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	I	S	10
12	S	S	R	S	R	S	R	R	R	R	R	S	S	S	R	S	R	9
13	S	S	R	R	R	R	R	R	R	R	R	R	S	R	R	S	S	12
14	S	S	R	S	S	S	S	R	S	S	I	S	S	S	R	S	S	3
15	S	S	R	S	R	R	R	R	R	R	R	S	S	S	R	S	S	9
16	S	S	R	S	R	S	R	R	R	R	S	R	S	S	R	R	R	10
17	S	S	R	R	R	R	S	R	S	S	S	S	S	R	R	S	S	7
18	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R	R	S	14
19	S	S	R	R	R	R	R	R	R	R	S	R	S	I	R	R	R	12

Abbreviations : R: Resistance, S:Sensitive, I : moderate resistance.

Ecoli isolates in the present study isolated from dummy sucking children have been shown to have multiple resistance to antibodies, especially Amoxicillin, tetracycline, kanamycin, trimetheprim,chloramphenicol and aztronam and other common treatments for intestinal infections and bacterial diarrhea figure(1).while (Shakya) pointed to *E.coli* isolates isolated from stool were percentage of resistanse to tetracycline (37%) ,amoxicillin and trimetheprime (29%) and no isolates were resistant impenem (0%).that This may be due to the ability of bacteria to repel or inhibition antibiotics,and this occur by of its defense mechanisms to put the antibiotics out or change the location of the target of antibiotics, decrease or reduce the permeability of the outer membrane of the given inappropriate antibiotics without laboratory identification for bacterial cell, which prevents the entry of the treatments, and the excessive expression of the efflux pump to drugs, in addition bacteria produced beta lactamase that have inhibitory effect of the commonly used betalactam antibiotics,which common using in large amounts in Iraqi society,and resistance of bacterial isolates to these antibiotics may be due to the production of active beta-lactamase that convert the molecule of penicillin and its derivatives of modern antibiotics, also cephalosporinase which makes it, antibiotic useless therapeutic, and this has been confirmed by many studies that the many species of bacteria such as *E.coli* possess these enzymes (Shakya) (Hungin et al., 2005. The randomly use of antibiotics, either quality or quantitatively by patients or microorganismes, errors in laboratory identification and antibiotics sensitivity

testing, leads to the persistence of bacteria living, and the emergence of new multidrug resistance bacterial strains. β-Lactam antibiotics were properties, which inhibits the construction of the cell wall ,as well as it interferes in the manufacture of peptidoglycan layer (Willie).



Figur 1.Antibiotics resistance of *E.coli* isolates isolated from pacifier using by children.

Ciprofloxacin and norfloxacin had a contrasting effect on *Ecoli* isolates with 31.6% and 52.6% resistance respectively, and its resistance ratios could be increased to improve the resistance of bacteria against the negative effects of antibiotics due to the continuous use of antibiotics at the expense of the other for the treatment of bacterial infections in addition to the selective pressure for treatments against the current bacterial isolates, as well as prescription the treatment of diarrhea in children by some doctors without the examination of antibiotics sensitivity of medication, and patients to use the treatments with personal diligence by the patient himself or nurses in the sector for the government or private within their clinics after the guidance of the patient to bring the treatment from pharmacies and without medical advice by specialists and the health control of such irregularities resulting from very simple information in the pharmacology and microbiology branch, and the goal is to make profit at the expense of patient health ,all this leads antibiotics, or not to allow the passage of antibiotics through pores deployed in the to genetic mutations in microorganisms and this is confirmed by (Truing) .

The results showed that the highest resistance of *E. coli* was for some antibiotics, 89.5% for amoxicillin and trimethheprim,respectively while to ticarcillin were 84.2%, these resistance possibly due to transport of R-plasmid or transposone or a piece of DNA from high resistant bacterial isolates to sensitive bacterial isolates for same antibiotics, in addition to the narrow canal through which the treatment is carried out, Multidrugresistance of *E. coli* have very narrow or closed corridors sometimes qualify it to be more resistant to treatments compared to other microorganisms, as well as the absence or decrease of antibiotic receptors in its cellular walls or changes in its permeability, as well as the characteristic of permeability distinguished by its plasma membrane and lipid found in huge quantities more than bacteria that sensitive to

outer membrane, the current results showed that Amikacin, Meropinem,

Impinem were bactericidal for almost *E.coli* isolates under study, This study is confirmed by the fact that Meropenim and Impenem of the carpenem group of antibiotics are the most effective in treating most bacterial infections, its Therapeutic importance for many bacterial infections, including intestinal infections and urinary tract infections, especially resistance to antibiotics. The current results revealed the effectiveness of amikacin compared with gentamicin in its effect against *E. coli* isolates. This is confirmed by the fact that its resistance to gentamicin is higher than amikacin,This may be due to the biochemically modified enzymes produced by bacteria. This is one of the strong reasons for the multiplicity of antibiotic resistance by these bacteria. Gentamicin and Amikacin belong to the aminooglycosides group, which inhibits protein synthesis by interfering with the work of protein-making sites(ribosomes) in

The bacterial cell,Antibiotic resistance may occur in the case of long-term treatment of chronic injuries, and this may indicate that (6, 14). *E.coli* bacteria may show complications in the incidence of newborns due to their low immunity, for treatment and prolongation of the therapeutic period. The results revealed that most of the *E coli* isolates isolated from dummy sucking and diarrheal children are resistant to many antibiotics including Aztronam, Cefalexin, Chloramphenicol and Cefipiem Fig1. The minimum inhibitory concentration of 10 antibiotics ,the method of estimating the bacterial growth turbid in the liquid medium was determined through the naked eye to determine whether or not bacterial growth was present(Table 6). **Cha** refers to minimum inhibitory concentration(MIC) for meropenem and ciprofloxacin in range (0.12-8 and 0.06-64**)** µg/ml on *E.coli* isolates, while the (MIC) to cefotaxim,trimethprim,gentamicin ,Amikacin and amoxicillin were 0.12-128,0.32-608 ,0.06-64 and 0.16-0.32 µg/ml

Antibiotics	% of resistance for *E. coli*	MICs (µg/ml) for *E.coli*
Ciprofloxacin	6 .31	64 -0.25
Trimethprim	89.5	128 ->64
Sefetriaxone	31.6	32 -0.12
Cefotaxime	36.8	128 ->0.5
Meropenem	0.0	8 -0.12
Amoxicillin	100	128 ->32
Amikacin	31.6	32 -0.12
Cloramphenicol	73.7	128 -16
Gentamicin	47.4	64 -0.5
Cefalexin	73.7	128 ->32

Table4.Minimum inhibitory concentration of *E.coli* isolates from children pacifier

Antibiotic Combinations

The combination of antibiotics is often used to treat bacterial infections, including diarrhea bacteria to the antibiotics, because genetic mutation by use of one antibiotic, and helps the doctor in the treatment of the patient quickly (43).as well as reduce the negative effects of antibiotics, especially chloramphenicol, which effect on caused by bacteria resistant to antibiotics. It reduces the burden on the patient, accelerates his recovery and eliminates the sudden resistance of the

bone marrow in children. The mixture of antibiotics does not always destroy or inhibit bacterial growth. Synergism and addition in antibiotics combination are the most clinically effective in the treatment of infections, including diarrhea, and

Antagonism and Indeferance are not clinically beneficial to bacterial infections.**Cha** refers to effectiveness and evaluated *in vitro* of different antibiotics alone and combination aginst 291 isolates extended- spectrum beta lactamase(ESBL), among the common antibiotics mixed in order to the reduction of acute bacterial and chronic bacterial infections, antimicrobial mixtures (amoxicillin and cefotaxime) with antiminoglycoside (amikacin) and (ciprofloxacin) had a significant synergistic effect against isolates 1 and 18 resistant to 15 and 13 antibiotics respectively, while trimethprime and cephalaxin showed antagonists effect. The synergistic effect of antimicrobial agents is due to increased antimicrobial effect of the antimicrobial agent used in the mix, leading to the complete eradication of bacteria. Betalactam antibiotics are effective in entering the amikacin to the bacterial cell by changes the permeability of the bacterial cell membrane by its binding to receptors within the cell wall (Pencillin Bending Proteins), and lead to inhibiting the formation of cell wall. The current results are agreement with what is indicated (cha) in the combination of aminoclycoside and betalactem, as well as it combination with qunolone(ciprofloxacin) were an effective treatment for the infection of multidrugresistant gram-negative bacteria such as *E.coli*. Amoxicillin mixture with aminoclycoside or ciprofloxacin is preferred for treatment of children with diarrhea resistant bacteria, **Cha** showed that ؤeftazidime, cefepime, and piperacillin in mixture with aminoglycosides (amikacin) were more activity than found in mix with ciprofloxacin. These mixtures of antibiotics as therapy against *E.coli* contaminated pacifier used by children suffering from diarrhea, especially beta lactam antibiotics with amikacin,ciprofloxacin and aminoglycosides with qunolone, may be considered for empirical treatment towards gram-negative bacteria ifections.

Table 5. Antibiotics Combination Effect on *E. coli* solate No.1

Antibiotics	Amx	Ctx	Tip	Cl	Ak	Cip
Amx	++	(+)	++	+	-	-
Ctx		-	(+)	-+	-	-
Tip			++	+	-	(+)
Cl				++	-	-
AK					-	-
Cip						+

++ :static effect only + :partial cidal effect
(+) :incomplete cidal effect - :fully cidal effect

Table 6Antibiotics Combination Effect on *E.coli* solate No.18

Antibiotics	Amx	Ctx	Tip	Cl	Ak	Cip
Amx	++	(+)	++	++	-	-
Ctx		-	(+)	-	-	-
Tip			++	+	-	-
Cl				++	+	+
AK					-+	-
Cip						-

++ :static effect only + :partial cidal effect
(+) :incomplete cidal effect - :fully cidal effect

References

1- Abubaker,A.;El- Ayis,A,A.; Elgaddal,A.A.and Almofti,Y.A.(2015). Isolation, Identification and Enterotoxin Detection of Escherichia Coli Isolated from Calf Diarrhea and their Virulence Characteristics. J. Appl. Indu. Sci.3 (4): 141-149.

2- Al-Dawmy,F.A.A.and Yousif ,A.A.(2013).Prevalence of E.coli O157:H7 in intestinal and Urinary tract infection in children.J.Inter. Adva. Rese. 1(8) :111-120.

3- Al- Hashemi,E.H.and Abud Al-Shaheed ,M.I.(2010).Isolation and identification of bacteria in dummy sucking children with gastroenteritis(Comparative study).J. Bagh. Colle. Dent.22(4).111-114.

4- Barati,S.;Boniadian,M.;Habibian,R.and Jostejou,T.(2012).Antibiotic resistance of enterotoxigenic and entroaggrigative Escherichia coli isolated from gastroenteritis cases.J. Asian Biomed. and Pharma.Sci.2.(14).54-58.

5- Canadian Paediatric Society. 2006 - Oral rehydration therapy and early refeeding in the management of childhood gastroenteritis. Paediatr. Child. Heal., 11(8):527-531.

6- Clinical and Laboratory Standars Institute(CLSI).(2010).Performance standars for antimicrobial susceptibility testing; 20 ed. approved standars, M 100-S20 and M100-S19, U.S.A.

7- Garrod ,L. P. ;Reeves, D. S. ; Phillips, I.; Williams, J. D. and Wise , R. (1978). Laboratory method in antimicrobial chemotherapy. Livingstone, New York.:31-49

8- Ifeanyi,C.L.C. ; Ikeneche,N.F.; Bassey,E.B.; Al-Gallas,N.; Aissa, R.B. an Boudabous,A.(20015). Diarrheagenic Escherichia coli pathotypes isolated from children with diarrhea in the Federal Capital Territory Nigeria.J. Abuja, Infect. Dev.Ctries. 9(2):165-174.

9- Hungin, A.,Jones, R.,Kumar,D.,Rubin G.,Trudgill,N.and Whorwll,P.J. (2005). "Irritable Bowel Syndrome in the United States: Prevalence, Symptom Patterns and Impact," Alimentary Pharmacology and Therapeutics 21(11):1365-1375.

10- Wong, C. S. (2012). "Risk Factors for the Hemolyic Uremic Syndrome in Children Infected with Escherichia coli O157:H7: a Multivariable Analysis"Clin Infect Dis first published online March 19, doi:10.1093/cid/cis299.

11- Willie,K.,Valerie,O.and David, S.(2012). Prevalence and Antibiotic resistance of bacterial pathogens isolated from childhood diarrhoea I four province of Kenya. Center for Medical Research institute, 54840-00200, Nairobi Kenya.J.infect Deo Ctries 6 (7): 572-578.

12- Truing,V.N.;Phuong,V.L.;Chinch, H.L.and Andrej,W.(2004). Antibiotic resistance in diarrhoeagenic escherichia coli and shigella strains isolated from children in Hanoi,Vietnam. , Swe.Inter.J.Pharma.Sci. 2319 – 6718.

13- Shakya ,P.Barrett ,P.Diwan,V.Marothi,Y.(2013) .Antibiotic resistance among Escherichia coli isolates from stool samples of children aged 3 to14 years from Ujjain,India.J.infect.dis. 13(477):2-6.

14- Bartoloni, A.; Pallecchi ,L. Benedetti, M. ;Fernandez ,C. ;Vallejos, Y. ;Guzman, E. Villagran, A.L.; Mantella ,A.; Lucchetti ,C.; Bartalesi, F.;(2006). Multidrug-resistant commensal Escherichia coli in children. Peru and Bolivia.J. Emerg. Infect .Dis.12(6): 907–913.

15- Sahuquillo-Arce, J.M.; Selva, M.; Perpinan, H.; Gobernado, M.; Armero, C.; Lopez-Quilez, A.;Gonzalez, F.and Vanaclocha ,H.(2011). Antimicrobial resistance in more than 100,000 Escherichia coli isolates according to culture site and patient age, gender,and location.J. Antimicrob. Age. Chemother. 55(3):1222–1228.

16- Seidman, J.C.; Anitha ,K.P.; Kanungo, R.; Bourgeois ,A.L.and Coles, C.L.(2009). Risk factors for antibiotic-resistant E. coli in children in a rural area. J.Epidemiol. Infect. 137(6):879–888.

17- Cha ,M.K. ; Kim, S.H.; Young Cho,S. .(2015). In Vitro Activities of 21 Antimicrobial Agents Alone and inCombination with Aminoglycosides or Fluoroquinolones against Extended-Spectrum-_-Lactamase-Producing Escherichia coli Isolates Causing BacteremiaJ. Antimicrob. Age. and Chemothe.9(59):5834-5