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Assessment of Children under than Five Ages toward the Diarrheal Cases with Antibacterial Effect of bacteria isolates in Babylon Province, Iraq

Amean A.Yasir

Community Heath Nursing Department, College of Nursing, Babylon University, Babylon Province, Iraq

Abstract : Background: Diarrheal disease is an infection caused by the presence and growth of microorganism in the intestine. **Objectives:** This study aims at determining the common organism that causes diarrhea in children less than 5 years and detection the most effective antimicrobial agents and plant extract that causative agents of diarrhea and select the best antibiotics to treat it. **Methods:** One hundred stool sample was collected from children have watery diarrhea in Al-Qassim hospital; during the period (January to April 2015).The microbial isolate was identified tested for antibacterial agents. **The results:** Sixty case From 100 samples have a bacterial infection (60%) represented by ***E. coli*** (75%) and ***Salmonella*** spp.(25%)).
Regarding Antibiotic sensitivity test, ***E. coli*** and ***Salmonella*** spp**.** showed highly sensitive to Imipenem (88.8%), (100%) respectively, followed by Amikacin (100%) for ***E. coli*** and Ciprofloxacin (100%) for ***Salmonella* spp.**, while Rifampin were not effective against tested isolates, whereas the plants extract result revealed that lemon extract more effect on diarrheal bacteria than other one.
Conclusions: in this study I conclude that rate of bacterial diarrheal infection among children younger more than 5 years was high. ***E. coli*** was predominant isolate and impeneim was the most effective antibiotics on bacterial isolates. Among plants extract lemon were more effect on diarrheal bacteria
Keywords : Antibacterial effect, Diarrheal Children antibiotics and plant extracts.

Introduction:

The Diarrhea define according the Organization of World Health (WHO) as having three or more loose or stools liquid per day, which can be caused by a variety of parasitic, viral, bacterial and organisms. Infection is spread through contaminated water and food, and from patient to patient (fecal oral rout) as a result of poor hygiene.

Diarrhea remains one of the leading causes of mortality and morbidity among children, which causing more than 1 billion episodes of illness and 3-4 million deaths annually.

The major causes of diarrheal illness include, among others, limited access to or poor quality of poor food, and water hygiene and sanitation[1]. The mechanisms of transmission of diarrheal pathogen are person to person through the fecal oral route or by ingestion of contaminated water or food [2].The bacterial pathogens usually responsible for diarrheal illness include *Shigella, Escherichia coli, Aeromonas, Campylobacter Salmonella,* and *Yersinia,* [1].

There are many risk factors that increase susceptibility to infection with enteric bacteria including; young age, immune deficiency, lack of breast feeding, malnutrition and others[29].Gastrointestinal tract of breastfed infant is colonized more easily with micro flora than are those of formula fed infants. Colonization is through to reduce infant diarrhea by inhibition of the growth of pathogenic organism[3],[35].

The reason for the extensive use of plants as drugs may be the fact that medicinal plants are available, wide range of medicinal plants and their distinct form and thus are procured without any trouble. Plants extracts have a long history of use and their use is widespread in both developing and underdeveloped countries [4]. The medicinal plants are very important to eradication of disease due to multidrug resistance bacteria and the plant extract without side effect to human[36, 37].

This study aims at determining the common organism that causes diarrhea in children less than five years and detection the most effective antimicrobial agents and plant extract against the causative agents of diarrhea and select the best antibiotics to treat it and to identify the care level of diagnosis of bacteria.

Materials and methods:

Patients and Sample collection: A total of 100 specimens of stool were collected from children younger than five years suffering from diarrhea (watery diarrhea) in Al-Qassim hospitals in Babylon province, during a period of three months (January to April 2015). Sample ofFecal was collected from each patient. The sample plated onto MacConkey agar and incubated anaerobically and aerobically at 37 °C overnight.

Plants collection*:*

Fresh fruits (Pomegranate, banana and lemon) were obtained from the Hilla market, Iraq, 2015. It washed in running distill water in laboratory, sterilized the surface with 70% alcohol.

Extracts of Pomegranate peels were prepared as 50% extract, hot water was primed, peel were added clean container and left to cool, the container was covered in order to keep all active elements. The mixtures were vigorously swirled by the blender. The mixtures were filtered using filter paper into a clean beaker.

After **Bananapeels**, sterilized, then peel was taken. Distilled water was boiled, peels were added to the distilled water and left to cool.

These contents were mixed by the blender and filtered to remove the large, UN homogenized particles to get clear aqueous extract,while **lemon** were cut open with a knife (sterile)and the juice pressed out into a sterile container and then filtered by filter paper (0.45Millipore) into another container (sterile)to remove the tissues plants and used as crude (freshly) without refrigeration. The Extract was stored at four °C until use.

Citrus lemon(dried fruits) extracts were according to [5,22.]

Spice Collection and Extraction:

The plant material (Curcuma, cinnamon and Sumac extract) were brought from markets of Babylon Province. Ten grampowders of dried plant were boiled in 500 mL of distilled water[6,34]

Methods:

After positive results of growth were appear, the bacteria recapture on EMB(eosin methylen blue agar) and SS(*Salmonella shigella* agar) then identified with Gram stain and Biochemical test[7].

Antimicrobial susceptibility testing:

Susceptibility to antibacterial agents for all isolates were determined by the diffusion of standard disk method on Muller-Hinton agar incubated for twenty hours at 37 ºC. The selection of discs of antibiotic was performed according to the guidelines recommended by [1]. The following disks of antibiotic were used as table 1. After twenty hours, the diameter of each zone of inhibition was measured with a pair of calipers& recorded in mm. The results then interpreted according to CLSI documentation [8].

In vitro antibacterial activities of plants extract testing using Agar diffusion well assay.

The screening of antimicrobial activities of each extract on the tested bacteria used in this investigation was determined on Muller Hinton agar media, by the agar diffusion techniques using agar well diffusion method. Six mm diameter of wells and 5 mm depth were made on the solid agar using a sterile glass borer [9,10,23,33]. Approximately 20µl of each extract was inoculated onto wells were made in the pouring culture of each microorganism isolates. The plate was then allowed to incubate at 37°C for 18 hrs. After 18 hrs of incubation, each extract was zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by millimeter (mm).

Table 1:-Inhibition Zone of Different Antibiotics According To CLSI

Antibiotic disc	*Symbol*	*Potency*	*Zone diameter nearest whole mm*		
			Sensitive	*Medium*	*Resistant*
Azithromycin	AZM	15 µg	>18	14-17	<13
Amikacin	AK	30µg	>17	15-16	<14
Cefotaxime	CTX	30µg	>23	15-22	<14
Chloramphenicol	C	30 µg	>18	13-17	<12
Gentamicin	CN	10µg	>15	13-14	<12
Cefoxitin	FOX	30µg	>18	15-17	<14
Rifampin	RA	5 µg	>20	17-19	<16
Imipenem	IPM	10 µg	>16	14-15	<13
Ciprofloxacin	CIP	5 µg	>21	16-20	<15

Results and discussion:

Rate of infection:

The rates of bacterial infection among diarrheal children were (60%) the rest (40%) maybe due to parasite or viral infection.

Bacterial isolates:

This study show that ***E. coli*** was predominant isolate in 75%, while ***Salmonella*** spp. in 25% as shown in fig (1). ***E. coli*** is the most commonly recognized bacterial pathogen in infantile gastroenteritis [11].

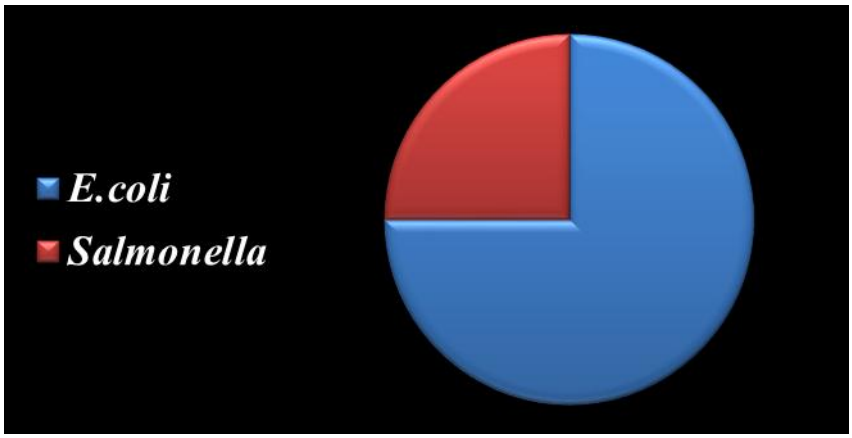

Fig (1): Rate of bacterial isolates among diarrheal children

Age group:

The high infection of diarrhea was recorded in age groups from 1 month to 1 year (table 2).

Theyare most groups exposed to this disease. The reason of higher infection in infants less than 6 months may be due to low immunity, as the amount of trance placental antibodies of the child starts dwindling after 6 months of age.

Those infants also may not have been breast-fed but bottle- fed instead, which is a source of infection and contamination [12].

Table (2): Number of diarrheal infections according to children's age

Age(years)	No. of infections	%
1month to1 year	40	40
1-2	10	10
2-3	20	20
3-4	25	25
4-5	5	5
Total	100	100

Antibiotics sensitivity:

In this study, Antibiotics sensitivity was studied. ***Salmonella*** spp**.** and ***E. coli*** showed highly sensitive to Imipenem(100%) and (88.8%) respectively. This results in agreement with [13], and [14]and [15] in Iraq, this study demonstrated that all (100%) ***E. coli*** isolates were susceptible to Imipenem (Fig: 2). Imipenem showed promising results to treat enteric fever [16].The high efficiency of these drugs may be the usage rarely in studied area and high cost and non-availability in oral forms [12] **and** [16].

Moreover, the result of sensitivity of Amikacin and Ciprofloxacin were studies. The bacterial isolates were highly sensitive to Amikacin and and Ciprofloxacin (100%) for ***E. coli*** and (100%) for Salmonella spp**.** (fig 2). Amikacin show more effect on ***E. coli***in 100% Sensitive rate that result similar to Baghdad study **(CLSI, formerly NCCLS, 2010)** and Ciprofloxacin is the first-line drug for the treatment of enteric fever [17]while the Rifampin were not effective against tested isolates. The reason of high resistance of antibiotics in area of study might be to the overuse of drug in the treatment of diarrhea could lead to an increase of antibiotic resistance[12]**.**

Antibacterial activity of plant extracts on diarrheal bacteria:

On the other hand, the present study investigated of the **antibacterial activity** of some plant products {**Curcuma**,**cinnamon, pomegranate peel, Limon juice**,**lemon (dry fruit)**and **banana peel}** against diarrheal bacterial isolates as result shown as **fig:3**.

The results indicate that the **Curcuma** aqueous extract have **antibacterial activity** against ***Escherichia coli*** and ***Salmonella*** [18] **and** [6].

Also **cinnamon** reveal antibacterial effect against bacterial isolates more than other spice on bacterial isolates. Several studies [19] **and** [31] have shown that cinnamon oil had good inhibitory effects against various pathogens as we found. The antibacterial activity has been due to the presence of active constituents in the oils. The study revealed cinnamaldehyde to be the major constituent of cinnamon oil [30].

Furthermore, the Sumac extract has less effect against bacterial isolates and its action of inhibiting gram negative bacteria related to its contain active component like alkaline and alcoholic toxic materials which inhibit and kill microorganism by destroy plasmic membrane, act on nucleic acid and protein denaturation[20].This result agree with [21] in effect on inhibition growth of ***E.coli***.

Moreover, the antimicrobial activities of juice Limon and lemon (dry fruit) on bacterial isolates were studied. **Limon juice** reveals high antibacterial activity of this extract against bacterial isolates; 30 and 35mm forE.coli and salmonella respectively. That result similar to [22]which found high antibacterial activity on gram

negative bacteria. Antibacterial activities of lemon (dry fruit) on bacterial growth were 22 and 28 mm for***E.coli*** and ***Salmonella*** respectively. This could be due to the acidic pH of this juice that will affect the charges of the amino acids that constitute the peptidoglycan, and it may affect the active sites of enzymes leading to defect in their activity [23].

Moreover, the result show that the **pomegranate peel extract** have active action on bacterial growth were 30 mm to both studied bacteria. This result agree with [24] who found that antibacterial effect on ***E.coli*** at 20mm and disagree with [22] which not found any effect of pomegranate peel on bacterial growth.

The consumption of **banana** was high contributed to nutritional value; its shell has been studied for treatment of gastrointestinal disorders [25]additionally, [26] who found banana peel gel inhibited the growth of enterobactericea. These studies support this result when found banana juice were effect on diarrheal bacterial growth at (18, 16 mm) to ***E.coli*** and ***Salmonella*** respectively.

The prevalence of resistance antibiotic is a continual problem due to the evolution of a potent defense mechanism against antibiotics.

Therefore, it is necessary to exploit and develop a novel inhibitory agent against those bacteria [27]. Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to plant extract various natural active products for screening antimicrobial activity [28].

The medicinal plants are very important to eradication of disease due to multidrug resistance bacteria and the plant extract without side effect to human[36, 37].

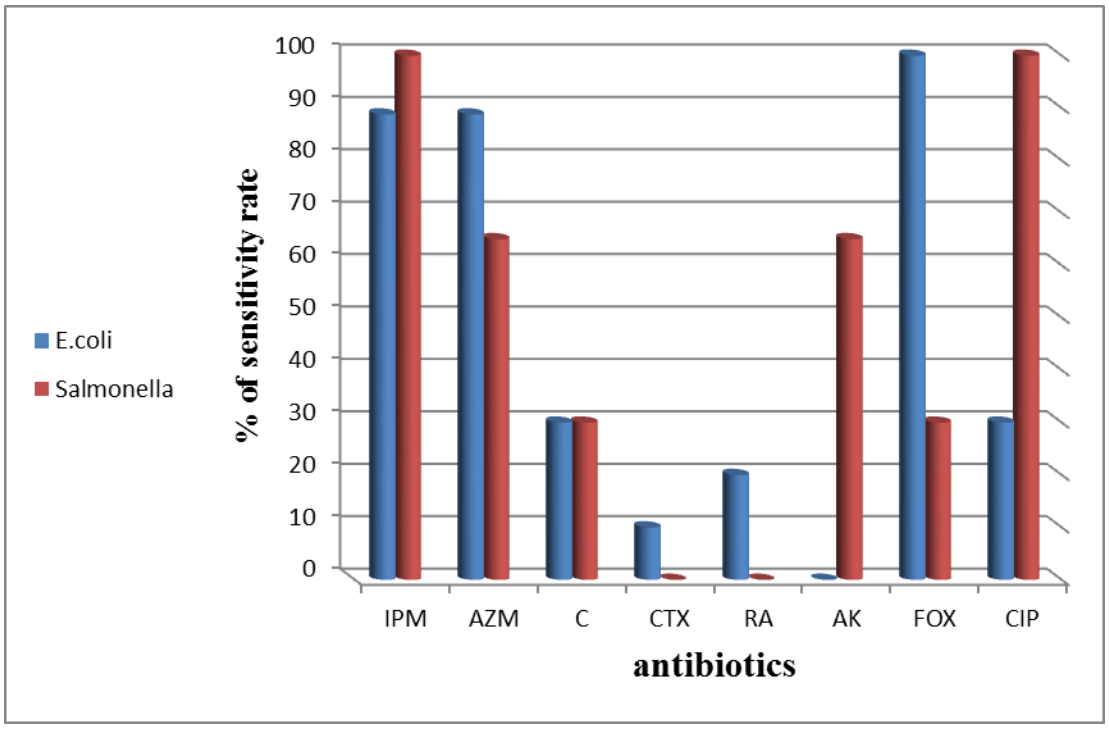

Fig 2: Sensitivity Rat of *E. coli* and *Salmonella* to different Antibiotics

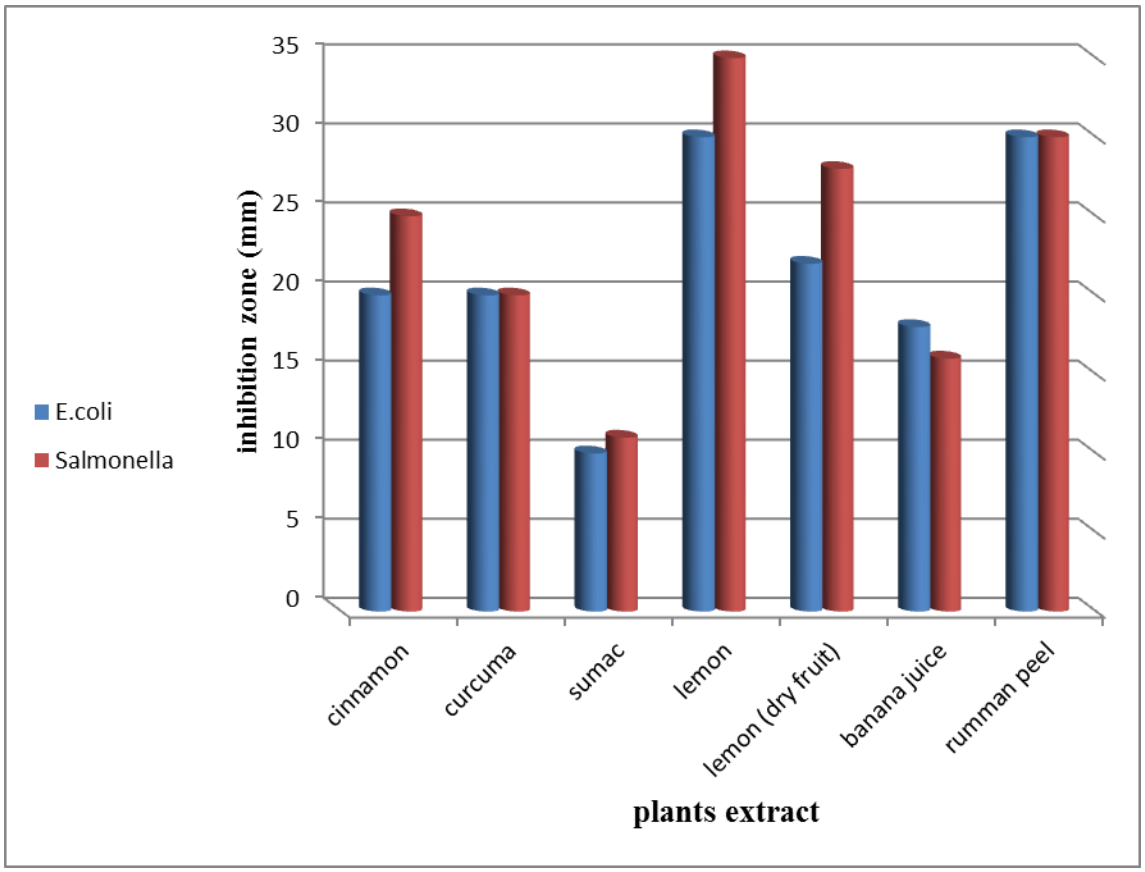

Fig 3: Sensitivity Rat of *E. coli* and *Salmonella*t o different plants extract

Conclusion:

In this study we conclude that rate of bacterial diarrheal infection among children younger than 5 years was high. ***E. coli*** was predominant isolate and impeneim was the most effective antibiotics on bacterial isolates. Among plants extract lemon were more effect on diarrheal bacteria.

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