Studying the causative agents of diarrhea among children under five years in Wasit Province

Adnan Kamel Shebeeb Technical Institute /Kut

دراسة مسببات الإسهال عند الأطفال تحت الخامسة من العمر في محافظة واسط

عدنان كامل شبيب المعهد التقني / كوت

المستخلص

الإصابات البكتيرية هي احدى الأسباب الشائعة لإسهال الأطفال التي تسبب الامر اضية و الوفاة في العالم. تم في هذه الدر اسة التعرف على البكتريا والطفيليات الشائعة عند الأطفال الذين بعانون من الإسهال .

جمعت 170 عينة براز من أطفال يعانون من الإسهال تحت سن خمس سنوات ، حيث جمعت العينات من مستشفى الكرامة التعليمي في مدينة الكوت، و مستشفى الزهراء التعليمي في مدينة الكوت ، ومستشفى الشهيد فيروز العام في مدينة الحي، ومستشفى العزيزية العام في محافظة واسط حيث جمعت العينات خلال فترة الأشهر (تشرين الأول، تشرين الثاني، وكانون الأول) من العام 2014 ، بعدها فحصت و زرعت العينات وعزلت الجراثيم بواسطة الطرق التقليدية ، وحساسيتها للمضادات الحيوية تم التعرف عليها بواسطة قرص الانتشار

أظهرت النتائج أن البكتيريا عزلت من 91 عينة بنسبة (53,529 %) من المجموع الكلي للعينات والبقية كانت إصابات طفيلية . كانت النسبة المئوية نتيجة الإصابة ببكتريا الاشيريشيا القولونية 91 عينة هي (53,529 %) ، أما النسبة المئوية نتيجة الإصابة بالمتحولة الحالة للنسيج 63 عينة هي 37,058 %، و الإصابة بالجيارديا اللمبلية 11 عينة هي 6,470 %، و الإصابة بفطريات المونيليا 3 عينة هي 1,764 % و الإصابة بالمحرشفة القرمة 2 عينة هي 1,176 %.

البكتيريا الأكثر شيوعا المسببة للاسهال في الأطفال كانت الاشيريشيا القولونية ، بينما الطفيلي الأكثر شيوعا المسبب للاسهال كان الجيارديا اللمبلية ، وتليها المتحولة الحالة للنسيج، وفطريات المونيليا ، والمحرشفة القزمة. وكذلك أظهرت النتائج ان المضاد الحيوي سيفتزوكسيم هو مضاد حيوي جيد الأكثر فعالية ضد الاشيريشيا القولونية ، بينما المضاد الحيوي الأقل فعالية هو الارثرومايسن .

Abstract

Bacterial infections are one of common cause of children diarrhea which causing mortality and morbidity worldwide. In this study One hundred – seventy stool samples were collected from children under five years old suffering from diarrhea. these samples were collected from AL-Karama Teaching Hospital, AL-Zahra'a Teaching Hospital in the Kut city, Al-Shahid Firoz General Hospital in Al-Hay City, and Al-Azizia General Hospital in Wasit Province, during period months from October, November, and December from 2014. Then these samples examined and cultured, and isolates were identified by conventional methods, and test their sensitivity for antibiotics by disc diffusion method.

The results showed that the bacteria are isolates from 91 samples from the total number of samples, while remaining are parasitic infections. The percentage of infections caused by *E. coli* (91 samples) is 53.529 %, While the percentage caused by *Entamoeba histolytica* (63 samples) is 37.058 %, and infection by *Giardia lamblia* (11 samples) is 6.47 %, and infection by Monillia fungi (3 samples) is 1.764 %, and infection by *Hymenolepis nana* (2 samples) is 1.176 %.

The most common bacterial caused diarrhea in children is *E. coli*, and then Klebsiella, while the common parasite caused diarrhea is *E. histolytica*, and then *Giardia lamblia*, Monillia fungi, and *H. nana*.

Also the results showed that Ceftizoxime is good antibiotic high effect against *E. coli*, while Erythromycin is less effect antibiotic against it.

Introduction

Acute diarrhea can be defined as the new onset of passage of three or more unformed stools in a 24-hour time period as passage of an increase number of stools of decreased form compared with the normal state. In any case the duration is less than 14 days. Acute diarrhea is frequently associated with one or more enteric symptoms like nausea, vomiting, increase in abdominal gas, abdominal pain or cramps, tenesmus (intense urge with straining but minimal or no bowel movement), fecal urgency or passage of stools containing gross blood and mucus (1).

Diarrhea is an important disease, and its complications worldwide cause of morbidity and mortality in children, especially in developing countries (2). The world Health Organization estimates that over 2.2 million deaths due to diarrheal infections, especially among children under five years of age (3), and without aggressive efforts to control diarrheal disease, Millennium Development Goal 4 which is to reduce childhood mortality by 2015 will remain out of reach. More than half of these cases are in Africa and South Asia where bouts of diarrhea are more likely to result in death or other severe outcomes (4).

Acute diarrhea as a gastrointestinal related symptom may have some different causes such as infection. diarrhea Infectious leads to approximately three million deaths worldwide and 516 deaths in Iranian children younger than 5 years per year (5, 6). The rate of entropathogen isolation in acute diarrhea varied in different studies depending on the sampling methods and microbiological techniques. Some of them, the most bacterial pathogen common is diarrheagenic E. coli (5, 7, 8).

When diarrhea lasts more than 14 days, it is considered to be persistent. The etiologic agents are likely to be different in these cases. The most important group of pathogens is the intestinal protozoa, including *Giardia lambelia*,

Cryptosporidium and E.histolytica

other well-known parasitic causes of persistent diarrhea are *Cyclospora, Isospora* and *Microsp oridium.* The bacterial enteropathogens can be implicated in a subset of persistently ill patients (9).

Infants attending day care centers may be exposed to enteropathogens secondary to environmental contamination when a day care center child develops diarrhea The most common causes of diarrhea outbreaks in day care centers are the lowinoculum pathogens including Shigella, Giardia,

Cryptosporidium and Rotavirus.

Immunity develops in high-risk day care centers by repeated exposure to prevalent enteric pathogens (10).

Travelers' diarrhea is defined as acute diarrhea acquired by persons during international trips, usually occurring in someone from an industrialized regions during visits to develop tropical and semitropical countries. Travelers' diarrhea is frequently caused by a bacterial pathogen. Poor sanitation of the host country is an important factor associated with enteric disease. The most important vehicle for transmission is food with water and ice being less important (11).

Food is an important vehicle for enteropathogens in all regions of the world. It is estimated that 76 million persons suffer from foodborne diseases each year in the United States .The illness leads to 325,000 hospitalizations and 5000 deaths per year, while enterotoxigenic E. coli (ETEC) is the important etiologic most agent in travelers' diarrhea, the organism occasionally causes foodborne outbreaks in the United States. Enterotoxigenic E. coli has caused extensive outbreaks of diarrhea secondary to contamination of recreational lakes or water parks where Cryptosporidiumis also implicated in outbreak disease. Surveillance data from the CDC demonstrated that 50% of waterborne gastroenteritis outbreaks related to treated water were due to Cryptosporidium and 25% of waterborne outbreaks from freshwater were due to ETEC and 25% were due to noroviruses (12).

The aim of study

The aim of this study is to detect causative agents of diarrhea among

children under five years in Wasit Province, and detect antibiogram of it.

Collection of samples

One hundred- seventy stool samples were collected from out patients (children) suffering diarrhea from AL-Karama Teaching Hospital, AL-Zahra'a Teaching Hospital in the Kut city, Al-Shahid Firoz General Hospital in Al-Hay City, and Al-Azizia General Hospital in Wassit Province.

Methods

All samples study was done on children with diarrhea in laboratories of AL-Karama Teaching Hospital, AL-Zahra'a Teaching Hospital in the Kut city, Al-Shahid Firoz General Hospital in Al-Hay City, and Al-Azizia General Hospital in Wasit Provence, and some information such as age, sex, and symptoms from each patient. Fecal specimen was collected in a sterile container, and also a rectal swab. All samples were collected tested for detection of bacterial, parasitic, and fungal agents.

Identification of bacteria

The stool samples was given a general stool examination with direct plating on blood agar, EMB and Mac Conkey agar, and inoculation into enrichment media

(peptone and tetrathionate) Identification of bacterial isolates were based on biochemical testes, using of the analytical profile index (Api 20 E) system(13) (Figure 1)



Figure (1): Analytical profile index system.

Also use Nutrient agar for sensitivity test to antibiotics discs which used (14). All samples were cultured and incubated at 37°C for 18 – 24 hours and incubated at 37 °C for 24 hr. Next day individual colonies were selected and identified on the bases of morphological, cultural and biochemical characteristics (15).

Determination antibiotic of resistance profile

screening by disc diffusion method. Amoxicillin, and Erythromycin. For this purpose bacterial isolates

from stool samples isolates was made on Muller-Hinton agar with help of commercially wire-loop then available antibiotic discs were placed on lawn of culture and plates were incubated at 37 ° C for 24 hr. Next day presence or absence of zone of inhibition around the antibiotic discs observed (16). and detect was sensitive or resistance of bacteria to antibiotics according to CLSI 2012 (17).

Bacterial isolates from stool samples Figure (2) Antibiotics were used Ampicillin, were subjected to antibiotic resistance Ciprofloxacin, Gentamycin, Ceftizoxime,



Figure (2): Antibiotics sensitivity test.

Results

The purpose of this research is to detect the causative agents of diarrhea in children, and the susceptibility or resistance profile of multi-drug resistant isolates from stool samples. One hundredseventy isolates from different pathological laboratories of Wasit Province were isolated and identified by routine methods. Identification of the causative organism and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages of diarrhea (18). Percentage and sex of children with diarrhea diagnosis is depicted in (Table 1).

T 11 (1)	D 4		6 1 9 1	• 41	1. 1	1
Table (1):	Percentage	and Gender	of children	with	diarrhea	diagnosis
= = = = = = = = = = = = = = = = = = = =						

Total No. Of Samples	Male		Female		
	No. of Diarrhea Diagnosis	Percentage	No. of Diarrhea Diagnosis	Percentage	
170	102	60 %	68	40 %	

The percentage of bacterial isolates was *E. coli* (53.529 %) from total samples. The percentage of parasitic infections includes , *E. histolytica* (37.058 %), followed by *Giardia* with (6.47 %), *Monillia* (1.764 %), and *H.nana* (1.176 %). Table 2.

Table (2):	percentage of	causative agents of	diarrhea o	of total s	amples
	percentage or	causante agento or	ului i iica (n total be	linpics

Causative Agents Of Diarrhea	Number Of bacterial isolates Or parasite	Individual % Of Bacteria and Parasites
E. coli	91	53.529
E. histolytica	63	37.058
Giardia	11	6.47
H. nana	3	1.764
Monillia	2	1.176
Total	170	100 %

All isolates of bacteria were screened for antibiotics sensitivity profile by disc-Diffusion method with commercially available disc of Ampicillin,Ciprofloxacin,Gentamycin,Ceftizoxime,Amoxicillin, and Erythromycin.

Table 3 indicates the resistance level against commonly used antibiotics in bacterial stool isolates. Nearly, all the isolates were found to be susceptible against most of the antibiotics, whereas isolates show more resistance to Erythromycin as compare to other antibiotics used (19).

Table (3): Total percentage efficacy of different	t antibiotics among bacterial stool
isolates (Total No. of E. coli isolates	91).

Antibiotic	Disc code	No. o sensitive isolates from total <i>E. coli</i>	No. of Resistant isolates from total <i>E. coli</i>	% Efficacy
Ampicillin	Am	2	3	2.197
Ciprofloxacin	Сір	27	3	29.670
Gentamycin	G	4	6	4.395
Ceftizoxime	CF	42	1	46.153
Amoxicillin	РВ	1	2	1.098
Erythromycin	Е	0	91	0
Total No. of E. coli	solates (91)			

Discussion

In this study, we found bacteria more than half of stool samples of children with diarrhea (60 %). Our findings are in agreement with the results of other studies from the developing countries

(5,8). In this study, the frequency of bacterial diarrhea in children was significantly higher than the others in agreement with the results of other studies (7, 19). This may be due to the kind of nutrition and high rate of breast milk feeding in infancy in our region.

E. coli was the most common cause bacterial diarrhea similar to many other previous studies (7, 20).

So we found parasitic infections (40%) from total stool samples of children with diarrhea. our findings are in agreement with the results of other studies as most important causative agent for diarrhea (21). In this study, the frequent of parasitic diarrhea in children was *E*. *histolytica*, then *Giardia lambrlia*, Monillia, and *H. nana* in agreement with AL-Najar, 1999 in Baghdad (22).

Ceftizoxime and Ciprofloxacin is more frequent in our study. It has been showed that there is a direct relation between the antibiotic used and the frequency and the kinds of antibiotic-resistant strains in human beings (23). The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements / plasmids (24).

In our studies, the most effective antibiotic for bacterial isolates from children with diarrhea is Ceftizoxime showing 46.153 %, then Ciprofloxacin which show 29.670 % efficacy, while Gentamycin 4.395 %, Ampicillin 2.197 %, then Amoxicillin 1.098 % efficacy, while Erythromycin didn't appear any effects on bacteria (Table 3), due to the Ceftizoxime and Ciprofloxacin are recent antibiotics and the good uses for them, so less frequent use take susceptibility to these antibiotics (25) while Erythromycin is used since far time and high frequent use it in bad using depends on the amount of dosage and time of dosage lead to induced mutation in this bacteria (Its generation gap is 15 minutes) take resistant for this bacteria and difficulty to treat because of their high frequency of drug reistance (26), and due to antibiotic resistance and limited antimicrobial activity of antibiotics some strains of disease causing bacterial diarrhea (27, 28).

Conclusion

Hence present study shows that the majority causative agents of diarrhea in children less than five years two types:

1-bacterial agents' especially E. coli.

2-parasitic agents especially *E. histolytica*, *Giardia lambelia*, *H. nana*, and *Monillia*.

3-Also showed their different susceptibility for the antibiotics usually used for the treatment of bacterial diarrhea.

References

1. Dupont HL.(1997).Guidelines on acute infectious diarrhea in adults.

ThePracticeParametersCommitteeoftheAmericanCollege of Gastroenterology. Am JGastroenterol; 92(11):1962-1975.

- **2.** Cooke ML. (2010). Causes and management of diarrhea in children in a clinical setting. South Afr J Clin Nutr, 23(1).
- 3. World Health Organization (WHO), (2012). The World Health Organization's infant feeding recommendation:Global Strategy on Infant and Young Child Feeding. Genevea/New York: WHO.
- 4. World Health Organization (WHO). (2004).United Nations Children Fund (UNICEF), 2004. Joint statement: clinical management of acute diarrhea. Geneva/New York: WHO/UNICEF.
- 5. Al jarousha AM, El Jarou MA, El Qouqa IA.(2011).Bacterial enteropathogens and risk factors associated with children diarrhea.Indian J Pediatr.; 78:65-70.
- 6. Kolahi AA,Nabavi M, Sohrabi MR.(2008).Epidemiology of acute diarrheal diseases among children under 5 years of age in Tehran, Iran. Iran J Chin Infect Diseases;3: 193-8.
- 7. Sabrina J Moyo, Njolstad Gro, Mecky I Matee, Jesse Kitundu, Helge Myrmel, Haima Mylvaganam, Samuel Y Maselle. (2011). Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania.... BMC Pediatrics; 11.
- 8. Kansakar P, Baral P, Malla S, Ghimire GR.(2011). Antimicrobial susceptibilities of enteric bacterial pathogens isolated in Kathmandu, Nepal, during

2002-2004. J Infect Devctries;5:163-8.

- 9. DuPont HL, Capsuto EG. (1996). Presistent diarrhea in travelers. Chil Infect Dis;22(1):124-128.
- 10. DuPont HL, Ericsson CD. (1993). Prevention and treatment of traveler's diarrhea. N Engl J Med;328(25):1821-1827.
- 11. Ekanem EE, DuPont HL, Pickering LK, Selwyn BJ, HawkinsCM.(1983).Transmission dynamics of enteric bacteria in day-care centers. Am J Epidemiol;118(4):562-572.
- 12. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM. (1999) .Tauxe RV. Food-related illness and death in the United States. Emerg Infect Dis;5(5):607-625.
- **13. Horan TC, Andrus M, Dudeck MA.CDC/NHSN.(2008).**Surveilla nce definition of health careassociated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 36:309–32.
- 14. Forbes BA, Sahm DF, Weissfeld AS, Bailey WR. Bailey & Scott's.(2007). diagnostic microbiology. 12 th ed.
- **15. Wikler MA, Cockerill FR, Crraig WA.(2007).**National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; 17th informational supplement M100-S17.
- **16. Cheesbrough, M. (2000).** Bacterial pathogens. In: District Laboratory Practice in Tropical Countries Vol. II. ELBS London, p.p.: 157-234.
- 17. Clinical an Laboratory Standards Institute (CLSI). (2012). Performance standards for antimicrobial susceptibility testing, 32(3).

- **18. Shafran, S.D.(1990).** The basis of antibiotic resistance in bacteria. J. Tolaryngol., 19: 158-168.
- **19. Nimri LF, Elnasser Z, Batchoun R.(2004).** Polymicrobial infections in children with diarrhoea in a rural area of Jordan. FEMS Immunal Med. Microbial; 42:255–9.
- 20. Nair GB, Ramamurthy T, Bhattacharya MK.(2010). Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. Gut Pathog;2.
- 21. Garcia PG, Silva VL, Diniz CG.(2011). Occurrence and antimicrobial drug susceptibility patterns of commensal and diarrheagenic Escherichia coli in fecal microbiota from children with and without acute diarrhea. J Microbial; 49:46–52.
- 22. AL-Najar, S. A., Mukhlis, F. A and Odisho, S. M. (1999). Intestinal parasites and rota virus in diarrhea J. fac.med. Baghdad 12(3):210.214.
- **23. Neu, H.C. (1994)**. Emerging trends in antimicrobial resistance in surgical infections. 573: 7-18.

24. Kupersztoch,

P.Y.M.(1981). Antibiotic resistance of gram negative bacteria in Mexico: Relationship to Drug Consumption. In: Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids (Levy, S.B., R.C. Clowes and E.L. Koenig, Eds.).Plenum Press, New York, p.p.: 529-537.

25. Karlowsky, J.A., M.E. Jones and C. Thornsberry, (2003). Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United State from 1998 to 2001. Antimicrob. Agents. Chemother., 47: 1672-1680 26. Gales, A.C., R.N. Jones, K.A. Gordon, H.S. Sader, W.W. Wilke, M. Beach, L. M.A. Pfaller and G.V. Doern, (1998). Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: Report from the second year of the sentry Antimicrobial (1998). J. Antimicrob. Chemother. 45: 295-303.

- **27. Tomasz, A,(1994).** Multipleantibiotic-resistant pathogenic bacteria-a report on the Rockefeller university workshop. The New England J. Med. 330: 1247-1251.
- 28. Hancock, L.E. and M.S. Gilmore, (2000). Pathogencity of Enterococci. Published In: Gram Positive Pathogens. A.S.M. Publications, pp: 251-258.