INCIDENCE OF INTESTINAL PATHOGENS AMONG HEALTHY FOODSTUFF WORKERS IN BABYLON PROVINCE[+]

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Abstract :

Out of the total seven hundred and seventy six (776) food handlers were examined by direct examination and of intestinal bacterial pathogens were isolated by stool culture the results show the total intestinal pathogens were 238 (30.66%) ,150(19.32%) of them were bacterial pathogens while the remaining 88(11.34 %) were intestinal parasites.

The main genus among bacterial pathogens was Salmonella 64(42. 6%) while Giardia lamblia was dominant among intestinal parasites and recoded 44 (5.86 %) food handlers infected with this parasites . The results of this study show the total mixed infection in food handlers was 3.47 % ,0.25 % of them showed double parasites , 1.28 % of them had more than one type of bacteria and 1.93 % had a bacterial and parasitic infection . The high significant difference was reported in the percentage of infection according to the prevalence in August . Bacterial isolations from food handlers were tested for their sensitivity to antibiotics and the results showed a multi resistance to more than three antibiotics in bacterial strain with various percentages.

المستخلص :

تم فحص ٧٧٦ نموذج خروج لعمال أصحاء للتحري عن الطفيليات المعوية و البكتريا المرضية أظهرت النتائج أن العدد الكلي للممرضات المعوية ٢٣٨ (٣٠,٦٦%) منها ١٥٠ (١٩,٣٢ %) ممرضات بكتيرية أما الباقي ٨٨ (١١,٣٤%) يعود إلى الممرضات الطفيلية.

تبين أن جنس *Salmonella* كان الأكثر انتشاراً ضمن الممرضات البكتيرية إذ تم عزل ٦٤ عزلة مثلت ٤٢,٦ % من الممرضات البكتيرية وإنّ الجيارديا اللمبيليا كانت الأكثر انتشاراً ضمن الممرضات الطفيلية إذ كان ٤٤عاملاً مصاباً بهذا الطفيلي بنسبة ٥,٨٦% و إن نسبة الإصابات المشتركة كانت ٣ ،٤ % منهم ٠,٢٥ % يحملون أكثر من طفيلي و١,٢٨% يحملون أكثر من نوع بكتيري و ٩٣ .١% يحملون بكتريا وطفيلي في آن واحد. ولوحظ أن هناك فرق معنوي عال بين نسبة إصابة عمال الأغذية بالممرضات المعوية خلال اشهر الدراسة إذ سجل أعلى نسبة إصابة في شهر آب. تم أجراء فحص

[+]Received on ٢٠٠٥/٣/٢٢ , Accepted on ٢٠٠٦/٢/٨ .

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الحساسية الدوائية لجميع العزلات البكتيرية و لوحظ وجود مقاومة لأكثر من ثلاثة مضادا ت حياتية بنـــسب مختلفة بين العزلات.

Introduction

Food is a good medium for microbial growth and it may go bad unless protected from the deleterious effects on them. Food borne diseases are leading global health problems. It was estimated that about 4 billion cases per year resulting in over 2.2 millions deaths [1]. The economic costs of food borne diseases are high in developed countries and even higher in developing countries, investigation of food borne disease helps in reducing that costs [1].

Many of the infectious diseases are spread through food contamination and handling of food can spread pathogenic microorganisms, food handlers may carry pathogens without experiencing any serious illness. There are about 250 known foods borne disease and the majority caused by bacteria followed by viruses and parasites [2]. The U. S. Center for Diseases Control and Prevention (CDC) estimates 76 million people suffering from food borne illnesses each year in United States only accounting for 325000 hospitalizations and more than 5000 deaths [3].Health experts in USA estimate that the yearly cost of all food borne diseases is about (5 to 6 billion $) in direct and indirect medical costs and Salmonella infection only account for 1 billion [2].

Ingestion of contaminated food with bacteria can lead to acute illness, (mild illness or infection without symptoms) any of these conditions can lead to symptomless carrier. Symptomless carriers are most likely to occur with organisms that belong to the family enterobacteriaceae which are adapted to gut environment, also if food handlers are carriers so they transmit the organism to another person via food and this may be a source of food poisoning outbreaks[4,5].

Materials and Methods

1- Specimens

Seven-hundred and seventy six stool specimens collected from a healthy food handlers who attended the Central Public Health Laboratory in Hilla during 2004 to be examined to get the permits for working as food handlers.

2- Direct stool examination (wet mount)

A small quantity of freshly passed stool was taken by the tip of a wooden applicator and thoroughly mixed with a drop of physiological saline or a drop of lugols Iodine solution on a glass slide, and then the slide was carefully examined under low power and high power lens.

3- Isolation and identification of pathogens standard methods were used to isolate and identify intestinal pathogens [6,7,8].and final identification by API 20 E Kit (Biomerieux) and serological test (the following Antisera were used 1-(Polyvalent (O and H phase 1 phase 2 (Murex .PRO. LAM.UK) for Salmonella 2- polyvalent Shigella Flexneri 1 and 6, 1—10 serums for Shigella dysenteriae , phase 1 & 2 serum for Shigella sonnei and 1-5 serums for Shigella boydii (Sifin Germany) for Shigella 3-polyvalent Vibrio cholera O1 and agglutinating serum Inaba and Ogawa (Murex Biotech limited U.K)for Cholera 4- E coli polyvalent 1,2,3, and 4 (Murex Biotech limited U.K and used the scheme in figure 1.

4- Antimicrobial testing: The Kirby – Bauer method (filter- paper disk method) was used for measurement of antibiotic sensitivity, the antibiotics used are Amoxicillin 25µg, Ampicillin10µg, Ampiclox30µg, Cefotaxime30µg, Cephaloridine30µg, Chloramphenicol30µg , Colistin10µg, Gentamicin10µg, Nalidixicacid30µg, Tetracycline30µg and Trimethoprim25µg.

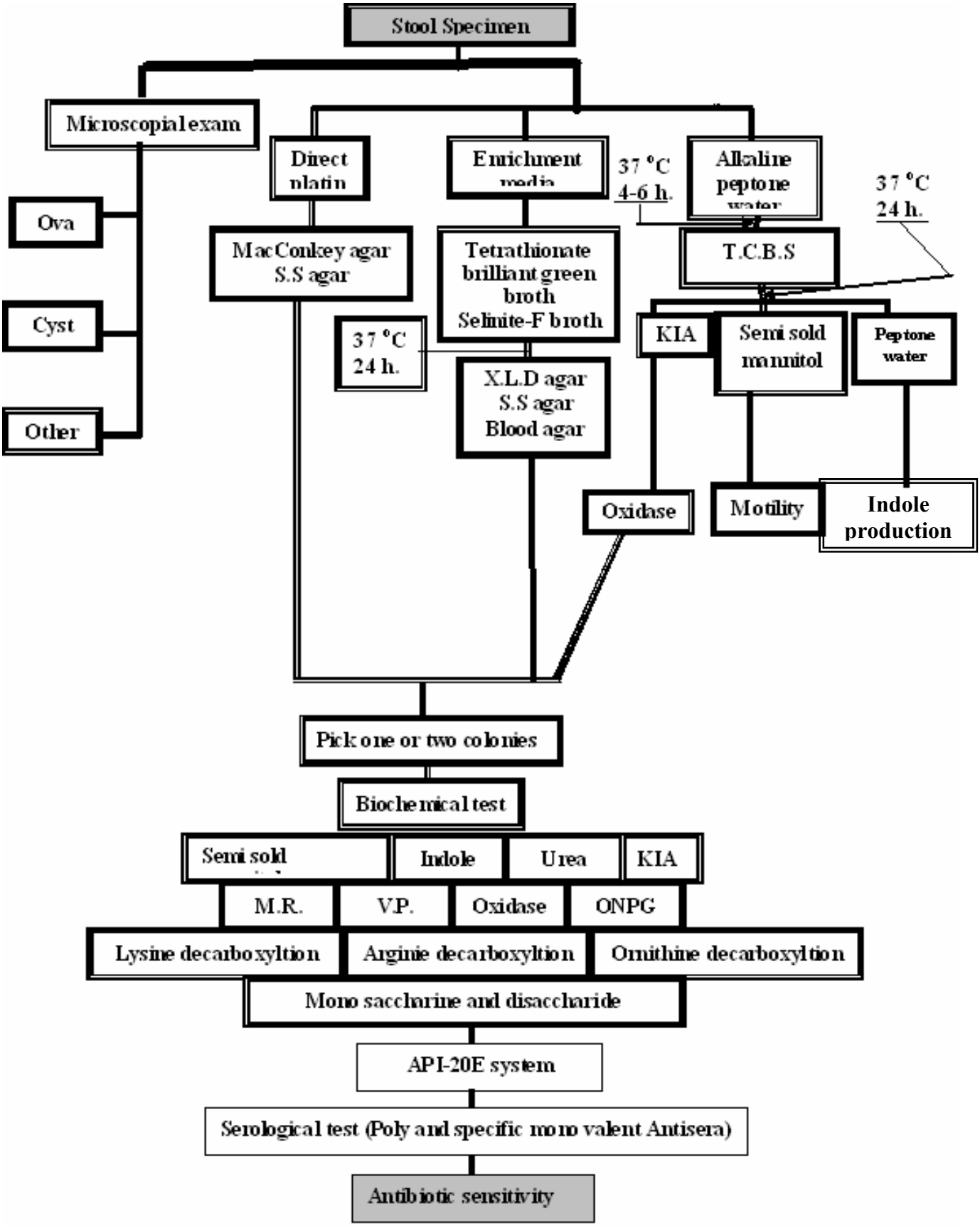


Figure (1): Scheme of diagnosis

Results:

Results of the total seven hundred and seventy six food handlers referred to the Central Public Health Laboratory in Hilla from march 2004 to end of August 2004, 211(27.19 %) showed a carriage of enteropathogens. Bacterial pathogens were dominant and isolated from 125 (16.107 %) of which 115(14.819 %) having one type of bacteria while the remainder 10 (1.288%) had more than one pathogenic species, intestinal parasites were isolated from 71 (9. 148 %), 69 (8.89 %) of them having one parasite , while the remainders 2 (0.257 %) had double parasites and 15 (1.932%) showed a mixed carriage state of both bacteria and parasite (Fig2). Stool specimens of food handlers examined for intestinal pathogens, showed the existences of 238 (30.66%) intestinal pathogens , 150 (19.32 %) of them were bacterial pathogens and the remainder 88 (11.34%)were intestinal parasite Fig (3). Table (1) shows that out of the 150 isolates, *Salmonella* were more frequently encountered (64 isolates, 42. 6 %) than the others, followed by *Aeromonas* (22 isolates, 14.6%) while the lowest frequency of isolation was noticed in Vibrio (5 isolates, 3.5 %),also Table (1) shows that out of the 88 intestinal parasite, *Giardia lamblia* were more frequently encountered 44 (50 %) than the others, followed by *E. histolytica* 30 (34.09%) while the lowest frequency of isolation was noticed in *E. vermicularis* 3 (3.4%). Table (2) shows that the mixed infection in this study was27 (3.47 %), 2 (0.25 %) of them showed more than one parasite , while 10 (1.28 %) showed more than one species of bacteria ,and the remainders15 1.93 % showed a mixed infection with bacteria and parasite . Table (3) shows the serotype of *Salmonella* in our study, reported according to the Kaufmanns classification, this table shows that the isolates are located in five serogroups which are: B, C, D, E, and G. Group B was dominant in its isolates 25 (39.06 %) followed by group C 14 (21.88%), and the less isolates was for group G 1 (1.65 %) within *Salmonella* groups. Table (4) shows the serotype and number of *E- coli*. Group 4 was more common and accounts 8 (40%) of species followed by group 2,7(35%), group 3,4 (20 %), and finally group 1, 1 (5%). In this study only two species, *Shigella sonnei* and *Shigella flexneri* were isolated in frequency of 7 (77.78 %) and 2 (22.22 %) Table (5). Table (6) shows that *Vibrio cholera* was isolated from stool culture of food handlers 5 times; two of them were *Vibrio cholera Inaba* (40%), while the remainder three isolates were Non-agglutinable Vibrio (NAG) (60 %). During our study from March 2004 to August 2004, it was found that 238 intestinal pathogens were identified, 150 of them were

bacterial pathogens while 88 of them were parasites, the number and percentage of intestinal pathogens in the positive carrier in food handlers according to the incidence on the month is shown in Figure (4). Figure (5) shows the age range to be from 15 to more than 50 years, maximal percentage, 40.5 % of positive cases were found in the age group of 40-49 years old. Figure (6) shows the highest number of positive carrier in food handlers in urban regions within the 30- 39 group and it was30 while the highest number was 45 in the 40- 49 group of age in comparison with rural regions. Antimicrobial sensitivity tests of 138 bacterial isolates from food handlers are shown in Table (7).Tetracycline ,Chloramphenicol , were the most effective drugs against *Vibrio cholera* 100% while Ampicillin was most effective drug against Citrobacter freundii 100% Colistin revealed the lowest effective drugs and gave the lowest inhibitory where only 26.5%, 22.2 % , 45 % and 40 % of strains of *Salmonella , Shigella , Citrobacter freundii ,* and *Enterobacter cloacae* were sensitive respectively, while *E coli, Vibrio cholera, Aeromonas hydrophila* were 100% resistance.

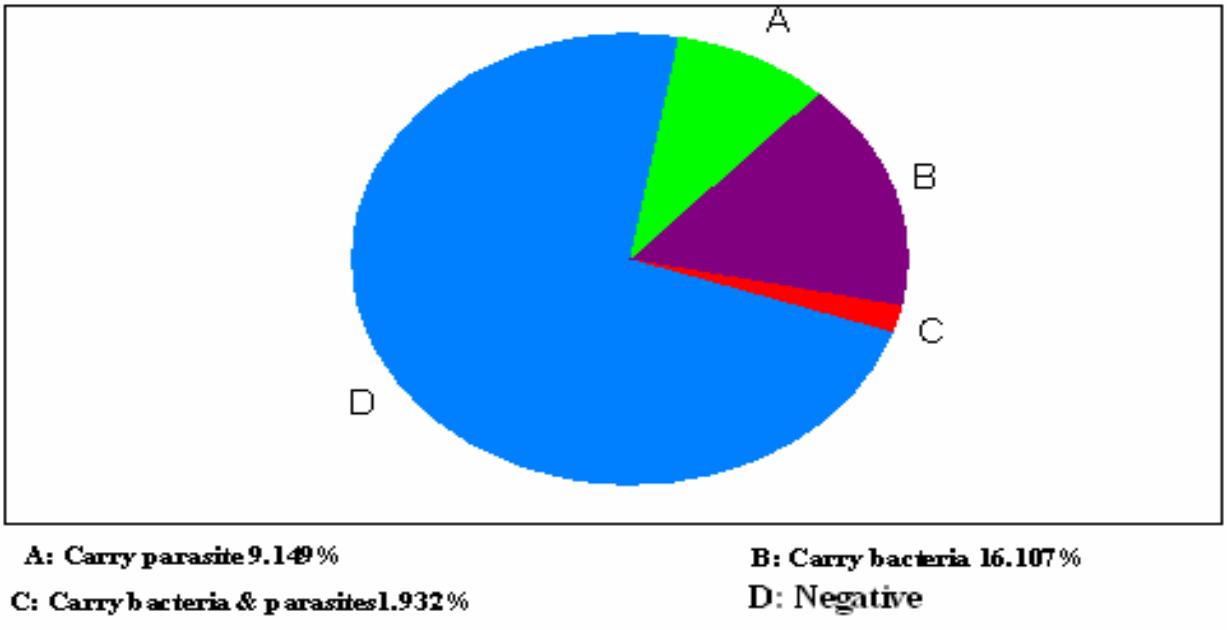
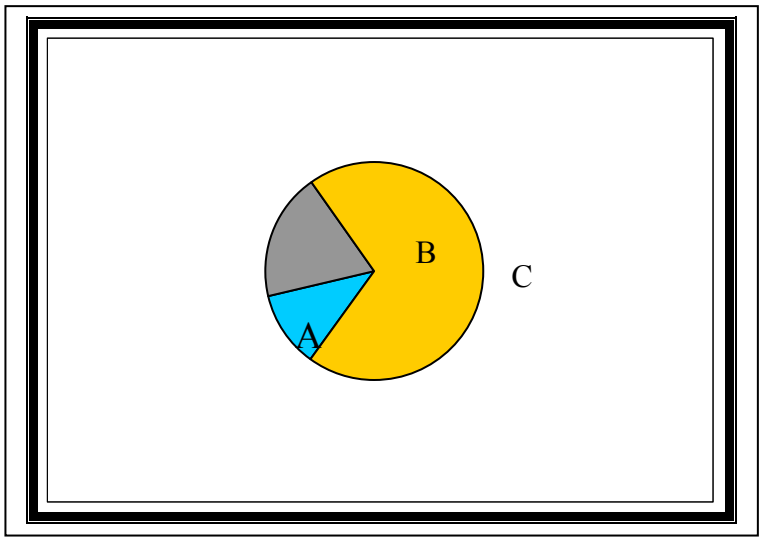


Fig: 2: The number of positive food handlers that carry intestinal pathogens



A: Parasites 11.34% **B: Bacterial pathogens 19.32 %** **C: Negative**

Fig: 3: The percentage of Enteropathogens isolated of 776 food handlers

Table :(1) The number and percentage of Bacteria and parasites isolated from stool specimens of 776 food handlers

parasites	**No**	**% of intestinal parasites No. 88**	**% of total food handlers examined No .776**	**Bacteria**	**No. of isolates**	**% of pathogenic bacteria No. 150**	**% of Total food handlers examined No.776**
Giardia lamblia	44	50	5.67	*Salmonella*	64	42.6	8.24
Entamoeba histolytica	30	34.09	3.86	*Aeromonas*	22	14.66	2.83
Total Protozoa	*74*	84.09	9.53	*Escherichia*	20	13.3	2.57
Ascaris lumbricoides	6	6.81	0.77	*Citrobacter*	20	13.3	2.57
Hymenolopis	5	5.68	0.644	*Enterobacter*	10	6.66	1.28

parasites	No	% of intestinal parasites No. 88	% of total food handlers examined No .776	Bacteria	No. of isolates	% of pathogenic bacteria No. 150	% of Total food handlers examined No.776
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Enterobius vermicularis	3	3.49	0.38	*Shigella*	9	6	1.15
Total helminthes	14	15.9	1.8	*Vibrio*	5	3.33	0.64
Total	**88**		**11.34**	**Total**	**150**	**-----**	**19.32**

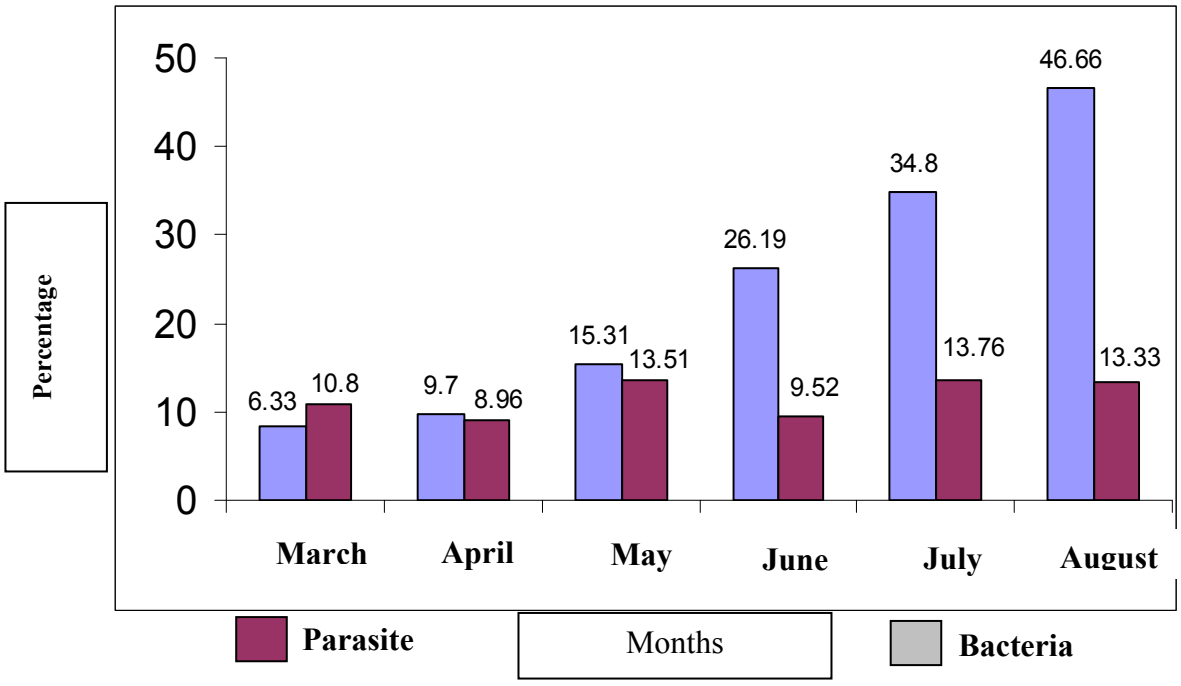

Fig4: The percentage of intestinal pathogens in food handlers according to incidence on the month.

Table (2) Mixed infection identified in food handlers examined.

Type of mixed infection	Number	Percentage
Parasite / parasite	**2**	**0.275**

Bacteria / bacteria	**10**	**1.288**
Bacteria / parasite	**15**	**1.932**
Total	**27**	**3.477**

Table: (3) Serotypes of Salmonella isolated from stool culture of 770 food handlers

group	Serotypes	No.	% of genus Salmonella	% of pathogenic bacteria	% of positive carrier in food handlers	% of Total food handlers examined
B	*S. Typhmurium*	15	23.4	10	7.1	1.93
	S. Chester	4	6.25	2.66	1.89	0.515
	S. Java	2	3.12	1.33	0.947	0.25
	S. agona	2	3.12	1.33	0.947	0.25
	S. Heidelberg	1	1.65	0.66	0.47	0.128
	S. derby	1	1.65	0.66	0.47	0.128
Total		25	39.06	16.66	11.84	3.22
C	*S. A bany*	1	1.65	0.66	0.47	0.128
	S. Virchow	4	6.25	2.66	1.89	0.515
	S. Muenchen	2	3.12	1.33	0.947	0.25
	S. Choleraeusis	2	3.12	1.33	0.947	0.25
	S. emek	2	3.12	1.33	0.947	0.25
	S. Thompson	1	1.65	0.66	0.47	0.128
	S. braenderup	1	1.65	0.66	0.47	0.128
	S. hadar	1	1.65	0.66	0.47	0.128
Total		14	21.8	9.33	6.63	1.8

D	*S. Typhi*	12	18.75	8	5.68	1.54
	S. Enteritides	1	1.65	0.66	0.47	0.128
Total		13	20.3	8.66	6.16	1.67
E	*S. Newington*	4	6.25	2.66	1.89	0.515
	S. senftenberg	2	3.12	1.33	0.947	0.25
	S. anatum	5	7.8	3.33	2.36	0.64
Total		11	17.18	7.33	5.21	1.41
G	*Worthington*	1	1.65	0.66	0.47	0.128
Total	-------	64	-----	42.66	30.33	8.24

Table (4): Escherichia coli serotype isolated from stool specimens of 776 food handlers**.**

Number of group	**serotype**	**No. of isolates**	**%**	**% of pathogenic bacteria No.150**	**% of positive carrier in food handlers No.211**	**% of Total food handlers examined No.776**
1	055,011,026	1	5	0.66	0.47	0.128
2	0125,0126,0128	7	35	4.66	3.31	0.4
3	0127, 086,0119	4	20	2.66	1.84	0.515
4	0114,0124,0142	8	40	5.33	3.79	1.03
Total	-	20	-	13.33	9.47	2.57

Table (5): Shigella serotype isolated from stool culture of 776 food handlers

Serotype	**No.**	**% of genus Shigella**	**% of pathogenic Bacteria No. 150**	**% of positive carrier in food handlers No .221**	**% of Total food handles examined No. 776**
Shigella Sonnei	7	77.78	4.67	3.31	3
Shigella Flexneri	2	22.22	1.33	0.49	0.25
Total	9	-----	6	4.26	1.15

Table (6): Vibrio cholera serotype isolated from stool culture of 776 foot handlers.

Serotype	**No**	**% of genus Vibrio No. 5**	**%of pathogenic Bacteria No. 150**	**% of Positive carrier in food handlers No.211**	**% of total food handlers examined No.770**
Vibrio cholera Inabn	2	40	1.33	0.94	0.257
*Vibrio cholera NAG**	3	60	2	1.42	0.386
Total	5	-	3.33	2.36	0.643

*NAG: non agglutinable cholera.

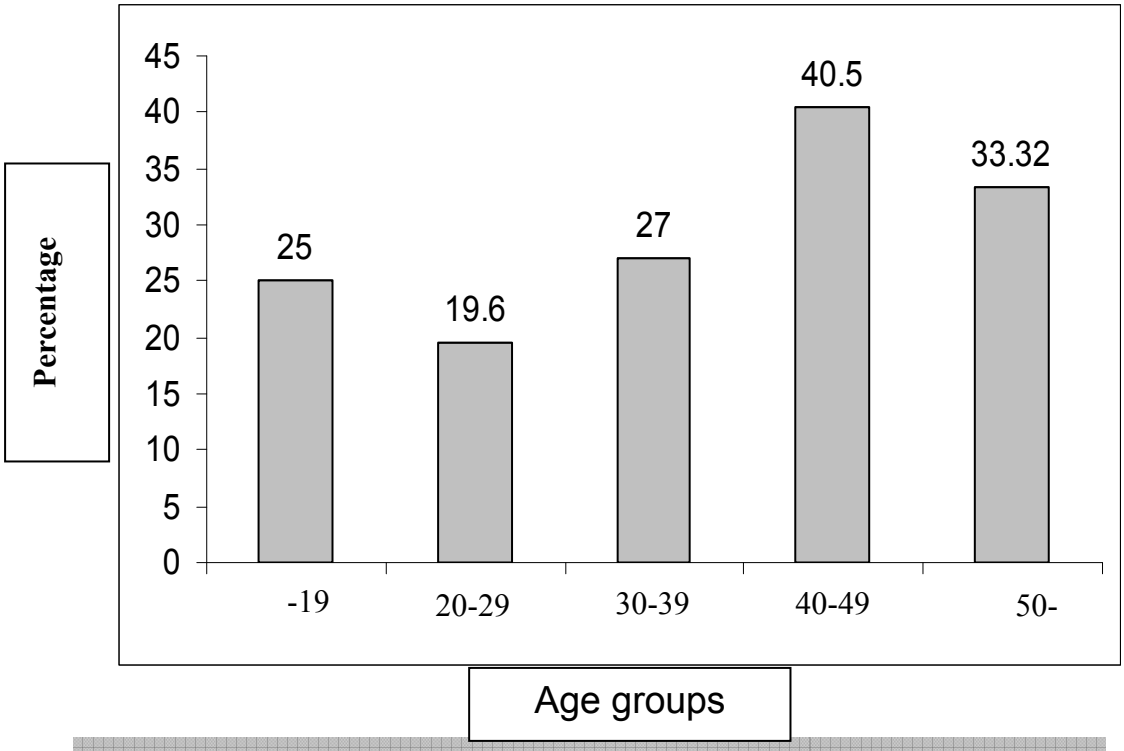

Fig. 5: The prevalence rate of positive carrier in food handlers according to the age.

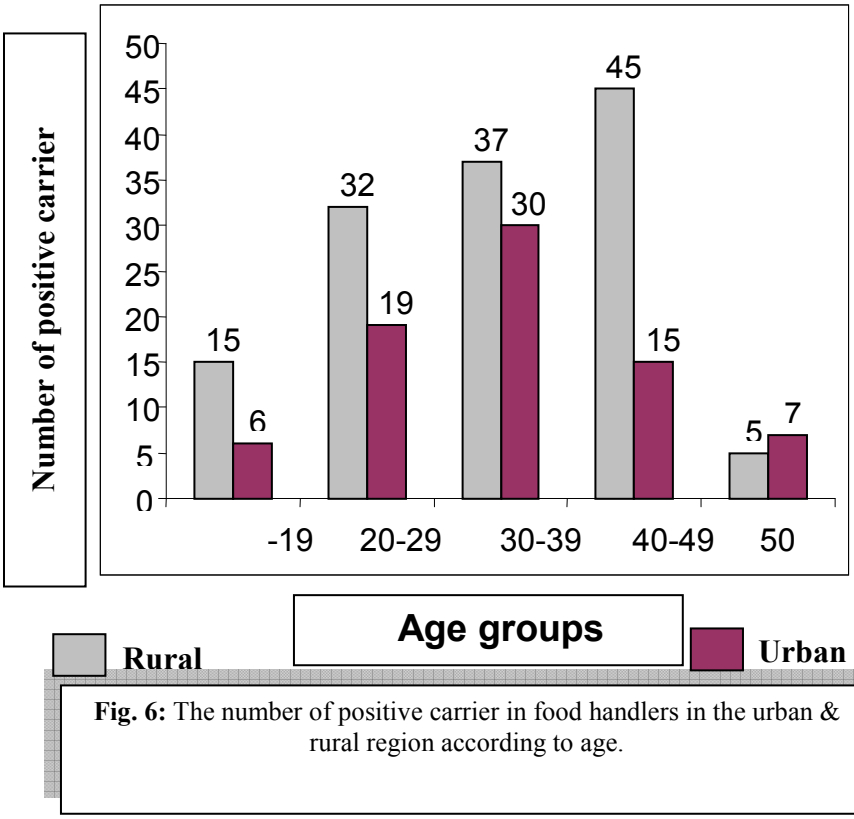

Fig. 6: The number of positive carrier in food handlers in the urban & rural region according to age.

Table (7): Antimicrobial susceptibility pattern of the different isolated species of bacteria.

Type of bacteria Antibiotics	No. & percentage of susceptible isolates													
	E-coli No.20		*Salmonella* No.64		*Shigella* No.9		*Citrobacter* No.20		*Enterobacter* No.10		*Cholera* No.5		*Aeromonas* No.10	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Amoxicillin	12	60	47	73.4	6	66.6	12	60	4	40	1	20	3	30
Ampicillin	10	50	29	45.3	7	77.7	20	100	3	40	3	60	3	30
Ampiclox	14	70	39	60.9	7	77.7	17	85	3	30	3	60	3	30
Cefotaxime	14	70	52	81.2	6	66.6	9	45	5	50	4	80	6	60
Cephaloridine	12	60	44	68.7	5	55.5	11	55	3	30	2	40	7	70
Chloramphenicol	13	65	54	84.3	7	77.7	15	75	8	80	5	10	7	70
Colistin	-	-	17	26.5	2	22.2	9	45	4	40	-	-	-	-
Gentamicin	15	75	50	78.1	6	66.6	17	85	7	70	4	80	7	70
Nalidixicacid	17	85	49	76.5	4	44.4	12	60	8	80	2	40	5	50
Tetracycline	12	60	40	62.5	4	44.4	13	65	7	70	5	10	7	70
Trimethoprim	15	75	45	70.3	7	77.7	16	80	8	80	4	80	8	80

Discussion :

There are a number of reports and studies which deal with intestinal parasite problems in food handlers in Iraq ,and many reports deal with Salmonella carrier in food handlers in Baghdad or in other part of Iraq , However no reports dealt with problems of healthy carriers of intestinal pathogens(Parasite and bacteria) in food handlers in Hilla city.

This study show that the total incidence rate with intestinal pathogens in food handlers were (27 .19 %).This result agrees with the report of [9] that recorded the prevalence rate of intestinal pathogen in food handlers between20 –50 %.

Bacterial pathogens were dominant in this study and isolated in a ratio of (16.107 %) and this result agrees with many literatures that indicate the intestinal bacterial pathogens to be the most common cause of food borne diseases and dominant in comparison with intestinal parasite [10,11,2,12].

In our study bacterial pathogens were dominant and isolated from 125 (16.107 %) food handlers, 115 (14.819 %) of them had one type of bacteria while the

remainder 1.288 % had more than one (DF =1, tabulated x^2 =3.84, calculatedx^2=0, P-value= 1).and the main genus among bacteria was Salmonella and the reasons for this variation may be due to the major mode of transmission for non typhoidal *Salmonella* that contaminated food and present enormous animal reservoirs and some studies deal with diarrheal patients or were done in countries with large numbers of tourists who may be carrier of the microorganism. The reason for high ratio may be due to variation in capabilities of the public health laboratories in isolation of *Salmonella* in that region .In this study the high rate of isolation of *Salmonella* in comparison with others may be due to the technique used.

This result shows no significant differences and does not agree with that of other studies such as [13,14] moreover they did not find any mixed bacterial infection. Intestinal parasite were isolated in a ratio of (9.148 %) from the total food handlers, (8.891%)of them carry single parasite and the remainders (0.275 %) have double infection, but there are no significant differences between cases(df 1, tabulated x^2= 3.84 ,calculated x^2=0,p-value= 0.09) and this result agrees with these of other studies which found the prevalence rate of intestinal parasite was in the range of (8.6%-9.2 %) [15], while others referred to a higher rates such as [16], 26.5 %, [17], 58.6 %, [18] 37-20 %, [19], 15.8 % , [20] 59.8 %. In this study, a significant difference is found between the infection rates of intestinal pathogens among food handlers of rural regions in comparison with food handlers of urban regions. (df 1, tabulated x^2 3.84, calculated x^2 84.2, P value 0) . The prevalence rate of the positive carrier in food handlers in rural region was recorded, as a 63.51% in comparison with urban region 36.49%. Several factors play an important role in the distribution of organism in rural regions for example village size, population density, source of water, the way of preparation of food, presence of domestic animals. Regarding the parasite infection in rural areas , domestic situation and habits affect the extent to which parasite egg , larva ,cysts, will come in contact with people. The variation of incidence in higher age group 40-49 and above 50 may be due to immunity and poor personal hygiene.

The variation in results of sensitivity to antibiotics of the intestinal pathogens isolated from food handlers may be due to the use of antibiotics in animal feeds and indiscriminant use of antibiotics in human, which may lead to the increase in antibiotics resistance by promoting transfer of R factors and genetic mutations.

References:

1-Besser, J., j. A. Beebe and S. Bala., *Manual of clinical Microbiology*. Volume 1, 8th ed, eds., M. Patrik, Washington American Society for microbiology USA,2003.

2-National Institutes of Health.. "Food borne disease" *Pakistan. J. Med. Res*. Vol.40. No.1, pp.300 – 310, 2003.

3-Pan American Health Organization, "Preventing food borne disease is relatively easy" *Indian Pediatrics*, Vol.47, No.4,pp. 398-400, 2002.

4-Garbutt, J.., *Essentials of Food Microbiology*, 1st ed, eds., Arnold London,1997.

5-Holmes, B., *Microbiology and Microbial infection,* 9th ed, eds., L. Collier, New York,1998.

6- Holt, J. G., et al, *Bergey's Manual of Determinative. Bacteriology* 9th ed, William & Wilkins in U.S.A,1994.

7-Benson, H. J., *Microbiological Applications,* 7th ed, McGraw Hill-Inc. New York,1998.

8-MacFaddin, F., *Biochemical Test for Identification of medical Bacteria,* 3rd ed, Lipincott, Williams & Wilkins, Philadelphia's, 2000.

9-Word Health Organization, "The food handlers" *New Engl. J. Med.* Vol. 335. No. 19,pp 1445-1453,2001.

10-Federal Registers, "Pathogen reduction, hazard analysis and critical control point system" *Fed. Regist*, 61pp. 38806-89, 1996.

11-Bean, N.H. & Griffin .P.M. "Food borne disease outbreak in the United States" *J. food. Port*, Vol.53, pp.804-817, 1998.

12-Young, A., "Food borne disease take toll on public health*" MMWR*, Vol. 12, No.4, pp. 1070-1074,2004.

13- Al-Magdi, E.J., *Diarrhea of multifactorial an etiology* MSc, Thesis, College of Medicine,. Baghdad, 1986.

14-Tawifiq, M.R.. *Isolation of Salmonella and Shigella from cases of diarrhea in Mosul and its vicinity*, M.Sc. thesis, College Medicine, University of Mosul,1988.

15-Shihab, K. & Sultan .M., "Parasitic disease among Egyptian workers in Baghdad city" *Bull. End. Dis, Vol.* 26, No.1, pp. 65-70,1985.

16-Arif, A.E. & A.S. Hassoun "An intestinal parasite survey amongst food handlers in Baghdad" *Bull. End. Dis*, Vol.11, No.4, pp. 7-27, 1969.

17-Aubaid, Q.N., *Workers in food establishment in Basrah: Microbiological and epidemiological study*, M .Sc. Thesis, College of Medicine, Univ. Basrah, 1990.

18-Al-Dulaimi, S.S., “Parasitic etiology of diarrhea in AL- Anabar province” *AL-Mustansiriyah J. Sci. Vol.* 7, No.2, pp.64-68,1996.

19-AL- Aamiri, R., “ Study on the prevalence of intestinal parasitic infestation in Basrah province” *Al-Buhooth AL-Techn. R.J. Vol. 14, No. 94, pp. 80-88, 2001.*

20-Al-Mussawi, K. and Mhaisen .F.T. “ Distribution of intestinal parasitic infection in Kerbala city” *Kerbala Uni .J*, Vol. 1No.4, pp.103-110, 2003