Characterization of some enteric bacteria isolated from some types of ready to eat fast foods with special reference to plasmid profile of *E. coli*

Ebtesam, M.M. Farid.; Dalia, A. Salim. and Eman, F. Sharaf
Serology (Bacteriology) and Food Hygiene Dep., Animal Health Research Institute, Dokki.

Abstract
Two hundred samples of ready to eat fast food (Kofta, Shawerma, beef burger and sausage) (50 of each) were collected randomly from different restaurants in kaluobia governorate and subjected to bacteriological examination for enteric bacteria. Overall the incidence of enteric bacteria in the examined samples was (75%) with the isolation of ***proteus vulgaris*** in an incidence of 10,18, 4 and 26 % from examined kofta, shawerma, beef burger and sausage samples, respectively. While 10% of beef burger samples was positive for ***proteus mirabilis. Klebsiella azaenae*** could be isolated from the examined shawerma and sausage samples with percentages reached 8 and 22, respectively. While the incidence of ***Klebsiella pneumonia*** in the examined kofta, shawerma and sausage samples was 4, 2 and 12%, respectively. ***Citrobacter diversus*** could be isolated from the examined kofta and sausage samples with percentages reached 22 and 16, respectively. While ***Citrobacter freundii*** could be isolated from the examined kofta and beef burger samples with percentage reached 4 and 12, respectively. ***Serratiamarcens*** and ***Enterobactera erogenes*** could be isolated from the examined kofta, shawerma and beef burger samples with percentages reached (2, 6 and 4) and (6, 8 and 2), respectively. ***E.coli*** could be isolated from the examined kofta, beef burger and sausage samples with percentages reached 28, 40 and 14%, respectively. ***Salmonella*** could not be isolated from any sample.The incidence of pathogenic ***E.coli*** serotypes in the examined ready to eat fast food samples revealed that6, 2, 6,12 and 2% of the examined kofta samples were belonged to O_{151}, O_{159}, O_{86a}, O_{111} and O_{128}, respectively. 14, 8, 8 and 16% of the examined beef burger samples were belonged to O_{20}, O_{159},O_{111} and O_{128}, respectively. While 2, 4, 4, 2 and 2% of the examined sausage samples were belonged to O_{151}, O_{20}, O_{86a}, $O_{111}$and O_{128}, respectively. The agarose gel electrophoresis of different isolated Plasmid from ***E. coli*** isolates showed that the number of plasmids ranged from 2-3bands.Where O_{151}, O_{159}, O_{86a}, $O_{111}$and O_{128}sharing the same band at 23 KBp.O_{151}, O_{20}, O_{159} and O_{128}sharing the same band at 8 KBp. O_{151}, O_{86a}, O_{111}, and O_{128}sharing the same band at 7 KBp at the same time O_{86a} and O_{111}sharing the same bands at 7 and 23 KBp. So plasmid present in all examined serotypes with percentage reached (100%).

Key words:
enteric bacteria, fast foods, plasmid profile.

Introduction

Fast foods are quick, reasonably priced, and readily available alternatives to home cooked food. While convenient and economical for a busy lifestyle (**Goyal and Singh, 2007),** the recent trends in eating out among upper soci-

ety, teenagers and youth have also increased and the fast food has won the palate of those groups. These are served as helpful purpose in official and private meeting, working people at lunch time and also Tiffin of students of different categories etc. But those fast foods may cause a serious problem when they are contaminated with pathogenic microorganisms due to lack of proper environmental and sanitary processing, lack of proper hygienic practices and storage mishandling (**CDC, 1990**).The transmission of human diseases through food is a global problem, particularly in developing countries where gastrointestinal diseases are one of the most important causes of morbidity and mortality. However, food habits adopted by populations maymitigate or increase the hazards (**WHO, 1968, WHO, 1976 and Ahmed *et al.,* 2008)**.Enteric bacteria are facultative aerobic, Gram-negative, non-spore forming rods that belong to the family *Enterobacteriaceae* (**Brenner, 1984**). This family is subdivided into a number of genera that include *Escherichia, Shigella, Salmonella, Citrobacter, Klebsiella, Enterobacter, Erwinia, Serratia, Hafnia, Edwardsiella, Proteus, Providencia, Morganella, Yersinia*. This classification primarily based on biochemical characteristics, genetic and antigenic properties and pathogenicity (**Blood and Curtis, 1995**). The human gut is the natural habitat for these bacterial community and these bacteria species participate in metabolic activities that salvage energy and absorbable nutrients protect the colonized host against invasion by alien microbes, and important trophic effects on intestinal epithelia and on immune structure and function. They thus play an essential role in the development and homeostasis of the immune system (**Guarner, 2006**). Although most enteric bacteria were previously considered to be harmless, there is evidence implicating them in certain pathological conditions and thus cause health problems including multisystem organ failure, colon cancer, diarrhea, enteritis, urinary tract infections and inflammatory bowl diseases in humans (**Cormican *et al.,* 1998, Goldstein, 2000**, **Dromigny *et al.,* 2002** and **Guenther *et al.,* 2010)**.

Diseases usually result through the production of enterotoxins of which two major types known as endotoxins and exotoxins have been identified (**Tornadijo *et al.,* 2001).** Moreover, there are other virulence factors that usually contribute to their pathogenicity. The capsular polysaccharide, adhesion factors, polysaccharide chains and surface antigens have been reported as putative virulence factors that facilitate attachment of bacteria to host cell wall (**Albert *et al.,* 1992 Frankel *et al.,* 1994**, **Ridell *et al.,* 1995Tschäpe *et al.,* 1995**, **Lai *et al.,* 2000** and **Mirold *et al.,* 2001**). The mode of transmission of these pathogens to humans is through the faecal – oral route hence a number of outbreaks have resulted from the consumption of contaminated food and/or water **(Borch and Arinder, 2002** and **Gudbjorndottir *et al.,* 2004)**. Plasmids are extrachromosomal genetic element which can replicate independently of bacterial chromosome and play an important role in the spread of antibiotics resistant genes (**Towner, 2000** and **Ahmadi *et al.,* 2007**). **Dharmalingam *et al.,* (2003)** indicated that many pathogenic bacteria can survive antibiotic treatment due to the existence of antibiotic resistant. Also plasmid analysis has proved a useful method for differentiating isolates **(Neuwirth *et al.,* 1996)**. The number and size of the plasmids present is used as the basis for strain identification. This strain typing technique has been used successfully for analysis of outbreaks of nosocomial infections **(Schaberg *et al.,* 1981)** and community acquired infections **(Fornasini *et al.,* 1992)** caused by a variety of species of Gram negative rods. So, the aim of this work to detect and identify the enteric pathogens which could be found in the examined ready to eat fast food samples (kofta, shawerma, beef burger and sausage), as well as detection of the plasmid profile of some ***E. coli*** isolates.

Material and Methods

Two hundred samples of ready to eat fast food including (kofta, shawerma, beef burger and sausage) (50 of each) were collected randomly

from different restaurants in kaluobia governorate. Each sample was packed in sterile plastic bag and transferred immediately to the laboratory in an ice box with a minimum period of delay to be examined bacteriologically.

1. Preparation of the samples. Samples were prepared according to the technique recommended by **ICMSF (1978)**.

2. Bacteriological examination.

Aliquots of 100µl were spread plated onto Salmonella-Shigella agar (SSA) (after pre-enrichment in non- selective medium as buffered peptone water followed by selective enrichment in Selenite-F-broth) and Xylose Lysine Deoxycholate agar (XLD) plates. This was basically to increase the chances of isolating different types of enteric bacteria. The plates were incubated aerobically at 37°C for 24 hours. Suspected colonies were purified by sub -culturing on SSA and XLD agar, the plates were incubated aerobically at 37°C for 24hours. Pure isolates were retained for presumptive identification and confirmatory biochemical tests.

The criteria used for confirmation of the retrieved isolates were based on microscopical examination of Gram stained smears according to **(*Cruickshank et al., 1975)***,biochemical profiling (Indole production, methyl red, Vogus Proskauer, Simmon's citrate utilization) and serological profiling **(FAO, 1992 and Garrity, 2001).**

Selective isolation of ***Salmonella*** species were done according to **(FAO, 1992)**. Full identification of the ***Salmonella*** suspect isolates were done after matching the achieved morphological, biochemical and serological results against standard methods reported by **(Garrity, 2001;Kerig and Holt, 1986)**.

3.Extraction of plasmid of *E.coli* isolated according to (Birnboim and Doly, 1979).

3.1. Harvesting of bacteria.

3.2. **Alkaline lysis.**

3.3. Precipitation by Ethanol.

3.4. RNase.

3.5. Electrophoresis.

Results

Table (1). The incidence of isolated species of Family Enterobacteriaceae in the examined ready to eat fast food samples (n=50).

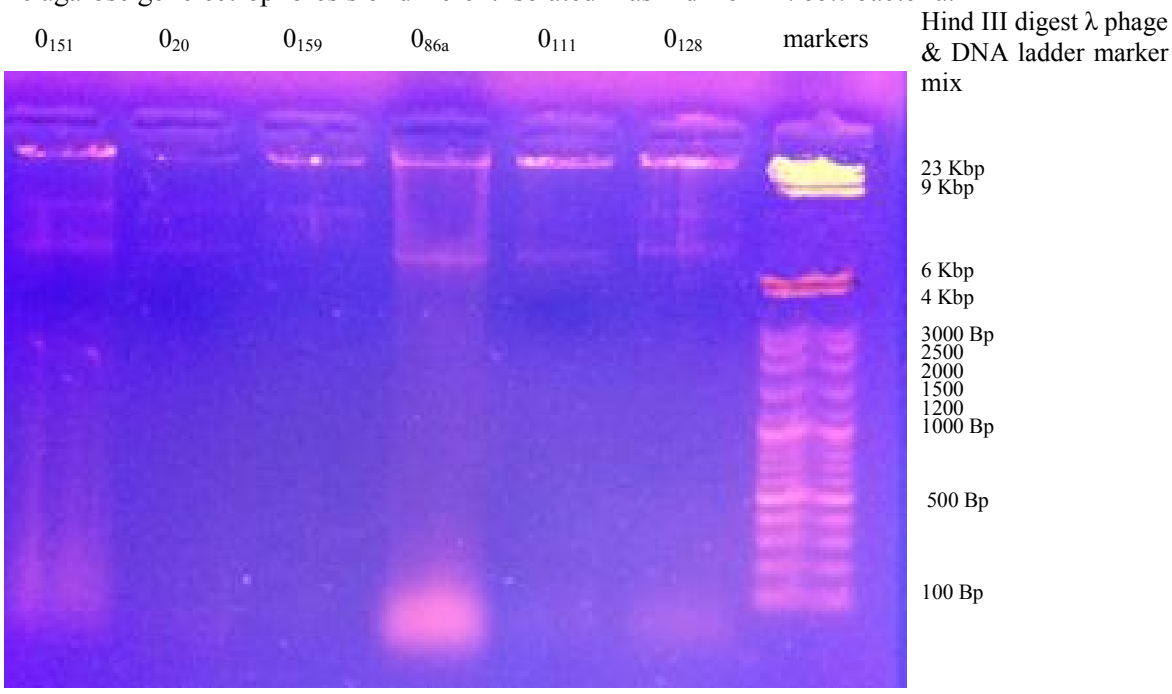
Fast food / Isolated Enteric bacteria	Kofta		Shawerma		Beef burger		Sausage	
	N	%	N	%	N	%	N	%
E.coli	14	28	-	-	20	40	7	14
Proteus vulgaris	5	10	9	18	2	4	13	26
Proteus mirabilis	-	-	-	-	5	10	-	-
Klebsiellaazaenae	-	-	4	8	-	-	11	22
Klebsiellapneumoniae	2	4	1	2	-	-	6	12
Citrobacterdiversus	11	22	-	-	-	-	8	16
Citrobacerfreundii	2	4	-	-	6	12	-	-
Serratiamarcens	1	2	3	6	2	4	-	-
Enerobacteraerogenes	3	6	4	8	1	2	-	-

Table (2). Incidence of pathogenic *E. coli* serotypes in the examined ready to eat fast food samples (n= 50).

Fast food / Serotypes	Kofta		Shawerma		Beef burger		Sausage	
	N	%	N	%	N	%	N	%
O_{151} (EPEC)	3	6	-	-	-	-	1	2
O_{20} (ETEC)	-		-	-	7	14	2	4
O_{159} (ETEC)	1	2	-	-	4	8	-	-
O_{86a} (EPEC)	3	6	-	-	-	-	2	4
O_{111} (EHEC)	6	12	-	-	4	8	1	2
O_{128} (ETEC)	1	2	-	-	5	10	1	2
Total	14	28	-	-	20	40	7	14

* Percentages were calculated according to the number of examined samples.

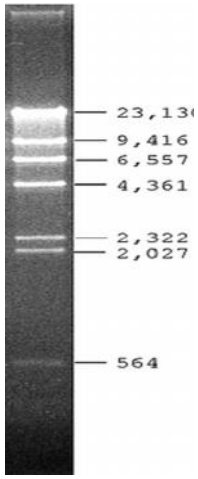
Fig (1). The agarose gel electrophoresis of different isolated Plasmid from *E. coli* bacteria.



E. coli type	0_{151}	0_{20}	0_{159}	0_{86a}	0_{111}	0_{128}
Plasmid approxi-mate size (Bp)	23 KBP 8 KBP & 7 KBP	7 KBP & 8 KBP	23 KBP & 8 KBP	23 KBP & 7 KBP	23 KBP & 7 KBP	23 KBP 8 KBP & 7 KBP

Note. The 7 and 8 KBP could be a result of the same plasmid that shows different states of supercoiling.

Photo of λ phage digest with Hind III restriction enzyme.



Discussion

Enteric bacteria have been found to cause diseases such as diarrhea, sepsis, meningitis systemic infections, necrotizing enterocolitis, bacteraemia, urinary tract infection, gastroenteritis, surgical wound infections in humans **(Alballaa *et al.,,* 1992, - Hoppe *et al.,,* 1993, Maraki *et al.,,* 1994, Oh and Tay, 1995, Funke and Rosner, 1995, Tschäpe *et al.,,* 1995 and FAO/WHO, 2004)**. These infections are considered to be opportunistic and would pose severe challenges in immunocompromised individuals **(Albert *et al.,* 1992, Frankel *et al.,* 1994; Ridell *et al.,* 1995 and; Tschäpe *et al.,* 1995 and Sofos, 2008**). Based on the aforementioned, it is very important to determine the incidence of contamination with these pathogens.

From this study, the incidence of the isolated enteric bacteria was 10, 18, 4 and 26 % for ***proteus vulgaris*** in the examined kofta, shawerma, beef burger and sausage samples, respectively. While 10% of beef burger samples were positive for ***proteus mirabilis*** (table1).

This agree with that reported by **Ahmed (1988)** who detect ***Proteus spp***. in (12.5%) of sausage samples and (10%) of hamburger samples. **Gobran (1985)** isolated ***Proteus vulgaris*** with an incidence of 4(16%) and **Al-Mutairi (2011)** who could isolate 6 *isolates* from a total of 75 meat product samples. Retrieved isolate incidence was distributed as 2 (8%) for each of kofta, sausage and shawerma. While Higher result was recorded by **(Ammar, 2005)** who found ***Proteus*** with an incidence of 42.7% from the total examined meat product samples.

Table (1) also, revealed that ***Klebsiella azaenae*** could be isolated from the examined shawerma and sausage samples with percentages reached 8 and 22, respectively. While the incidence of 4, 2 and 12(%) of the examined kofta, shawerma and sausage samples, respectively was recorded for ***Klebsiella pneumoniae.***

The recorded data in table (1) agree with that recorded by **Roushdy (1971)** who isolated ***Klebsiella*** **spp** with an incidence of (12%). **El-Khateib (1982)** isolated ***Klebsiella*** **spp** from sausage in incidence of (47.23%). Furthermore **(Gobran, 1985)** who isolated ***Klebsiella*** **spp** from sausage and basterma samples in incidence of (20%). **Lofti *et al.,* (1990)** isolated ***Klebsiella*** with an incidence of (12%) and**Al-Mutairi (2011)**could isolate ***Klebsiella*** in different rates of incidence. These incidences were (16%) in kofta, (4%) in sausage and (12%) in shawerma.

Citrobacter diversus could be isolated from the examined kofta and sausage samples with percentages reached 22 and 16, respectively. While ***Citrobacter freundii*** could be isolated from the examined kofta and beef burger samples with percentage reached 4 and 12%, respectively (Table1).

Serratia marcens and ***Enterobacter aerogenes*** could be isolated from the examined kofta, shawerma and beef burger samples with per-

centages reached (2, 6 and 4%) and (6, 8 and 2%), respectively (table 1).

E. coli could be isolated from the examined kofta, beef burger and sausage samples with percentages reached 28, 40 and 14%, respectively(table1).

E. coli is a common flora organism in the gastrointestinal tract of humans and animals. Most of them are harmless; however, some are pathogenic and can cause disease. Diarrhoeagenic ***E. coli*** strains are categorised into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O: H serotypes **(Meng *et al.,* 2001).** The six main categories include enteropathogenic ***E. coli*** (EPEC), enterotoxigenic ***E. Coli*** (ETEC), enteroinvasive ***E. coli*** (EIEC), enteroaggregative ***E. Coli*** (EAEC), enterohemorrhagic ***E. Coli*** (EHEC), diffuse-adhering ***E. coli*** (DAEC) **(Nataro and Kaper, 1998).** There are regional differences in the prevalence of the different diarrhoeagenic ***E. coli*** categories **(Cravioto *et al.,* 1988, Albert *et al.,* 1995, Ratchtrachenchai *et al.,* 2004 and Nguyen *et al.,* 2006).**

Table (2) declared the incidence of pathogenic ***E. coli*** serotypes in the examined fast food samples in which 6, 2, 6,12 and 2% of the examined kofta samples were belonged to O_{151}, O_{159}, O_{86a}, O_{111} **and** O_{128}, respectively. 14, 8, 8 and 16% of the examined beef burger samples were belonged to O_{20}, O_{159},O_{111} and O_{128},respectively. While 2, 4, 4, 2 and 2(%) of the examined sausage samples were belonged to O_{151}, O_{20}, O_{86a}, O_{111} and O_{128}, respectively.

ETEC **(** O_{128}**)** is considered the common cause of traveler's diarrhea and or / children diarrhea. It can produce either heat labile (LT) and / or heat stable (ST) toxins which are mainly attributed to the colonization factors that are specific for the host animal species and enable the organism to adhere to the epithelium of small intestine **(David *et al.,* 1990)**. ETEC may contaminate ready to eat food through asymptomatic carrier, a person who recovers from an ETEC infection and continue to excrete the organism for several months. In developing countries, also it is revealed that ETEC is often present in faeces of symptomatic human carriers **(Cliver, 1990)**. The enterotoxigenic properties were found to be mediated by a plasmid which may be transmited to other strains of ***E.coli***, even to other members of Enterobacteriaceae **(Brill *et al.,* 1979).** While, **Evans *et al.,* (1979)** mentioned that enterotoxigenic E.coli serotypes involved O_{20}, O_{26}, O_{44}, O_{55}, O_{78}, O_{114}, O_{119}, O_{124}, O_{125}, O_{127}, O_{128}, O_{142}, O_{158} and O_{159}.

On the other hand, EHEC (O_{111}) was implicated in outbreaks of diarrhea in young children and infants **(Evans *et al.,* 1979)**. Illness caused by EHEC is typically quite severe crampy abdominal pain followed by watery diarrhea, which later on becomes grossly bloody. Typically, there is little or no fever and the duration of illness is 2 to 9 days death rate in some reported outbreaks may reach 36%. Since 1982, more than 10650 outbreaks of EHEC were reported in USA **(WHO, 1997).**

O_{86} serotype was recorded as enteropathogenic ***E. coli*** (EPEC) while O_{111} is recognized as enterohaemorrhagic ***E. coli*** (EHEC) **(Varnam and Evans., 1991).** In general, EPEC strains are the major cause of many infantile diarrhea, symptoms appear within 12 to 26 hours and characterized by fever, nausea, vomition and watery stools, which occasionally contain mucous, but without gross blood **(Toledo *et al.,* 1983).** Furthermore, EPEC was implicated in cases of human gastroenteritis, cystitis, colitis, pyelonephritis, peritonitis and puerperal sepsis as well as food poisoning outbreaks **(Doyle, 1990)**. EPEC showed to be the first bacterial cause of diarrhea in infants and its proportion may reach 54% **(Varnam and Evans, 1991).**

The first time infections by ***E. coli*** O 151: K-:H10 were reported from Japan and the Soviet Union. In 1972 it was included into the international antigenic scheme. Since 1976 these germs were occasionally demonstrated in sporadically occurred acute enteritis in the G.D.R. In autumn 1979, 60 ***E. coli O 151*** strains were isolated from accumulating cases of gastroenteritis in the Rostock district. These infections not only concerned young children, but also adults. Microbiological properties of 87 iso-

lates of this serogroup are described. Biological examination of these strains did not result in symptoms for invasive activity or enterotoxin production. An indirect reference to the existence of fimbrial antigens could be obtained by the Evans' method of direct hemagglutination. A corresponding hemagglutination pattern was found out in 60 ***E. coli O 151*** strains tested: mannose resistant hemagglutination of bovine and chicken erythrocytes. Hemagglutination pattern, association of these strains with enteritis and antigenic relations of Salmonellae motivated the recommendation to integrate these germs into the group of facultatively enteropathogenic *E. coli* described by Czirók and Evans***. E. coli O 151*** strains show biochemical particularities which can be useful for identification of these strains **(Steinrück *et al.,* 1980).**

The identification of pathogenic enteric bacteria species in any food product indicates that proper hygiene practices should be implemented during slaughter, processing and handling of these food products. Moreover, it has been proven that if the hygiene standards implemented during these processes are compromised, there is bound to be contamination **(Dickson and Anderson, 1992).** The presences of these bacteria in meat pose a health risk on consumers especially those that may be immune- compromised, the elderly and young children if not Properly prepared **(Sofos, 2008)**.

In the present study out of 4 isolates of O_{151}, 9 isolates of O_{20}, 5 isolates of O_{159}, 5 isolates of O_{86a}, 11 isolates of O_{111} and 7 isolates of O_{128} we took randomly 6 isolates ***(O_{151}, O_{20}, O_{159}, O_{86a}, O_{111} and O_{128})*** to determine their plasmid profile analysis.

Many important bacterial genes defined as bacterial extra chromosomal DNA elements that replicate autonomously this called plasmids which have different properties such as resistance to antibiotics, production of exotoxins or formation of neoplasm in plants **(Quinn *et al.,,* 2002 and Enany *et al.,* 2010).**

Fig (1) showed the agarose gel electrophoresis of different extracted Plasmid from ***E. coli*** isolates. The number of plasmids ranged from 2-3bands. The current study found that O_{151}, O_{159}, O_{86a}, O_{111} and O_{128} sharing the same band at 23 KBp.O_{151}, O_{20}, $O_{159}$and O_{128}sharing the same band at 8 KBp . O_{151}, O_{86a}, O_{111}, and $O128$sharing the same band at 7 KBp at the same time O_{86a} and O_{111}sharing the same bands at 7 and 23 KBp. So plasmid present in all examined serotypes with percentage reached (100%).This agree with the result reported by **Reddy *et al.,* (2012)** who proved that Plasmid profiling of all isolates of ***E. coli*** contain plasmid DNA. Most of the isolates have from 1 to 3 plasmid bands. On the other hand **Adziety *et al.,* (2013)** recorded that plasmid DNAs were detected in 93% (51/55) of the ***E. coli*** isolates. Thus 4 isolates or 7% of the ***E. coli*** did not harbor plasmid DNAs. Furthermore, 17 ***E. coli*** isolates (31%) harbored 3 plasmid DNAs, 11 isolates (20%) harbored 2 plasmid DNAs, 10 isolates (18%) harbored 1 plasmid DNA, 6 isolates (11%) harbored 4 plasmid DNAs, 5 isolates (9%) harbored 5 plasmid DNAs, 1 isolate (2%) each harbored 6 and 7 plasmid DNAs. Plasmid DNA sizes vary among the ***E. coli*** isolates. The largest plasmid DNA size was 81.5 kb and was detected in only one ***E. coli*** isolate. Thirty three (33) ***E. coli*** isolates harbored one or more plasmid DNAs that were >23.13 kb. Plasmid DNAs of the smallest size <2 kb were present in two isolates.

In this study the 7 and 8 KBP could be a result of the same plasmid that shows different states of supercoiling.

In plasmid profile analysis the number and size of plasmids is away for better identification of bacterial strains. Application of plasmids patterns for strains containing multiple plasmids is more useful. When the number of plasmids was fewer than three, the power of plasmid pattern for distinction of strains decreased **(Tobatabaei and Mehrabian, 2011)**. In this study the number of plasmid in isolated strains was few but plasmid profile analysis indicated that most serotypes contained a plasmid with size 23 KBp which can be considered as a common characteristic of ***E. coli*** serotypes isolated from fast food in this area.

Conclusion and recommendations

1-Different enteric bacterial species were isolated and positively identified in all examined ready to eat fast food samples (kofta, shawarma, beef burger and sausage) so different precautions should be applied as:

-Thorough cooking of food to an internal temperature at least 74^{o}C to prevent food borne illness.

-Hygienic awareness should be applied for personnel whom involved in handling and preparing of food at factories, home or restaurants.

-Utensils and equipments used in cooking should be cleaned and sterilized.

-Washing hands and utensils, as well as surfaces, using clean cloths or towels after each contact with raw food.

-Periodical medical examination of persons sharing in handling and cooking.

2-Plasmid profiling analysis supplies a quick and relatively easy method to fingerprint bacterial strains and to study their spread.

References

Adzitey, F.; Ali, G.R.R.; Huda, N. and Ting, S.L. (2013). Antibiotic resistance and plasmid profile of *Escherichia coli* isolated from ducks in Penang, Malaysia. International Food Research Journal 20(3): 1473-1478.

Ahmadi, M.; Ayremlou, N. and Saie, H.D. (2007). The effect of heat stress on the antibacterial resistance and plasmid profile in *Escherichia coli* isolates. Pakistan Journal of Biological Science 10: 4261-4265.

Ahmed, J.; Lokman Hossain, M.L.; Abdul Malek, M. and Begum, F. (2008). Assessment of Bacteriological Quality of Fast Foods and Soft Drinks in Relation to Safety and Hygiene .Bangladesh J Microbiol, 25 :73 -75.

Ahmed, S. (1988). *Salmonella* in locally manufactured meat products. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Cairo University, Egypt.

Alballaa, S.R.; Qadri, S.M.; al-Furayh, O. and Qatary, K. (1992). Urinary tract infection due to Rahnella aquatilisin a renal transplant patient. J. Clin. Microbiol.: 30:2948-2950.

Albert, M.J.; Faruque, S.M.; Ansaruzzaman, M.; Islam, M.M.; Haider, K.; Alam, K.; Kabir, I. and Robins-Browne, R. (1992). Sharing of virulence associated properties at phenotypic and genetic levels between enteropathogenic *Escherichia coli* and *Hafnia alvei*. *J. Med. Microbiol.* 37: 310-314.

Albert, M.J.; Faruque, S.M.; Faruque,A.S.; Neogi, P.K.; Ansaruzzaman, M.; Bhuiyan, N.A.; Alam, K. and Akbar, M.S. (1995). Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. J. Clin. Microbiol. 33:973-977.

Al-Mutairi, M.F. (2011). The Incidence of Enterobacteriaceae Causing Food Poisoning in Some Meat Products. Advance Journal of Food Science and Technology 3(2): 116-121.

Ammar, M.A.M. (2005). Spoilage and pathogenic microorganisms in traditional meat products in Assuit. M. V. Sc. Thesis, (Meat Hygiene), Faculty of Veterinary Medicine, Assiut University, Egypt.

Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res*. 7: 1513-23.

Blood, R.M. and Curtis, G.D.W. (1995). Media for 'total' *Enterobacteriaceae,* coliforms and *Escherichia coli. Int. J. of Food Microbiol.* 26: 93-115.

Borch, E. and Arinder, P. (2002). Bacteriological safety issues in red meat and ready-to -eat meat products, as well as control measures. *Meat Sci.* 2002:62: 318-390.

Brenner, D.N. (1984). In: Bergey's Manual of Systematic Bacteriology, Vol. 1. Williams and Wilkins, Baltimore, MD; 408-420. Life Science Journal, 2011:8 (S2)http://www-.lifesciencesite.comhttp://www.sciencepub.net/life.

Brill, B.M.; Wasilauskas, B.L. and Richerdson, S.H. (1979). Adaptation of Staphylococcal coagulation technique for detection of heat labile enterotoxins of *E. coli*. J. Clin. Mcrobiol. 9:49-55.

CDC (Centers for Disease Control and Pre-

vention) (1990). Foodborne hepatitis A - Alaska, Florida, North Carolina, Washington. Morb Mortal Wkly Rep. **39**(14): 228-232.

Cliver, D.O. (1990). Foodborne disease. 4th ed. Academic press Inc. San Diego, USA.

Cormican, M; Morris, D; Corbett-Feeney, G. and Flynn, J. (1998). Extented Spectrum Beta-Lactamase production and flouroquinoloe resistance in pathogens associated with community acquired urinary tract infection. *Diagn. Microbiol Infect. Dis.* 32: 317-319.

Cravioto, A.; Reyes, R.E.; Ortega, R.; Fernandez, G.; Hernandez, R. and Lopez, D. (1988). Prospective study of diarrhoeal disease in a cohort of rural Mexican children: incidence and isolated pathogens during the first two years of life. Epidemiol. Infect. 101:123-134.

Cruickshank, R.; Deguid, J.P.; Marmion, B.P.and Swain, H.A. (1975). Medical microbiology. The practice of medical microbiology. 12th, Vol. II. Churchill, Edinbourgh.

David, G.W.; Richard, C.B. and Slank, J.F. (1990). Medical microbiological 4th ed. Chrichill Livengstone England.

Dharmalingam, S.; Rao, U. A.; Jayaraman, G. and Thyagarajan, S. P. (2003). Relationship of plasmid profile with the antibiotic sensitivity pattern of Helicobacter., IFRJ 20 (3):1473-1478.

Dickson, J.S. and Anderson, M.F. (1992). Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. J. Food Prot.55, 133-140.

Doyle, M.P. (1990). Pathogenic *E. coli*, The Lancet 336: 1111-1115.

Dromigny, J.A.; Nabeth, P. and Perrier Gros Claude, J.C. (2002). Distribution and susceptibility of bacterial urinary tract infections in Dakar, Senegal. Int. J. Antimicrob. Agents. 20, 339-347.

El-Khateib, T.S. (1982). Sanitary condition of sausage in Assiut. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Assiut University, Egypt.

Enany, M.E.; Soheir, S.E. and Heba, E. Farhan. (2010). Plasmid profile of Pseudomonas aeruginosa strains of different sources. J. Egypt. Vet. Med. Assoc. 70 (2):267-274.

Evans, D.; Evans, O. and Doupont, H. (1979). Haemagglutination patterns of enterotoxigenic and enteropathogenic *E. coli* determined with human, bovine, chicken and guinea pig erythrocytes in the presence and absence of mannose. J. Food Prot. 5:1051.

FAO (1992). Food and Agriculture Organization of United Nation, Manual of Food Quality Control.

FAO/WHO (2004). Enterobacter sakasakii and other microorganisms in powder infant formula. Microbiological risk assessment series. No. 6.Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). Rome, Italy.

Fornasini, M.; Reeves, R.R; Murray, B.E; Morrow, A.L. and Pickering, L.K. (1992). Trimethoprim-resistant *Escherichia coli* in households of children attending day care-centers. J. Infect. Dis.; 166:326-330.

Frankel, G.; Candy, D.C.; Everest, P. and Dougan, G. (1994). Characterization of C-terminal domains of intimin-like proteins of enteropathogeneic and enterohemorrhagic *Escherichia coli,* Citrobacterfreundii and Hafnia alvei. Infect. Immun.62, 1835-1842.

Funke, G. and Rosner, H. (1995). Rahnella aquatilisbacteremia in an HIV-infected intravenous drug abuser. Diagn. Microbiol. Infect. Dis.22, 293-296.

Garrity, G.M. (2001). Bergey's Manual of Systematic Bacteriology. 2nd Edn., Springer-Verlag, New York.

Gobran, R.A. (1985). Enterobacteriaceae in meat products in Upper Egypt. M. V. Sc., Thesis, Assiut University, Egypt.

Goldstein, F.W. (2000). Antibiotic susceptibility of tract of bacterial strains isolated from patients with community-acquired urinary infections in France. Eur. J. Clin. Microbiol. Infect. Dis. 19, 112-117.

Goyal, A. and Singh, N.P. (2007). Consumer perception about fast food in India: an exploratory study .British Food Journal, 109(2): 182-195.

Guarner, F. (2006). Enteric flora in health and disease. Supplement. 73(1):5-12.

Gudbjorndottir, B.; Suihko, M.L; Gustavsson, P.; Thorkelsson, G.; Salo, S.; Sjoberg, A.M.; Niclassen, O. and Bredholt, S. (2004). The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in Nordic countries. Food Microbiol. 21, 217-225.

Guenther, S.; Filter, M.; Tedin, K.; Szabo, I.; Wieler, L.H.; Nöckler, K.; Walk, N. and Schierack, P. (2010). *Entrobacteriaceae* population during experimental *Salmonella* infection in pigs. Vet. Microbiol.142, 352-360.

Hoppe, J.E.; Herter, M.; Aleksic, S.; Klingebiel, T. and Niethammer, D. (1993). Catheter-related Rahnella aquatilisbacteremia in a pediatric bone marrow transplant recipient. J. Clin. Microbiol.31, 1911-1912.

ICMSF (1978). Microorganisms in Foods. Their significance and Methods of Enumeration, 2nd Edn., University of Toronto, Press, Toronto and Buffalo, Canada.

Kerig, N.R. and Holt, J.G. (1986). Manual of Systemic Microbiology. 8th Edn., Williams and Wilkins, London.

Lai, Y., Yang, S., Peng, H. and Chang H. (2000). Identification of Genes Present Specifically in Virulent Strain of Klebsiella pneumonia. Infect. Immun. 68(12), 7149-7151.

Lofti, A.; Youssef, H.; Hefnawy, Y.; El-Timawy, A. and Nasser, A. (1990). Sanitary status of meat meals in Assiut University hospitals. J. Assiut Vet. Med., 23(46): 126-131.

Maraki, S.; Samonis, G.; Marnelakis, E. and Tselentis, Y. (1994). Surgical wound infection caused by Rahnella aquatilis J. Clin. Microbiol.33, 2372-2376.

Meng, J.; Doyle, M.P.; Zhao, T. and Zhao, S. (2001). Enterohemorrhagic *Escherichia coli*, p. 193-213. In Doyle, M. P., M. P. Beuchat, and T. J. Montville (ed.), Food microbiology: Fundamentals and frontiers. ASM Press, Washington D. C.

Mirold, S.; Ehrbar, K.; Weissmüller, A.; Prager, R.; Tschäpe, H.; Rüssmann, H. and Hardt, W.D. (2001). *Salmonella* hot cell invasion emerged by acquisition of a mosaic of separate genetic elements, including *Salmonella* pathogenicity island1 (SPI1), SPE5, and sopE2. J. Bacteriol. 183, 2348-2358.

Nataro, J.P. and Kaper, J.B.(1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:142-201.

Neuwirth, C.; Siebor, E.; Lopez, J.; A. Pechinot, A. and Kazmierczak, A. (1996). Outbreak of TEM-24-producing *E. aerogenes* in an intensive care unit and dissemination of the extended--spectrum beta-lactamase to other members of the family Enterobacteriaceae, J. Clin. Microbiol.34: 76 –79.

Nguyen, T.V.; Van, P.L.; Huy, C.L.; Gia, K.N. and Weintraub, A. (2006). Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. Int. J. Infect. Dis. 10:298-308.

Oh, T. and Tay, L. (1995). Bacteremia caused by Rahnella aquatilis: report of two cases and review. Scand. J. Infect. Dis. 27, 79-80.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C.; Leonard, F.C. and Maguire, D. (2002). Veterinary microbiology and microbial disease P.18, by Black well science Ltd, registered at the United Kingdom.

Ratchtrachenchai, O.A.; Subpasu, S.; Hayashi, H. and Ba-Thein, W. (2004). Prevalence of childhood diarrhoea-associated Escherichia coli in Thailand. J. Med. Microbiol. 53:237-243.

Reddy, M.V.B.; Sasikala, P. and Karthik, A. (2012). Identification of *Escherichia coli* isolates from meat samples by antibiotic susceptibility, plasmid DNA and whole cell protein fingerprinting.(IJPCBS) 2(4), 705-712.

Ridell, J.; Siitonen, A.; Paulin, L.; Lindroos, O.; Korkealla, H. and Albert, M.J. (1995). Characterization of *Hafnia alvei* by biochemical tests, random amplified polymorphic DNA PCF, and partial sequencing of 16 S rRNA gene. J. Clin. Microbiol. 33, 2372-

2376.

Roushdy, S.A. (1971). Studies on market minced meat. M. V. Sc. Thesis, Cairo University, Egypt.

Schaberg, D.R.; Tompkins, L.S. and Falkow, S. (1981). Use of agarose gel electrophoresis of plasmid deoxyribonucleic acid to fingerprint gram-negative bacilli. J. Clin. Microbiol., 13: 1105-1110.

Sofos, J.N. (2008). Challenges to meat safety in the 21stcentury. Meat Sci.18, 3-13.

Steinrück, H.; Mochmann, H.; Kontny, I.andWerner, U. (1980). *Escherichia coli* O151:K-:h10 ("krim") as etiologic agent of diarrhoea (author's transl)]. Zentralbl Bakteriol A.; 248(3):335-344.

Tobatabaei, R.R. and Mehrabian, S. (2011). Comparison of different typing methods in *Salmonella* strains. World Applied Science Journal 14(6): 914-917.

Toledo, M.R.E.; Alvariza, M.C.B.; Murahovschi, J.; Sramos, S.R.T. and Trabulsi, L.R. (1983). Enteropathogenic Escherichia coli serotypes and endemic diarrhea in infants. Infect. Immun. 39:586-589.

Tornadijo, M.E.; Garcia, M.C.; Fresno, J.M. and Carballo, J. (2001). Study of *Enterobacteriaceae* during the manufacture and ripening of San Simón cheese. Food Microbiol.18, 499-509.

Towner, K.J. (2000). Resistance to antimicrobial agents. Antimicrobial Chemotherapy.4th Edition, Oxford University Press Inc., New York.

Tschäpe, H.; Prager, R.; Streckel, W.; Fruth, A.; Tietze, E. and Bohme, G. (1995). Verotoxinogenic Citrobacter freundii associated with severe gastroenteritis and cases of heamolytic uraemic syndrome in a nursery school: green butter as the infection source. Epidemiol. Infect. 114, 441-450.

Varnam, A.H. and Evans, M.G. (1991). Food borne pathogens. Wolfe publishing Ltd., London.

WHO. (1968). World Health Organization. Microbiology aspects of food hygiene. Technical Report, Series No. 399. World Health Organization (WHO), Geneva.

WHO. (1976). World Health Organization**.** Microbiological aspects of food hygiene. Technical Report, Series No. 598. Geneva.

WHO. (1997). World Health Organization. Consultation on prevention and control of EHEC infections. World Health Organization, Geneva, Switzerland.

توصيف بعض الميكروبات المعوية فى بعض الوجبات السريعة الجاهزة للأكل مع إجراء البلازميد بروفيل للميكروب المعوى

ابتسام محمد محمود فريد - داليا عاطف سالم - ايمان فريد شرف

قسم السيرولوجى (البكترولوجى) و صحة الأغذية بمعهد بحوث صحة الحيوان- الدقى

الملخص العربى

تم تجميع عدد مائتين عينة عشوائية من الوجبات السريعة الجاهزة للأكل (الكفتة – الشاورما-البيف برجر- السجق) (50 عينة من كل نوع) من بعض مطاعم الوجبات السريعة بمحافظة القليوبية حيث تم ارسال هذه العينات مباشرة إلى المعمل لفحصها بكتريولوجيا لمعرفة مدى تواجد الميكروبات المعوية و كانت نتائج العزل تواجد هذه الميكروبات بنسب مختلفة فى هذه الوجبات و الميكروبات المعزولة شملت على البروتيس فيلجارس, البروتيس ماريبلس, الكليبسيلا اذينى, الكليبسيلا نيمونى, السيتروبكتر ديفيرسيس, السيتروبكتر فريندى, سيراتيا مارسينس, انتيروبكتر أيروجينس و أى كولى ولم يتم عزل ميكروب السالمونيلا من آى من العينات المفحوصة. و بالتصنيف السيرولوجى للمعزولات من أى كولى أمكن تحديد تواجد (O_{151}, O_{20}, O_{159}, O_{86a}, O_{111},O_{128})كما تم تحليل الصفة البلازميدية لهذه المعزولات من أى كولى و اثبت التحليل البلازميدى للعترات المعزولة تواجد عدد من البلازميدات فى كل عترة واشتراك المعزولات ((O_{151}, O_{159}, O_{86a}, O_{111}, O_{128})فى الوزن الجزيئى 23130, قاعدة نيتروجينيةو اشتراك ((O_{151}, O_{20}, O_{159},O_{128})فى الوزن الجزيئى 8 قاعدة نيتروجينية و((O_{151}, O_{86a}, O_{111}, O_{128})فى الوزن الجزيئى 7 قاعدة نيتروجينية