Journal of Genetic and Environmental Resources Conservation, 2016, 4(2):109-113.
www.jgerc.com

Isolation and identification of toxigenic strains of *E. coli* from local cheese and evaluate their resistance to antibiotics

Mohammed M. Jaafar[1]*, Hussein O. Kready[2], Salwa Kh. Khalid[3] and Anaam Mahmood[4]

[1,2,4]Agricultural Research Service, Ministry of Science and Technology and [3]Al-Mustansiriyah University, Iraq.
*Corresponding author: mohammedmosa1@yahoo.com

Abstract

Raw milk cheese is considered a rick for food borne shiga-toxin-producing *E. coli* (STEC) contamination .The aim of the study is to isolation and identification of isolated strains by using serological and molecular methods. In this study 60 raw milk cheese samples were collected from different local of Baghdad city (10 sample from each region of Baghdad) such as Algadeda Baghdad, Gesser Diyala, Fudhaliyah, Sader city, Kadhimiyh area and Rashid district. The isolated samples were cultured in selective media for identification and biochemical tests by using API 20 E and also by PCR technique for the presence of (STEC) by using bfp gene markers (stx1 and stx2). The results showed the presence of *E. coli* 0.26 haemorrhagic type (EHEC) and the Enteropathogenic strain of *E. coli* (0.86, 0.127 and 0.128) and the Enteroaggregative strain of *E. coli* (0.55). On the level of serological the isolates gave positive result that indicate presence of shiga toxin (stx1 and stx2)in raw milk cheese .The result also showed multiple resistant of certain antibiotic; amicillin 100%, nalidixic acid and tetracycline and gentamicin 80%, while was observed sensitive to amikacin 100% and trimethoprim and cefotaxin 80%.

Keywords: *Escherichia coli* strains, Toxin, Cheese, Antimicrobial resistance.

Introduction

By a high water content 50.0% and low pH 5.1-5.6 (Freitas *et al.,* 1993). Recovery and counting of *Escherichia coli* is used as an index of recent fecal contamination and indicate that other microorganisms of fecal origin may be The production of local one of the most popular cheeses produced in Iraq. This soft white cheese is made from local pasteurized or raw milk. It is characterized present (Portaria, 1997). After an outbreak of foodborne disease caused by *E. coli* strains (Marier *et al.,* 1973), the presence of these microorganisms in cheese acquired additional significance toxigenic *E. coli* of bovine origin has been classified into five categories: Enterotoxigenic *E. coli* (ETEC), Enteropathgein, *E. coli* (EPEC). Enterohemorrhgic, *E. coli* (EHEC). Enteroinvasive *E. coli*(EIEC) and Enteroaggregative *E. coli* (EAEC) (Quinto and Cepeda, 1997). The first group constitutes of the most important vectors of *E. coli* diarrhea and is considered the major cause of diarrhea in children in developing countries. It is also the most frequently etiological agent responsible for diarrhea (Decludt *et al*., 2000). ETEC causes diarrhea by adhering to the intestinal mucosa by their unique colonization factors, producing either heat-labile enterotoxins (LT-I and LT-II), heat-stable enterotoxins (STa and STb), or both (Nataro and Kaper, 1998). Enteropathocenic *E. coli* , like ETEC and EPEC also causes diarrhea, but the molecular mechanisms of colonization and aetiology are different. EPEC lack fimbriae, ST and LT toxins, but they use an adhesion known as intimin to bind host intestinal cells. This virotype has an array of virulence factors that are similar to those found in shigilla, and may possess a shiga toxin (Bertin, *et al*., 2001). Adherence to the intestinal mucosa causes a rearrangement of actin in the host cell, causing significant deformation. EPEC cells are moderately invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in intestinal cell ultrastructure due to "attachment and effacement is likely the prime cause of diarrhea in those. The most infamous member of this virotype is strain 0157.H7 which causes bloody diarrhea without no fever (Schuller and Frankel, 2004). EHEC can cause haemolytic uraemic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment (*E. coli* common pilus, ECP), is moderately invasive and possesses a phage-encoded shiga toxin that can elicit an intense

inflammatory response. Effect with verocytotoxin (VT)-producing *E. coli* (VTEC) are a well-recognized cause of severe disease in human beings (Fernandez *et al*., 2006). While numerous outbreaks have been related to *E. coli* serotype O157:H7, several other VTEC serotypes have been associated with human diseases (Paton and Paton, 1998). Outbreaks and sporadic cases of illnesses were also traced to consumption of VTEC-contaminated cheese (Deschenes *et al*., 1996). Enteroinvasive (EIEC) infection causes a syndrome that is identical to shigellosis with profuse diarrhea and high fever. Enteroaggregative (EAEC) named because they have fimbriae which aggregate tissue culture cells, EAEC bind to the intestinal mucosa to cause watery diarrhoea without fever. EAEC are noninvasive, they produce a hemolysin and an ST enterotoxin similar to that of ETEC. Cattle are an important reservoir of toxigenic *E. coli*, and have been implicated as a source of *E. coli* that infect and cause disease in human beings (Hornitzky *et al.,* 2002). The aims of the present study were to determine the presence of *E. coli* strains in soft cheese made of raw milk in Baghdad, and also to verify the resistance of *E. coli* strains to antimicrobial agents.

The present study aimed to possibility of using serological and molecular technique to identify certain isolates from raw milk cheese and multiple resistance to certain antibiotic of bacteria.

Materials and Methods

Sixty samples of soft cheese made from raw milk were purchased from local source. All samples were kept at 4 ºC in plastic bag information about dates of production and of assigned shelf life were not presented. Samples were collected over a period of six months between February and July of 2013, and were analyzed for the day of purchasing. Samples were transported at (4-6ºC) in ice boxes containing ice packs and were tested immediately after collection. Twenty five gm of sample were blended with 225ml of nutrient broth for two min at normal speed, using a stomacher lab blender and incubated at 37ºC for 24hrs.1ml of the nutrient broth culture was mixed with 9ml of MacConkey broth and further incubated at 37ºC for 24hrs. One loop of each tube was streaked on MacConkey agar deep red colonies were produced, as the organism lactose-positive . Four colonies from each plate with typical *E. coli* morphology were selected and examined by biochemical tests, including indole-positive (red ring) and methyl red-positive (bright red), but VP-negative (no change-colorless) and citrate-negative (no change-green color) (Koneman *et al*., 1997). Five *E. coli* strains were isolated from sixty cheese samples, and one isolated from each cheese sample was used for further characterization except kadhimiya. Five samples were studied for the determination of O antigens, All *E. coli* colonies were tested for slide agglutination with commercial polyvalent and monovalent antisera and conformed by API 20E . The antimicrobial susceptibility patterns of *E. coli* strains isolated from cheese was done by the disk diffusion method using commercial disks according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLSS, 2002), testing the susceptibility against 7 antibiotic agents tested, loaded on the disks were the following: ampicillin (AMP-10) amikacin (AMI-30) nalidixic acid (NAL-30) cefotaxime (CTX-30) gentamicin (GEN-10) tetracycline (TET-30) trimethoprim (TRI-5). Using Mueller Hinton Agar. Bacterial strains were overnight grown in nutrient broth at 37^{o}C. 100µl sample of the culture was centrifuged and the pellet was resuspended in distilled water. After boiling the suspension for 10min, the supernatant was used as a template for PCR. Used primer for comparison of the company (promega) The base sequences annealing temperatures and predicted sizes of the amplified products for the specific oligonucleotide primers used in this study. The amplified product was visualized by ethidium bromide staining after gel electrophoresis of 10µl of the final reaction mixture in 1.5% agarose.

Results and Discussion

Bacteria were detected in biology laboratory microorganisms and the Ministry of Science and Technology and the diagnosis of strains in the Ministry of Health of the Central Public Health Laboratory by vitek compact-2 (biomero). *E. coli* was isolated from five (8.3%) out of the sixty tested cheeses. Five strains were isolated and were identified by the slide agglutination serological test using available polyvalent and monovalent antisera as pertaining to serogroups O.26, O55, O86 and O127 .O.128 all commonly involved in human diseases (Nataro and Kaper, 1998). Similar results were reported by Araujo *et al.* (2002) who found that 97.0% of soft cheese samples from Rio de Janeiro, Brazil, contained *E. coli* of the same serogroups, among others. Serogroups O111 and O119 have been recognized as important pathogens in Brazil (Gomes et al, 1991) and also have been isolated as STEC strains from diarrheic and non-diarrheic calves in Brazil (Leomil *et al*., 2003).

Table (1): Five sample serogroups of *Escherichia coli* strain isolates from cheese made of raw milk in Baghdad (n= 5) all of them involved in human it big chance diseases.

E. coli serogroups	No. of isolates %
0.26	1 (20%)
0.55	1 (20%)
0.86	1 (20%)
0.127	1 (20%)
0.128	1 (20%)

PCR showed that isolate carried the VT2 gene, and isolate the VT1 gene, a value much higher than 0.4% registered by Quinto and Cepeda (1997) in soft cheese in Spain, but less than the 13.0% reported by Vernozy-Rozand *et al.* (2005) in French cheese. PCR of heat labile (LT-I and LT-II) and heat stable (STa and STb) enterotoxins showed that only one isolate carried the LT-II gene while the ST gene was not found. Frank *et al*. (1984) reported the presence of 3.2% of ETEC strains in milk and milk products. Soft and semi-soft cheese have been previously associated with disease outbreaks involving pathogenic strains of *E. coli* (MacDonald *et al*., 1985; Deschenes *et al*., 1996) which demonstrated that contamination occurs at some point during cheese production and processing. Experimental studies have already shown that *E. coli* O157 can survive during the manufacturing process of soft Hispanic-type cheese (Kasrazadeh *et al*., 1995). These findings indicate that food of animal origin may be a significant source of pathogenic species of *E. coli*. Result showed both isolated were multiple resistance to ampicillin 100%, nalidixic acid, tetracycline and gentamicin 80%.While was observed sensitive to amikacin 100% and trimethoprim and cefotaxim 80%. Most frequent resistance was observed to the following antimicrobials: nalidixic acid (40mm), tetracycline (31mm) and ampicillin (29mm). Zhao *et al*. (2001) examined VTEC strains from humans, animals and food, and reported a high antimicrobial sensitive to amikacin (48mm), trimethoprim (48mm) and cefotaxim (33mm), and some of them were similar to those found in this study. Resistance to at least one of a series of tested antimicrobial agents was found in 83.0% of the examined isolates. Khan *et al*. (2002) reported resistance to one or more antimicrobials 49.2% of the VTEC strains in India, moreover, some of those strains showed multidrug-resistance. The high level of resistance may be a consequence of the abusive use of antimicrobials in animal therapeutics as well as in food additives used to promote animal growth.

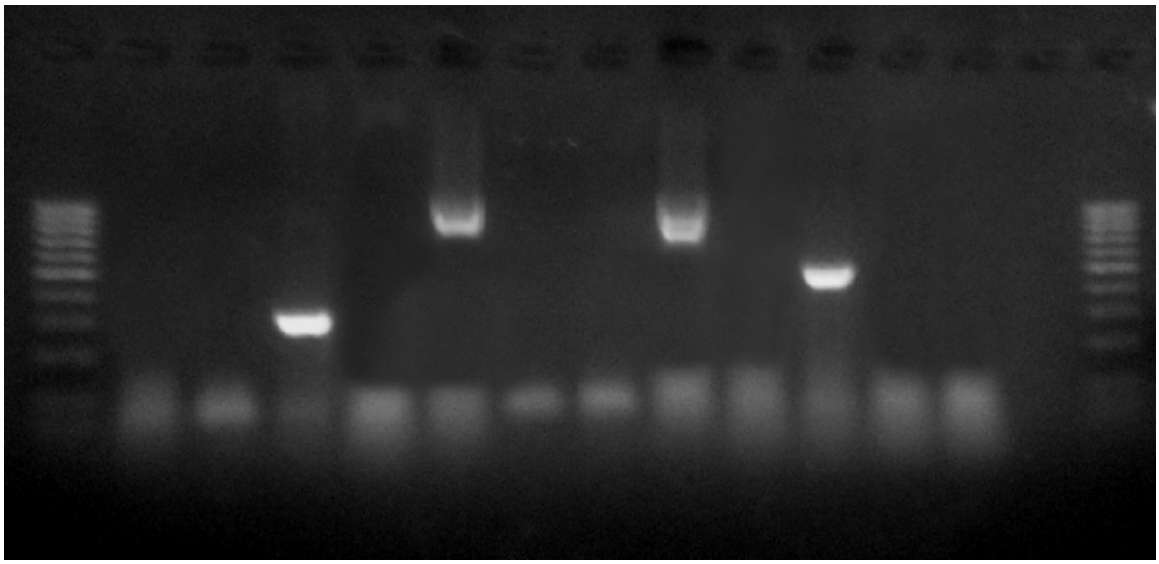

Figure (1): Gel electrophoresis of amplified *bfpA* (326bp), SLT I (894bp) and SLT II (478bp) from *E. coli* DNA isolated from cheese specimens. Agarose (1.5%), 5V/cm for 2 hrs, stained with ethidum bromide and visualized on a UV transilluminator.
Lane 1 and 14 . 100 bp DNA ladder.
Lane 2-5. Amplified *bfp* gene of Serogroups 55, 86, 127 and control respectively.
Lane 6-10. Amplified SLT I gene of Serogroups 26, 55, 86, 128 and control respectively.
Lane 11-13. Amplified SLT II gene of Serogroups 55, 86 and control respectively.

Table (2): The table below shows sensitivity of the test *E. coli* strain measured in millimeter(mm) zone of inhibition according to (NCCL)*

		Serogroups				
Antibiotic	Inter mediate	0.26	0.55	0.86	0.127	0128
Ampicillin	16-14	R	R	R	R	R
Trimethoprim	15-11	S 37 mm	R	S 30 mm	S 25 mm	S 20 mm
Nalidixic Acid	18-14	R	R	R	S 19 mm	R
Amikacin	16-15	S 35 mm	S 33 mm	S 30mm	S 30 mm	S 29 mm
Tetracycline	19-16	R	R	R	S 22 mm	R
Cefotaxine	22-15	R	S 30 mm	S 30 mm	S 24 mm	S 26 mm
Gentamicin	19-16	R	R	S 22mm	R	R

Resistant(R), Sensitive(S), zone of inhibition millimeter(mm), Control (zero), *National Committee for Clinical Laboratory Standards (NCCL).

Conclusion

In conclusion, that toxigenic *E. coli* may pass to the milk destined results of other authors (Quinto and Cepeda, 1997; Araujo *et al.,* 2002; Vernozy-Rozand *et al.,* 2005). They represent a health hazard and this suggests that soft cheese should be considered a vehicle for the transmission of potentially pathogenic bacteria to manufacture cheese, surviving in soft cheese made of raw milk, confirming the.

References

Araujo, V.S.; Pagiares, V.A.; Queiroz, M.L.P. 2002. Occurrence of *Staphylococcus* and enteropathogens in soft cheese commercialized in the city of Rio de Janeiro, Brazil. J. Appl. Microbiol., 92: 1172-1177, .

Bertin, Y.; Boukhors, K.; Pradel, N.; Livrelli, V. and Martin, C. 2001. Stx2 subtyping of Shiga toxin production *Escherichia coli* isolated from cattle in France: detection of a new Stx2 subtype and correlation with additional virulence factors. J. Clin. Microbiol., 39: 3060-3065

Deschenes, G.; Casenave, C. and Grimont, F. 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurise cheese. Pediatr. Nephrol., 10: 203-205.

Decludt, B.; Bouvet, P.; Mariani-Kurkdjian, P.; Grimont, F.; Grimont, P.A. and Hubert, B. 2000. Haemolytic uraemic syndrome and shiga toxin-producing *Escherichia coli* infection in children in France. Epidemiol. Infect., 124: 215-220.

Fernandez, G.C.; Lopez, M.F.; Gomez, S.A.; Ramos, M.V.; Bentancor, L.V. and Fernandez-Brando, R.J. 2006. Relevance of neutrophils in the murine model of haemolytic uraemic syndrome: mechanisms involved in shiga toxin type 2-induced neutrophilia. Clin. Exp. Immunol., 146: 76-84.

Frank, V.; Hahn, G. and Tolle, A. 1984. Incidence and identification of enterotoxigenic *Escherichia coli* strains in milk and milk products. Zentral. Bakteriol. Microbiol. Hyg., 257:51-59.

Freitas, A.C.; Nunes, M.P. and Milhomem, A.M. 1993. Occurrence and characterization of *Aeromonas* species in pasteurized milk and white cheese in Rio de Janeiro, Brasil. J. Food Prot., 5: 62-65.

Gomes, T.A.T.; Viera, M.A.M. and Wachsmuth, I.K. 1991. Enteropathogens associated with acute diarrheal diseases in urban infants in São Paulo, Brazil. J. Infect. Dis., 164: 331-337.

Hornitzky, M.A.; Vanselow, B.A. and Walker, K. 2002. Virulence properties and serotypes of shiga toxin-producing *Escherichia coli* from healthy Australian cattle. Appl. Environ. Microbiol., 68: 6439-6445.

Kasrazadeh, M. and Genigeorgis, C. 1995. Potential growth and control of *Escherichia coli* O157: H7 in soft hispanic type cheese. Int. J. Food Microbiol., 25: 289-300.

Khan, A.; Das, S.C. and Ramamurthy, T. 2002. Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. J. Clin. Microbiol., 40: 2009-2015.

Koneman, E.W.; Allen, S.D. and Dowell, V.R. 1997. Diagnóstico microbiológico. 2nd ed., São Paulo: Panamericana, 61-132pp.

Leomil, L.; Aidar-Ugrinovich, L. and Guth, B.E.C. 2003. Frequency of shiga toxin-producing *Escherichia coli* (STEC) isolates among diarrheic and non-diarrheic calves in Brazil. Vet. Microbiol., 97: 103-109.

Macdonald, K.L.; Eidson, M. and Strohmeyer, C. 1985. A multistate outbreak of gastrointestinal illness caused by enterotoxigenic *Escherichia coli* in imported semi soft cheese. J. Infect. Dis., 151: 716-720.

Marier, R.; Wells, J.G. and Swanson, R.C. 1973. An

outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. Lancet, 1376-1378.

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

National Committee for Clinical Laboratory Standards, (NCCLSS). 2002. Performance standards for antimicrobial disk susceptibility testing. Wayne, P.A. Document M-100-S12, 22, n°1.

Paton, J.C. and Paton, A.W. 1998. Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev., 11: 450-479.

Portaria nº 451 de 19 de setembro de 1977. Princípios gerais para o estabelecimento de critérios e padrões microbiológicos para alimentos. Brasília: Secretaria de Vigilância Sanitária – MS, 1997.

Quinto, E.J. and Cepeda, A. 1997. Incidence of toxigenic *Escherichia coli* in soft cheese made with raw or pasteurized milk. Lett. Appl. Microbiol., 24: 291-295.

Schuller, S.; Frankel, G. and Phillips, A.D. 2004. Interaction of shiga toxin from *Escherichia coli* with human intestinal epithelial cell lines and explants: Stx2 induces epithelial damage in organ culture. Cell Microbiol., 6: 289-301.

Vernozy-Rozand, C.; Montet, M.P. and Beradin, M. 2005. Isolation and characterization of Shiga toxin-producing *Escherichia coli* strains from raw milk cheeses in France. Lett. Appl. Microbiol., 41: 235-241.

Zhao, S.; White, D.G.; Ayers, S. 2001. Identification and characterization of integron-mediated antibiotic resistance among shiga toxin-producing *Escherichia coli* isolates. Appl. Environ. Microbiol., 67: 1558-1564.