MOLECULAR CHARACTERIZATION OF SOME GRAM-NEGATIVE BACTERIA ISOLATED FROM MILK.

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ABSTRACT

Milk isn't only a perfect food, but also an ideal media for microbes, thus it is a major pathogen vehicle. This study was conducted to characterize molecularly some of Gram-negative pathogens in random150 raw milk samples (100 from healthy dairy farm animals and 50 from milk vendors) collected from ElmahallaElkobra and Mansoura cities, Egypt, then 20 farm samples with subclinical mastitis were excluded. Gram-negative bacteria were isolated and identified biochemically from the other 130 samples; the predominant species percentages were Escherichia coli 25.4, Serratiamarcescens 13.8, Kluyveraascorbata 8.5, Citrobacterdiversus 7.7, Klebsiellaoxytoca 5.4, and Klebsiellapneumoniae3.8. 18 shigatoxin producingE. coli (STEC) strains were classified serologically to 8 non-O157 serotypes, and the predominant was O26:H11 (27.78%). By PCR;16S-23S rDNA was identified in 3 K. pneumoniae, and shiga toxins (stx1 and 2), α-haemolysin (hlyA) and intimin (eaeA) genes were detected in 15, 13, 7 and 9 STEC respectively. Conclusively, raw milk still contributes a public hazard,so, better effective control measures are required.

INTRODUCTION

Milk has a marvelous nutritional value, but is also a perfect medium for growth of bacteria,and it can be contaminated from various sources; within the udder, exterior to the udder and from the surface of milk handling and storage equipment **(*Panigrahiet al., 2018*)**, due to poor handling and storage **(*Ogotet al., 2015*)**. It is a staple food in epidemiology linked to zoonotic pathogens, as it acts as a dangerous source of pathogenic bacteria (mainly GNB) such as *Salmonella* spp., *E. coli*, and other coliforms that have a public human health hazard **(*Panigrahiet al., 2018*)**, and also a reservoir for a diverse group of microorganisms that can cause spoilage**(*O'Sullivan and Cotter 2017*)**. Despite all these dangers,there is an increasing trend toward raw milk consumption as its health benefits are believed to be destroyed upon heating **(*Claeys et al., 2013*)**, and the demand of safe and high quality milk urges for more studies to ensure these demands, therefore this study aimed to characterize molecularly by PCR some of the pathogenic GNB isolated from raw milk samples.

MATERIAL AND METHODS

Sampling: A total of 150 random milk samples; 100 quarter milk samples from apparently healthy dairy animals (buffalo and cow), and 50 from milk vendors, were collected aseptically in sterile container from Mansoura and ElmahallaElkobra cities in Egypt, from November 2016 to June 2017, and farm samples were collected after cleaning, drying udders and scrubbing the tip of each teat with 70% ethanol, then discarding the first few streams. All samples were transferred to the laboratory of Microbiology department, faculty of Vet. Med., Kafrelsheikh University, in icebox under aseptic conditions, and examined as soon as possible. **California mastitis test (CMT):** CMT was carried out as described in ***A.P.H.A.(2004)*** on farm samples using Schalm reagent, and 20 positive samples were excluded. **Isolation and biochemical identification of GNB:** After mixing, 1 ml of each sample was nourished in 9 ml nutrient

broth, at 37ºC for 24 hours, then a loopful was streaked on MacConkey's agar, and incubated at 37ºC for 24-48hrs.Purified single colonies were identified based on Gram stain reaction, KOH test, cultural characters and biochemical tests (IMViC, TSI, LIA and urease test) **(*Quinn et al., 2002*)**. Suspected *E. coli* and *K. pneumoniae*isolates were streaked on EMB agar, and subjected to additional confirmatory tests (gelatin hydrolysis, oxidation–fermentation, nitrate reduction, ODC, ADH, ONPG and sugars fermentation tests) **(*Kreig and Holt, 1984*)**. **Serotyping of *E. coli* isolates:** *E. coli* isolates were transferred on semi-solid agar to Food Analysis Center, Faculty of Veterinary Medicine, Benha University for serotyping of somatic (O) and flagellar (H) antigens by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) **(*Kok et al., 1996*)**. **Identification of STEC and *K. pneumoniae* isolates by PCR:** Bacterial DNA was extracted from the isolates according to ***Shah et al. (2009)*** by QIA amp kit. *E. coli* isolates were tested by multiplex PCR for *stx1*, *stx2*, *eaeA* and*hlyA* genes, using Pharmacia Biotech primers (Table 1), and the amplification reaction was performed on a thermal cycler using 25 *µl*as described by ***Fagan et al.(1999)***; initial denaturation at 95°C/3 min, followed by 35 cycles of amplification (denaturation at 95°C/20 sec, annealing at 58°C /40 sec, and extension at 72°C/90 sec) and final extension at 72°C/5 min. The control positive was *E. coli* O157:H7 Sakai strain. Amplified products were analyzed by 2% agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TAE buffer stained with ethidium bromide to be visualized under UV transillumator **(*Sambrook et al., 1989*)**.*K. pneumoniae*isolates were tested for 16S-23S rDNA internal transcribed spacer (specific for identification of *K. pneumoniae*) (Table 1) **(*Liu et al., 2008*)**. Amplification of PCR; initial denaturation at 94°C/10 min, followed by 35 cycles of denaturation at 94°C/30 sec, annealing at 57°C/20 sec and extension at 72°C/20 sec, followed by a final extension at 72°C/10 min. The control positive was*K. pneumoniae*ATCC 700721 (MGH 78578)strain.Amplified products were analyzed by 1% of agarose gel electrophoresis.

RESULTS

Isolated Gram-negative: Out of 130 non-repeated Gram-negative bacterial isolates; 113 belonged to 20 species were identified according to ***Farmer et al. (2007)***, in which *E. coli* predominated (Table 2).**Serotyping of STEC:** 18 STECisolates classified with in 8 different non-O157 serotypes (Table 3), and O26:H11 (5) was the predominant. **PCR results:** *stx*1, *stx*2, *eaeA* and *hlyA* were detected in 15, 13, 7, and 9 STEC respectively (Fig. 1, and Table 3), and 3 *K. pneumoniae*isolates tested positive for 16S-23S rDNA(Fig. 2).

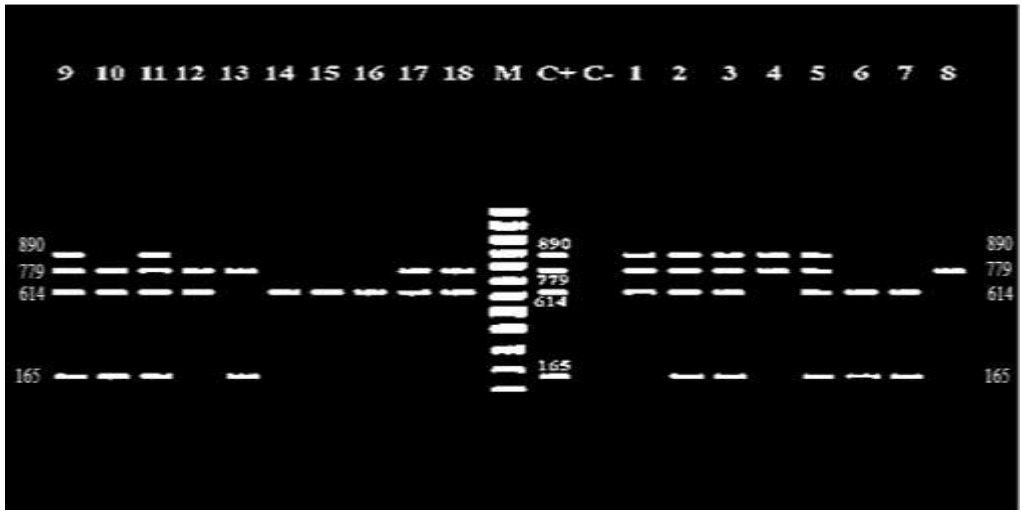

Fig. (1): Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA*(165 bp) genes: **M:** 100 bp ladder as molecular size DNA marker, **C+:** Control positive *E. coli* for*stx1*, *stx2*,*eaeA*and *hlyA* genes, **C-:** Control negative: **1** (O26): Positive for *stx1*, *stx2* and*eaeA*; **2**, **3**, **5** (O26), **9**&**11** (O111): Positive for *stx1*, *stx2*,*eaeA*and *hlyA*; **4** (O26): Positive for *stx1*and *eaeA*; **6**&**7** (O55): Positive for *stx1*and *hlyA*; **8** (O86): Positive for stx1;**10** (O111): Positive for *stx1*, *stx2*and *hlyA*; **12** (O119), **17**&**18** (O146): Positive for stx1 and *stx2*; **13** (O121): Positive for *stx2*and *hlyA*; **14**, **15**&**16** (O128): Positive for *stx1*.

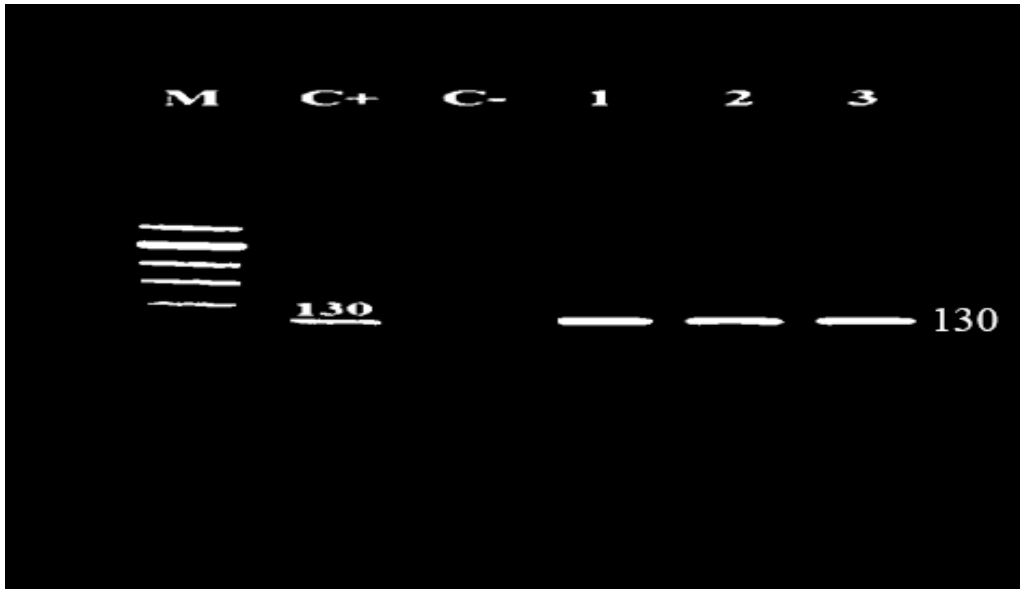

Fig. (2): Agarose gel electrophoresis of PCR of 16S-23S rDNA"ITS" (130 bp) specific for detection and identification of *Klebsiellapneumoniae.* **M:** 100 bp ladder as molecular size DNA marker; **C+:** Control positive *K. pneumoniae* for 16S-23S ITS; **C-:** Control negative. **1**, **2**&**3**: Positive *K. pneumoniae* strains.

Table (1): Sequences of identification primers

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
*stx*1 (F)	**5′** ACACTGGATGATCTCAGTGG **′3**	614	***Dhanashree and Mallya(2008)***
*stx*1(R)	**5′** CTGAATCCCCCTCCATTATG **′3**		
*stx*2(F)	**5′** CCATGACAACGGACAGCAGTT **′3**	779	
*stx*2 (R)	**5′** CCTGTCAACTGAGCAGCACTTTG **′3**		
eaeA (F)	**5′** GTGGCGAATACTGGCGAGACT **′3**	890	***Mazaheri et al. (2014)***
eaeA (R)	**5′** CCCCATTCTTTTTCACCGTCG **′3**		
hlyA (F)	**5′** ACGATGTGGTTTATTCTGGA **′3**	165	***Fratamico et al. (1995)***
hlyA (R)	**5′** CTTCACGTGACCATACATAT **′3**		
16S-23S ITS (F)	**5′** ATTTGAAGAGGTTGCAAACGAT **′3**	130	***Liu et al. (2008)***
16S-23S ITS (R)	**5′** TTCACTCTGAAGTTTTCTTGTGTTC **′3**		

Table (2): Incidence of Gram-negative bacteria in milk samples

Isolated bacteria	Farm samples NO. %	Vendors samples NO. %	Total NO. %
Buttiauxellagaviniae	2 (2.41)	0	2 (1.54)
Citrobacterdiversus	10 (12.05)	0	10 (7.69)
Citrobacterfreundii	2 (2.41)	0	2 (1.54)
Citrobacter group 137	2 (2.41)	0	2 (1.54)
Citrobactermurliniae	2 (1.205)	0	2 (1.54)
Enterobacteraerogenes	0	4 (8.51)	4 (3.07)
Escherichia coli	26 (31.33)	7 (14.89)	33 (25.38)
Escherichia vulneris	0	1 (2.13)	1 (0.77)
Klebsiellaoxytoca	2 (2.41)	5 (10.63)	7 (5.38)
Klebsiellaozaenae	0	1 (2.13)	1 (0.77)
Klebsiellapneumoniae	0	5 (10.63)	5 (3.85)
Kluyveraascorbata	5 (6.02)	6 (12.77)	11 (8.46)
Kluyvera Georgiana	0	3 (6.38)	3 (2.31)
Moellerellawisconsensis	1 (1.20)	0	1 (0.77)
Morganellamorganii	0	1 (2.13)	1 (0.77)
Salmonella species	3 (3.61)	1 (2013)	4 (3.08)
Serratiafonticola	1 (1.205)	0	1 (0.77)
Serratiamarcescens	8 (9.64)	10 (21.28)	18 (13.85)
Serratiarubidaea	1 (1.205)	1 (2.13)	2 (1.54)
Tatumellaptyseos	1 (1.205)	0	1 (0.77)
Non*Enterobacteriaceae*	14 (16.87)	0	14 (10.76)
Un identified	3 (3.61)	2 (4.26)	5 (3.85)
Total	83/130 (63.85)	47/130 (36.15)	130 (100)

Table (3): Distribution of Serotypes and virulence genes in STECisolates

serotypes	Farm samples NO. %	Vendor samples NO. %	Total NO. %	*stx*1 NO. %	*stx*2 NO. %	*eaeA* NO. %	*hlyA* NO. %
O26:H11	5 (33.32)	0	5 (27.78)	4 (80)	5 (100)	5 (100)	3 (60)
O55:H7	1 (6.67)	1 (33.33)	2 (11.11)	2 (100)	0	0	2 (100)
O86	1 (6.67)	0	1 (5.56)	0	1 (100)	0	0
O111:H2	3 (20)	0	3 (16.67)	3 (100)	3 (100)	2 (66.7)	3 (100)
O119:H6	1 (6.67)	0	1 (5.56)	1 (100)	1 (100)	0	0
O121:H7	0	1 (33.33)	1 (5.56)	0	1 (100)	0	1 (100)
O128:H2	3 (20)	0	3 (16.67)	3 (100)	0	0	0
O146:H21	1 (6.67)	1 (33.34)	2 (11.11)	2 (100)	2 (100)	0	0
Total	15/18(83.33)	3/18(16.67)	18 (100)	15 (83.33)	13 (72.22)	7 (38.89)	9 (50)

DISCUSSION

Despite milk is sterile at secretion, it can be contaminated, and consumption of raw milk is involved with food-poisoning and zoonotic outbreaks **(*Sulaiman and Hsieh, 2017*)**. In this study non-repeated 130GNB isolates were obtained in variable incidences (Table 2)andthe predominant species was *E. coli*.GNB are a major causative agents of several types of infections **(*Hidron et al., 2008*)**, and their occurrence in raw milk in general is an indicator of faecal contamination, but it might be originating from other environmental sources, so it may indicates low sanitation program and unhygienic environmental conditions. There is to some extent similarity betweenthese results and ***Ntuli et al. (2016)***in which*E. coli* (22.8%) predominated in raw milk, while lower incidences werereported by ***AbdEl Aalet al.(2016)***.

*E. coli*is a major food-borne bacteria,and the main microbe responsible for ~2–4 billion diarrheal episodes/year, and a main cause of morbidity and mortality in children **(*Johnson, 2018*)**. Previous studies proved that it is a reliable indicator of faecal pollution, poor hygiene and sanitary conditions during milking and handling. The incidence of *E. coli* in this study (25.38%) was almost the same as ***Eid (2014)***(26.67%) in Gharbia, and near ***Zeinhom and Abdel-Latef (2014)*** (21.35%) in Beni-Suef, however they detected higher incidence in market samples than farm samples. Higher incidenceswere reported in Egypt by ***Youssef and Mohamed, (2015)***, and in many studies in other countries such as***Nema (2014)***, while lower incidences were reported by ***Merwad et al. (2014)***in Sharkiaand ***Younis et al. (2018)*** in Mansoura city.

Serotyping detected 18 STEC in this study within 8 serotypes (Table 3), in which the predominant was O26 (27.78%), and most of

these serotypes were identified in Egypt by ***Merwad et al.(2014)***, and ***Younis et al.(2018)***with different percentage. Non-O157 serotypes can cause diseases not less severe than O157 **(*Brooks et al., 2005*)**, and O26, O86 and O111 were identified in mastitis in Egypt **(*Osman et al., 2012*)**.

All serotypes were positive for at least one virulence gene (Table 3) of; *stx*1 and 2;the key factor and *hlyA*; a major factor in the pathogenesis of many diseases in human and animal all over the world mainly haemorrhagic colitis and haemolytic uremic syndrome (HUS) **(*Bielaszewskaet al., 2013*;*Villyssonet al., 2017*)**, and *eaeA* encoding intimin that perform the characteristic "attaching and effacing" (A/E) phenomena as a main mechanism of the bacterial colonization **(*Leo et al., 2015*)**, in which5 serotypes (27.78%) harbored the 4 virulence genes within O111 and O26. Lower incidences of *stx* and *eaeA* were obtained by ***Merwad et al. (2014)***,and of *hlyA*by ***Younis et al. (2018)***.The combinations of the four genes have also been reported by ***Rashidet al. (2013)***.

K. pneumoniae(an important opportunistic GNB and a leading human nosocomial pathogen **(*Wang et al., 2017*)**), was identified in 3.85% of all the samples, that is near to the results of ***Abdel Hameed (2017)***(2.92%) in Assiut and Qena cities, however lower than ***Badri et al. (2017)***. By PCR, 3 *K. pneumoniae* isolates were positive for 16S-23S rDNA, and this wasn't an aspect of the previously mentioned studies.

These variations might be regarded to variation in geographical location, season, farm size, animal health and feed, number of animals on the farm, management practices, type of milking and hygiene status, the type and number of samples, time of sampling, isolation and testing detection methods **(*Nema, 2014*)** and sources of contaminations, whichmake the comparisons of different studies difficult **(*Xia et al., 2010*)**.

CONCLUSION

The obtained results prove that raw milk in the examined area was highly contaminated with GNB, even the pathogenic one such as STEC harboring at least one virulence gene and *K. pneumoniae*, which constitutes a health hazard for the consumers.

Therefore, the applied hygienic measures should be reconsidered and its application must be checked, more strict preventive hygienic measures is needed, and on-site pasteurization should be applied to improve the quality of milk, and ensure its safety.

REFERENCES

- ***A.P.H.A. (American Public Health Association) (2004):*** Standard Methods For Examination of Dairy Products. 17thEd. USA. www.apha.org.
- ***Abd El Aal, S. F. A.; Amer, I. H.; Mansour, M. A. H. andAlgendy, R. M. M. (2016):*** Bacteriological Studies of Raw Cow's Milk in Zagazig Markets, In 3rd International Conference of Food Safety "Environmental Hazards and Food Safety" Damanhour University, Egypt 10th October 2016.
- ***Abdel Hameed, K. G. (2017):*** Widespread acquisition of antimicrobial resistance of Klebseillapneumoniae isolated from raw milk and the effect of cinnamon oil on such isolates. International Food Research Journal, 24(2): 876-880
- ***Badri, A. M.; Ibrahim, I. T.; Mohamed, S. G.; Garbi, M. I.; Kabbashi, A. S. and Arbab, M. H. (2017):*** Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing Escherichia coli and Klebsiellapneumoniae Isolated from Raw Milk Samples in Al Jazirah State, Sudan. Molecular Biology, 7(1).

- ***Bielaszewska, M.; Rüter, C.; Kunsmann, L.; Greune, L.; Bauwens, A.; Zhang, W.; Kuczius, T.; Kim, K.S.; Mellmann, A.; Schmidt, M.A. and Karch, H. (2013):*** Enterohemorrhagic Escherichia coli hemolysin employs outer membrane vesicles to target mitochondria and cause endothelial and epithelial apoptosis. PLoS pathogens, 9(12), p.e1003797.

- ***Brooks, J. T.; Sowers, E. G.; Wells, J. G.; Greene, K. D.; Griffin, P. M.; Hoekstra, R. M. and Strockbine, N. A. (2005):*** Non-O157 Shiga toxin–producing Escherichia coli infections in the United States, 1983–2002. The Journal of infectious diseases, 192(8): 1422-1429.

- ***Claeys, W. L.; Cardoen, S.; Daube, G.; De Block, J.; Dewettinck, K.; Dierick, K.; De Zutter, L.; Huyghebaert, A.; Imberechts, H.; Thiange, P.; Vandenplas, Y., and Herman, L. 2013.*** Raw or heated cow milk consumption: Review of risks and benefits. Food Control, 31(1): 251-262.

- ***Dhanashree, B. and Mallya, S. (2008):*** Detection of shiga-toxigenic Escherichia coli (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Indian Journal of Medical Research, 128: 271-277.

- ***Eid, A. M. 2014.*** Molecular identification of some contagious microorganisms causing food poisoning from bulk tank milk in Gharbia Governorate. Benha Veterinary Medical Journal, 27(2): 29-47.

- ***Fagan, P. K.; Hornitzky, M. A.; Bettelheim, K. A. and Djordjevic, S. P., 1999.*** Detection of Shiga-like toxin (stx1 and stx2), intimin (eaeA), and enterohemorrhagic Escherichia coli (EHEC) hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR. Applied and Environmental Microbiology, 65(2), pp.868-872.

- ***Farmer III, J. J.; Boatwright, K. D. and Janda J. M. (2007):*** Enterobacteriaceae: Introduction and Identification, p. 649-669, in P. R. Murray, E. J. Barron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller, Manual of Clinical Microbiology, 9th Edition. ASM Press, Washington, DC.

- ***Fratamico, P. M.; Sackitey, S. K.; Wiedmann, M. and Deng, M. Y. (1995):*** Detection of Escherichia coli O157: H7 by multiplex PCR. Journal of Clinical Microbiology, 33(8): 2188-2191.

- ***Hidron, A. I.; Edwards, J. R.; Patel, J.; Horan, T. C.; Sievert, D. M.; Pollock, D. A. and Fridkin, S. K. (2008):*** Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infection Control and Hospital Epidemiology, 29(11): 996-1011.

- ***Johnson, D. I. (2018):*** Escherichia spp. In Bacterial Pathogens and Their Virulence Factors, Springer, 209-239.

- ***Kok, T.; Worswich, D. and Gowans, E. (1996):*** Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.

- ***Kreig, N. and Holt, J. (1984):*** Bergey's Manual of systemic bacteriology,Vol.1.William and Wilkins,Baltimore,M.D. 21202, USA.

- ***Leo, J. C.; Oberhettinger, P.; Schütz, M. and Linke, D. (2015):*** The inverse autotransporter family: intimin, invasin and related proteins. International Journal of Medical Microbiology, 305(2): 276-282.
- ***Liu, Y.; Liu, C.; Zheng, W.; Zhang, X.; Yu, J.; Gao, Q.; Hou, Y. and Huang, X. (2008):*** PCR detection of Klebsiellapneumoniae in infant formula based on 16S–23S International journal of food microbiology, 125(3): 230-235.
- ***Mazaheri, S.; Ahrabi, S. and Aslani, M. (2014):*** Shiga Toxin-Producing Escherichia Coli Isolated From Lettuce Samples in Tehran, Iran. Jundishapur journal of microbiology, 7 (11): 1-6.
- ***Merwad, A.; Gharieb, R. and Saber, T., (2014):*** Occurrence of shiga toxin-producing Escherichia coli in lactating cows and in contact workers in Egypt: serotypes, virulence genes and zoonotic significance. Life Science Journal, 11(5): 563-571.
- ***Nema, P. (2014):*** Prevalence and molecular characterization of Escherichia coli from milk and its products (Doctoral dissertation, NanajiDeshmukh Veterinary Science University Jabalpur).
- ***Ntuli, V.; Njage, P. M. K. and Buys, E. M. (2016):*** Characterization of Escherichia coli and other Enterobacteriaceae in producer-distributor bulk milk. Journal of dairy science, 99(12): 9534-9549.
- ***O'Sullivan, O., and Cotter, P. D. (2017):*** Microbiota of Raw Milk and Raw Milk Cheeses. In: P. L.H. McSweeney, P. F. Fox, P. Cotter and D. W. Everett, Cheese (4th Ed), Elsevier Ltd: 301-316.

- ***Ogot, H. A.; Ochuodho, H. O. and Machoka, R., 2015:*** Microbial analysis of raw and boiled milk sold at Baraton center in Nandi County, Kenya. Baraton Interdisciplinary Research Journal, (5): 113-117.

- ***Osman, K. M.; Mustafa, A. M.; Aly, M. A. and AbdElhamed, G. S. (2012):*** Serotypes, virulence genes, and intimin types of Shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli isolated from mastitic milk relevant to human health in Egypt. Vector-Borne and Zoonotic Diseases, 12(4): 297-305.

- ***Panigrahi, S.; Devi, B.; Swain, K. and Priyadarshini, P., 2018.*** Microbiology of milk: Public health aspect. The Pharma Innovation Journal, 7(1): 260-264.

- ***Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donelly, W. J. C. and Leonard, F. C. (2002):*** Veterinary Microbiology and Microbial diseases. 2ed. Blackwell Science Ltd. NdBodmin, Cornwall, UK.

- ***Rashid, M.; Kotwal, S.K.; Malik, M. A. and Singh, M. (2013):*** Prevalence, genetic profile of virulence determinants and multidrug resistance of Escherichia coli isolates from foods of animal origin. Veterinary World, 6(3): 139-142.

- ***Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989):*** molecular cloning: A laboratory manual (Ed. 2). Cold spring Harbor Laboratory Press. New York.

- ***Shah, D.; Shringi, S.; Besser, T. and Call, D. (2009):*** Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA, 369-389.

- ***Sulaiman, I. M., and Hsieh, Y. H. (2017):*** Food borne Pathogens in Milk and Dairy Products: Genetic Characterization and Rapid Diagnostic Approach for Food Safety of Public Health Importance. In Dairy in Human Health and Disease Across the Lifespan, 127-143.
- ***Villysson, A.; Tontanahal, A. and Karpman, D. (2017):*** Microvesicle Involvement in Shiga Toxin-Associated Infection. Toxins, 9(11): 376.
- ***Wang, J.; Shao, Y.; Wang, W.; Li, S.; Xin, N.; Xie, F. and Zhao, C. (2017):*** Caspase-11 deficiency impairs neutrophil recruitment and bacterial clearance in the early stage of pulmonary Klebsiellapneumoniae infection. International Journal of Medical Microbiology, 307(8): 490-496.
- ***Xia, X.; Meng, J.; McDermott, P.F.; Ayers, S.; Blickenstaff, K.; Tran, T.T.; Abbott, J., Zheng, J. and Zhao, S. (2010):*** Presence and characterization of Shiga toxin-producing Escherichia coli and other potentially diarrheagenic E. coli strains in retail meats. Applied and environmental microbiology, 76(6): 1709-1717.
- ***Younis, G.; Awad, A. and Ghabour, R. (2018):*** Prevalence and virulence determinants of Escherichia coli isolated from raw cow's milk. African Journal of Microbiology Research, 12(9): 225-229.
- ***Youssef, A. I. and Mohamed, S. E. (2015):*** Raw Milk as a Potential Source of Some Zoonotic Bacterial Diseases in Ismailia, Egypt. Global Veterinaria, 14 (6): 824-829.
- ***Zeinhom, M. M., and Abdel-Latef, G. K. (2014):*** Public health risk of some milk borne pathogens. Beni-Suef University Journal of Basic and Applied Sciences, 3(3), 209-215.

الملخص العربي

اللبن ليس فقط غذاء متكامل، لكنه أيضاً بيئة مثالية لنمو الميكروبات، لذا فإنه يُعتبر حامل رئيسي للميكروبات المُمرضة، و تهدف هذه الدراسة للتوصيف الجزيئي لبعض البكتريا سالبة الجرام المعزولة من 150 عينة لبن خام (100 من حيوانات مزرعة سليمة و 50 من الباعة الجائلين) تم تجميعها عشوائياً من مدينتي المحلة الكبرى والمنصورة بمصر، ثم تم إستبعاد 20 عينة لإصابتها بإلتهاب ضرع تحت إكلينيكي. تم عزل البكتريا سالبة الجرام من ال130 عينة المتبقية و التعرف عليها بواسطة الإختبارات الكيميائية الحيوية، فوُجد أن ميكروب الإيشريشيا القولونية هو الأكثر عزلاً بنسبة 25.4، يليه سيراتيا مارسيسينس 13.8، ثم كليوفيرا اسكورباتا 8.5، ثم سيتروباكتر دايفيرسس 7.7، ثم كليبسييلا أوكسي توسا 5.4، ثم كليبسييلا نيموني 3.8. بعمل تصنيف سيرولوجي لمعزولات الاي كولاي، وُجد أن كل العينات التي تم اختبارها تنتمي لعترات أخرى بخلاف O157، والأكثر عزلاً كان عترةO26:H11بنسبه 33.32. بإجراء تفاعل إنزيم البلمرة المتسلسلعلى معزولات كليبسييلا نيموني وُجد أن ثلاثة منها تحوي جين 16S-23S rDNA ، بينما وُجدت الجينات الأربعة stx1 و stx2 و eaeA و hlyA في 15 و 13 و 7 و 9 من معزولات الإي كولاي على التوالي. مما سبق نستنتج أن اللبن الخام مازال يشكل خطراً على الصحة العامة، لذا يجب إتخاذ إجراءات وقائية أفضل.