Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Molecular characterization and microbial resistance of different bacterial isolates in some dairy products

W.K. Alkhafaje[1], Z.A. Olama[1] and S.M. Ali[2]

[1]Botany and Microbiology Department, Faculty of Science, Alexandria University, [2]Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological applications, Alexandria, Egypt

Article information

Article history:
Received June 10, 2021
Accepted August 14, 2021
Available online March 1, 2022

Keywords:
Multidrug resistance
Gene
Bacteria
Milk
Dairy products

Correspondence:
S.M. Ali
safaa.mohamedali@yahoo.com

Abstract

Bacterial contamination of milk and dairy products is a common problem. In the last two years, the foodborne diseases caused by the intake of milk and dairy products have been mostly disturbed with *Salmonella entertica*, *Listeria monocytogenes Escherichia coli* 0157:H7 and *Campylobacter jejune*. The study aims to isolate multidrug resistance (MDR) bacteria in dairy products and study of the molecular characterization of that isolates. MDR bacteria were found in 30 out of the 131 bacterial isolates. The incidence of MDR bacterial isolates revealed the abundance of *Staphylococcus* sp. with 43.3%, *Bacillus* sp 16.7%, *Salmonella* 13.3%, *E. coli* 10 %, *Enterococcus* 6.7 % *Psedoumonas* 3.3 %, *Shegella* 3.3 % and *Proteous* 3.3 %. Molecular studies of genes presence or absence for class A contain TEM, CTX and BSHV, class B contain VIM, IMP, KPC and NDM, class C contain FOX and class D contain OXA-10, OXA-24 and OXA-58 were tested. NDM, TEM, CITM and OXA -10 genes were the most abundant the selected bacterial isolates. The results of this study indicate that cheese made from unpasteurized milk can pose a significant risk to consumers. Product manufacturing processes should be subject to health control-to-control pathogens. The novelty in this work depend on screening of gene responsible of the resistance from the bacteria isolated from dairy product using the molecular technique.

DOI: 10.33899/ijvs.2021.130206.1764,

Introduction

The spoiling milk products worldwide is a huge economic problem. The microbial capacity and incidence of the bacterial pathogens in foods are indicators of the quality and safety of food. In addition, the education of the food handlers about the personal hygiene is importance from the food safety point of view (1). The highly nutritious nature of the dairy products makes them especially good media for the growth of microorganisms (2). The microbial contamination is one of the leading causes of the food spoilage worldwide (3). The contamination of food with microbes can occur at any stage of the food chain (4). The large number of diseases caused by food borne pathogens with significant effects on economy and human health. The bacterial pathogens use a variety of different motility modes, including swimming, twitching, and swarming (1). *Staphylococcus. aureus* is commonly food born pathogen of the great importance of animal and human concern. It is responsible for contaminate dairy products, kariesh cheese and ice cream from the different sources during their production, processing and handling that make them unfit for human consumption or even a dangerous source of the infection among customers establishing a potential health hazard (5). Also, *S. aureus* known for its ability to secrete a lot of toxins to aid in host tissue infiltration and a acquire nutrients (6). *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Bacillus cereus*, *Campylobacter*, *Clostridium*, *Cronobacter*, *Escherichia (E.) coli*, *Staphylococcus (S.) aureus*, *Vibrio*, *Yersinia (Y.) enterocolitica*, viruses such as hepatitis A and Noro viruses,

and parasites *Cyclospora (C.) cayetanensis*, *Toxoplasma (T.) gondii*, and *Trichin* (7). The antimicrobial resistance (AMR): has the ability of kill microbes or limit their growth to grow in the presence of a drugs (8). The AMR complicates infection treatment and linked to increased mortality and morbidity. The emergence and spread of resistant and multidrug-resistant (MDR) bacteria has the enormous implications for worldwide healthcare delivery and the population health (9). The virulence functions encoded on the large extrachromosomal plasmids by pathogenic bacteria. These plasmids maintained at the low copy number to reduce the metabolic burden on their host (10). The widespread use of extended-spectrum cephalosporin creates a reservoir of resistant bacteria. Moreover, multi-resistance frequently associated with strains carrying (Extended-spectrum beta-lactamase) ESBLs, which could dramatically reduce the treatment options. The increasing number of *Enterobacteriaceae* with ESBLs that also contain (Metallo-β-lactamases) MBLs or AmpCs and other new mechanisms of resistance to fluoroquinolones or aminoglycosides indicate that recent increase of ESBLs -producing bacteria in Europe constitutes a complex problem (11).

ESBLs are worthy of the scientific community's attention over the past decades among the β-lactamases. ESBL's older and classical definition includes TEM-1, TEM-2, or SHV derivatives. ESBL divided into three main groups by the most recent definition. (I) ESBLA (class A ESBLs): CTX-M, SHV and TEM enzymes. (II) ESBLM (miscellaneous ESBLs) are sectioned into ESBLM-C (class C, plasmid mediated AmpC) and ESBLM-D (class D). (III) ESBLCARBA (ESBLsthat degrade carbapenems) are divided into ESBLCARBA-A, ESBLCARBA-B, and ESBLCARBA-D. More than 500 β-lactamases reported to date produced by diverse bacteria. Beta-lactamases thought to be the most common resistance mechanism that contributes to widespread resistance among Gram-negative microbes also transmission of resistance occur between microorganisms (12). NDM-1 producing by *E. coli* infects the host by commonly invading sites like, urinary tract, blood, lungs, and wounds, leading to the urinary tract infections, septicemia, pulmonary infections, diarrhea, peritonitis, device-associated infections and soft tissue infections (13). The study's goal is to identify MDR bacteria from dairy products and examine their molecular characteristics.

Materials and methods

Sample collection and analysis of microbiology

Dairy products: samples from different locations in Alexandria collected during 2018: white cheese, white cheese produced, old cheese and milk. The sample collected for further use in sterile containers. To evaluate the incidence of isolates of bacterial ESBL and CR isolated from the certain dairy products at the various locations in Alexandria, Egypt. 100 sample were collected that were distributed as Kareish cheese, Industrial white cheese, Old cheese, Roomy cheese and milk 40, 28, 15, 10 and 7% respectively.

Assessment of isolated bacteria's resistance prevalence

According to the modified Kirby-Bauer Disc Diffusion method, all the isolated bacteria subjected to the antibiotics resistance by using the disk diffusion method.

Microorganisms and molecular identification

The antibiotic resistant isolates subjected to phenotypic identification using the cultural characteristics in a trail to identify. Gram staining and analysing biochemistry. The region of 16S rRNA was amplified by using the universal primers F: AGAGTTTGATCMTGGCTCAG and R: TACGGYACCTTGTTACGACTT. The PCR reaction, was performed for 4 min at 95ºC followed by 40 cycles each of (Denaturation: 40 second at 94ºC, annealing: 50 second at 58ºC and extension: 50 second at 72ºC), followed by a supplementary 10min at 72ºC. Sequences of the 16S rRNA genes obtained from the NCBI database. Multiple alignments based on the most closely related sequences and the similarity levels carried out by using the BLAST program1. A phylogenetic tree reconstructed using the Mega 5 software.

Bacterial resistance determination using molecular techniques

The fresh bacterial cells used to extract DNA by using the GeneJET Genomic DNA Purification Kit. GEBRI kit removed plasmid from the bacteria's selected isolates.

Virulence gene detection using PCR assay

Eighteen primers used in this study; the genus used for screening of ESBL class A were TEM, CTX BETA and BSHV. ESBL class B carbapenemase encoding genus were CITM, VIM, IMP, KPC and NDM. ESBL class C encoding genus ACCM. ESBL class D encoding genus was OXA-10, OXA-55, OXA58, OXA 60 and OXA 69. Screening for coding sequence of genes DHAM, EBCM, MEC A, VAN A, and TOHO1 evaluated. PCR reaction achieved for 4 min at 95ºC followed by 35 cycles each of (Denaturation: 40 second at 94ºC, annealing: 50 second at 50-60ºC and extension: 50 second at 72ºC), followed by a supplementary 10min at 72ºC. After amplification by PCR assay, the products checked in 2% agarose gel electrophoresis. The genetic miscellany was determined as the experimental number of differentiation (Table 1).

Results

Assessment of isolated bacteria's resistance prevalence

The multiantibiotic resistance shown by the biggest promising isolates. Seven bacteria isolates were isolated from milk but show sensitive effect towards the antibiotic used for detection of MDR bacteria. All the selected MDR isolates were resistance to metronidazole (MTZ). Incidence

of antibiotic sensitivity of multi-antibiotic resistance bacterial isolates (Table 2). Distribution of the MDR bacterial isolates were 14 isolates from Kariesh cheese (out of 52 bacterial isolates), 11 isolates from industrial white cheese (out of 19 bacterial isolates), 3 isolates from old cheese (out of 37 bacteria isolates) and 2 isolates from roomy cheese (out of 13 bacteria isolates) (Figure 1).

Table 1: primers used in the work-study

Gene	Primer	Sequence (5- 3)	Size [bp]	Pcr program	Reference
NDM	NDM-1 NDM-2	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	580	55	14
KPC	KPC 1 KPC 2	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	750	56	15
VIM	VIM-1 VIM-2	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	780	54	16
TEM	TEM-1 TEM-2	AAAATTCTTGAAGACG TTACCAATGCTTAATCA	1000	60	17
DHAM	DHAM-1 DHAM-2	AACTTTCACAGGTGTGCTGGG TCCGTACGCATACTGGCTTTGC	500	60	18
MECA	MECA-1 MECA-2	AAAATCGATGGTAAAGGTTGGC AGTTCTGCAGTACCGGATTTTG	550	59	19
CTX_beta	CTX beta-1 CTX beta-2	TTTGCGATGTGCAG(C/T)ACCAG CGCGATATC(A/G)TTGGTGGTGCCATA	650	58	Designed
OXA 58	OXA 58-1 OXA 58-2	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	600	58	20
CITM	CITM-1 CITM-2	TTT CTC CTG AAC GTG GCT GGC TGG CCA GAA CTG ACA GGC AAA	500	55	21
CTX	CTX-1 CTX-2	TTAGGAAATGTGCCGCTGTA CGATATCGTTGGTGGTACCAT	900	56	22
ACCM	ACCM-1 ACCM-2	AACAGCCTCAGCAGCCGGTTA TTCGCCGCAATCATCCCTAGC	750	53	23
OXA-10	OXA-10-1 OXA-10-2	GCCATGAAAACATTTGCCGC GCCACCAATGATGCCCTCAC	350	53	24
IMP	IMP-1 IMP-2	GTGGTTCTTGTAAATGCTGAGG CCGCCTGCTCTAATGTAAGT	650	55	25
EBCM	EBCM-1 EBCM-2	TCGGTAAAGCCGATGTTGCGG CTTCCACTGCGG CTG CCA GTT	500	56	26
OXA-69	OXA-69-1 OXA-69-2	CTAATAATTGATCTACTCAAG CCAGTGGATGGATGGATAGATTATC	500	60	27
TOHO-1	TOHO1-1 TOHO1-2	GCGACCTGGTTAACTACAATCC CGGTAGTATTGCCCTTAAGCC	600	60	28
BSHV	BSHV-1 BSHV-2	ATGCGTTATATTCGCCTGT TGCTTTGTTATTCGGGCCAA	500	56	29
VAN A	VANA-1 VANA-2	GGGAAAACGACAA TTGC GTACAATGCGGCCGTTA	750	50	30

Identification of the bacterial isolates

The most commonly isolated pathogens were evaluated for phenotypic characterization and molecular identification using 16s rDNA. The amplified fragment sequences of 1500 bp identified. A dominant tool for identifying and classifying prokaryotes was the sequences of the different types of the strains from the gene bank. GenBank deposited the sequence of the most promising isolates and had the accession. From all the examined samples were *staphylococcus spp, Bacillus spp, Salmonella spp, E. coli, Enterococcus spp, Pseudomonas spp, Shigella spp* and *Proteous spp.* with 43.3, 16.7, 13.3, 10, 6.7, 3.3, 3.3 and 3.3 % respectively. 23.1% out of the total isolates 130 were pathogens.

Resistance determination using molecular technique

The variation between the isolated MDR bacteria shown by plasmid extraction of the selected bacterial isolates. The results showed that isolates 2, 9, 16, 19, 20, 24, 25, 27, 29, 30, 39, 40 and 41 were highly antibiotic-resistant positive plasmid with high copy numbers (Figure 2).

Table 2: Resistance prevalence of the bacterial isolates

Antibiotics	Resistance %	Antibiotics	Resistance %
Colistin (CT)	66.7	Ceftazidime (CAZ)	90
Ampicilin +Sulbactam (A/S)	90	Amikacin (A/K)	70
Penicilin (P)	83.3	Clindamycin (DA)	93.3
Chloramphenicol (C)	76.7	Rifampine (RA)	86.7
Ofloxacin (OFX)	83.3	Nitrofurantion (F)	76.7
Levofloxacin (LEV)	86.7	Cefoperazone (CFP)	86.7
Cefepime (CPM)	93.3	Azetreonum (ATM)	96.7
Meropeneme (MEM)	70.7	Tetracycline (TE)	83.3
Metronidazole (MTZ)	100	Streptomycine (S)	83.3
Doxycycline (DO)	83,3	Trimethoprim-sulfamethoxaz (STX)	80

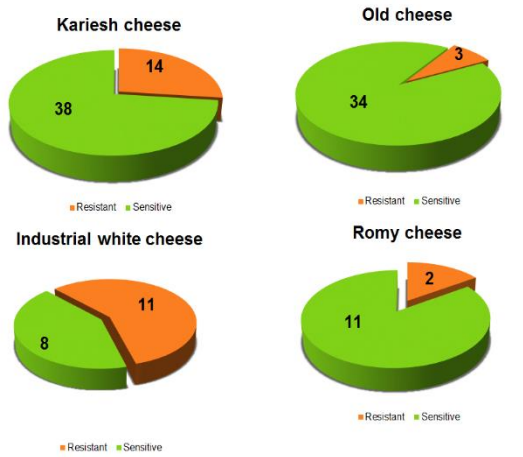

Figure 1: MDR incidence distribution in dairy product samples under test.

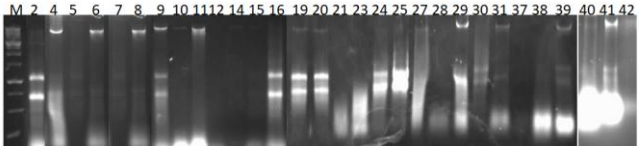

Figure 2: Agarose gel electrophoresis plasmid profiles of different bacterial isolates.

Detection of the virulence gene using PCR assay (Detection of β-lactamase gene class A, B, C and D)

Collected data of genes presence or absence for class A (TEM, CTX and BSHV) (Figure 3), class B (CITM, VIM, IMP, KPC and NDM) (Figure 4), class C (ACCM) (Figure 5), and class D (OXA-10, OXA-55, OXA-58 and OXA 69) (Figure 6). Screening of DHAM, EBCM, MEC, VAN and TOHA gene tested by using the selected bacterial isolates (Figure 7). The fragment size of amplified PCR product was calculated by using the software of Gel Documentation Analysis System (Alpha Imager TM 1220). In addition, the results of the present study showed that the average percentage of positive genes in all the selected MDR (30 isolates). Distribution of presence or absence of the 18 tested virulence genes among the MDR bacterial isolates (Figure 8).

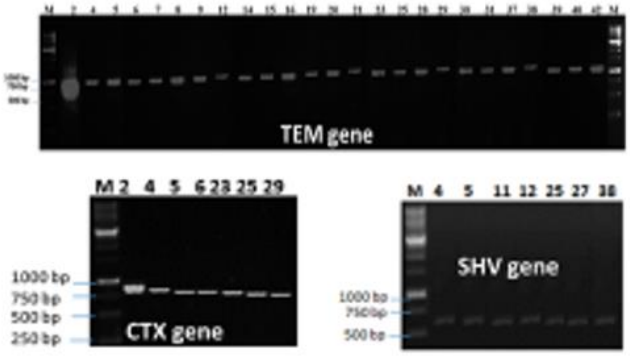

Figure 3: Agarose gel electrophoresis of the amplified PCR fragment for Class A, no. refers to the code of positive isolate.

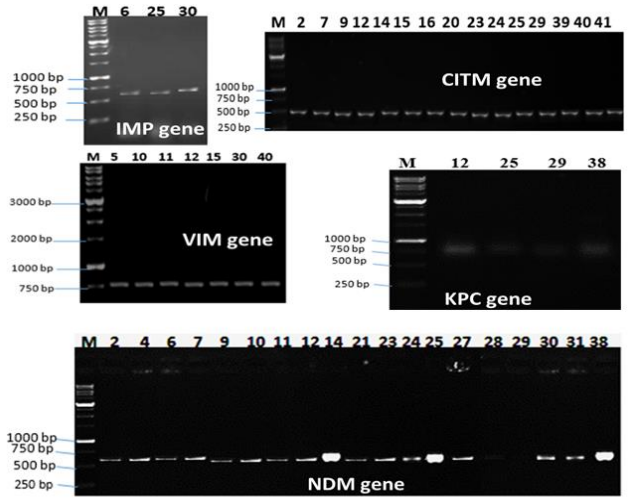

Figure 4: Agarose gel electrophoresis of the amplified PCR fragment for Class B, no. refers to the code of positive isolate.

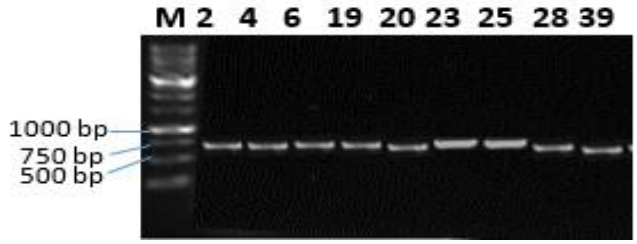

Figure 5: Agarose gel electrophoresis of the amplified PCR fragment for Class C (ACCM gene), no. refers to the code of positive isolate.

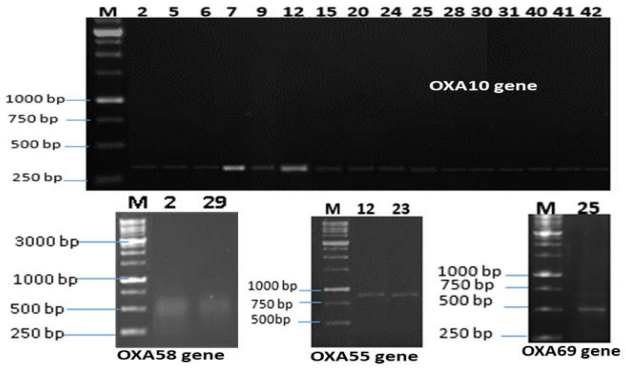

Figure 6: Agarose gel electrophoresis of the amplified PCR fragment for Class D, no. refers to the code of positive isolate.

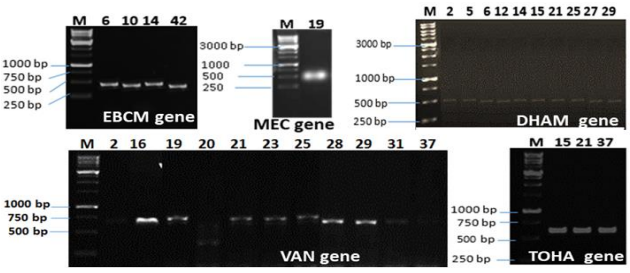

Figure 7: Agarose gel electrophoresis of the amplified PCR fragment for (EBCM, MEC, DHAM, VAN AND TOHA gene), no. refers to the code of positive isolate.

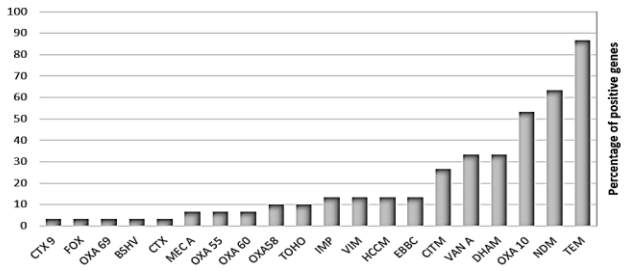

Figure 8: Average percentage of positive genes in all the selected MDR (30 isolates).

Discussion

Studies previously conducted in different countries revealed a wide *S. aureus* diversity in milk and dairy products. The prevalence of *S. aureus* was 43.3% and it was differing than which carried out from Iran and Italy on different dairy products revealed a lower *S. aureus* percentage. Lower prevalence noted in studies which detected *S. aureus* with 10, 11.3, 5 and 9.1% respectively for the kariesh cheese (31). Greater incidence of *S. aureus* was 72, 50, 70, 93 and 68 % respectively for the kariesh cheese (32). Percentage of *Bacillus spp.* in our study was 16.7% which were isolated from the industrial white cheese and the kareish cheese where it was higher percentage in industrial white cheese. It was differed than the study by EFSA 2005 which appeared the incidence of *B. cereus* was highest in karish cheese 25 to 80% during all the seasons followed by koshary 45 to 70% and cornsnacks 20 to 50%. Percentage of *Salmonella sp.* in our study was 13.3% that were isolated from the industrial white cheese and the kareish cheese 10 and 3.3%. Relatively lower results were obtained by Abo Zeed (33,34) where *Salmonella spp.* could not be identified in the surveyed of the white cheese samples. Ghada and colleagues (35) identified two samples polluted with *Salmonella spp.*

In our study, the *E. coli* percentage was 10% isolated from roomy, kareish and industrial white cheese compared to other bacterial isolates. In study by Abdelrahman *et al.*, (36) found that, the incidence of *E. coli* in Kariesh, and Domiati cheese were 37.1% and 2.8%, respectively. While, could not be detect in Tallaga cheese. Naj and Ghanbarpour (37) who isolated *E. coli* with percentage of 98.7 % from the domestic soft cheese. A common principal of the resistance markers in all varieties estimated and associated antimicrobials such as tetracycline, B-lactams, sulfonamide and quinolones were detected (38). An aggregate number of extended-spectrum β-lactamases (ESBLs) have been predictable in Enterobacteriaceae through the latest few years. SHV types of enzymes shown to carry the SHV-1 gene within the chromosome (39). NDM-1 producers were resistant to imipenem, meropenem, ertapenem, gentamicin, amikacin, tobramycin, and ciprofloxacin, whereas, isolates were susceptible to colistin (40). The high prevalence of tetracycline- and penicillin-resistant *S. aureus*, *Salmonella* and *E. coli* observed in the current study, is in agreement with earlier findings. Gram-negative bacteria mostly preset TEM, almost 90% of the resistance against ampicillin in Gram-negative bacteria are due to TEM encoded genes (41).

Conclusion

The results of this study indicate that cheese made from unpasteurized milk can pose a significant risk to consumers. This risk varies depending on the geographical location of the study area, the state of education of the population, the extent of attention to hygiene and the method of preparing

and packaging dairy products. Product manufacturing processes should be subject to health control-to-control pathogens. Dairy markets monitored for pathogens. Reducing the surface area exposed to air reduces harmful microbial growth in dairy products.

Conflict of interest

No conflict.

Acknowledgments

The authors acknowledge City of Scientific Research and Technological Applications.

References

1. Mahendra P, Selamawit M, Muluken T, Suneeta VP, Prajapati JP. Bacterial contamination of dairy products. Beverage Food World. 2016;43:40-43. [available at]
2. Pal M, Bekele T, Feleke A. Public health significance of pasteurized milk. Beverage Food World. 2012;39: 55-56. [available at]
3. Pal M. Spoilage of dairy products due to fungi. Beverage Food World. 2014;41: 37-38. [available at]
4. Vrdoljak J, Dobrani V, Filipovi I, Zdolec N. Microbiological quality of soft, semi-hard and hard cheeses during the shelf-life. J Macedonian Vet Rev. 2016;39:59-64. DOI: 10.1515/macvetrev-2015-0068
5. Khalil II, AlDabbagh SYA, Shareef AM. Isolation, identification and detection of some virulence factors in yeasts from local cheese in Mosul city. Iraqi J Vet Sci. 2018;32(1):81-85. [available at]
6. Kong C, Neoh H, Nathan S. Targeting *Staphylococcus aureus* toxins: A potential form of anti-virulence therapy. Toxins. 2016;8(3):72. DOI: 10.3390/toxins8030072
7. Bintsis T. Foodborne pathogens. AIMS Microbiol. 2017;3(3):529. DOI: 10.3934/microbiol.2017.3.529
8. World Health Organization. Antimicrobial resistance: Global report on surveillance. Switzerland: World Health Organization; 2014. [available at]
9. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infectious Dis. 2016;16(2):161-168. DOI: 10.1016/S1473-3099(15)00424-7
10. Mustafa JY. Study the effect of cloned pET-32a (+) plasmid by Lysostaphin gene against *Staphylococcus aureus*. Iraqi J Vet Sci. 2020;35(2):233-238. DOI: 10.33899/ijvs.2020.126698.1362
11. Urumova V. Extended spectrum beta lactamase producing animal enterobacteriaceae isolates as potential risk to public health - review. Valentina. 2015;166:192-207. [available at]
12. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. Lancet Infect Dis. 2011;11:355-62. DOI: 10.1016/S1473-3099(11)70059-7
13. Hu H, Hu Y, Pan Y, Liang H, Wang H, Wang X, Hao Q, Yang X, Yang X, Xiao X. Novel plasmid and its variant harboring both a bla (NDM-1) gene and type IV secretion system in clinical isolates of *Acinetobacter lwoffii*. Antimicrob Agents Chemother. 2012;56:1698-1702. DOI: 10.1128/AAC.06199-11
14. Nor Zanariah Z, Anita S, Hanafiah A, Najihan AS. Molecular detection of the New Delhi metallo-b-lactamase-1 genein Enterobacteriaceae isolates in a tertiary medical centre. Malaysian J Pathol. 2015;37(3):227-232. [available at]
15. Patrice, N, Thierry, N, Laurent, P. Global Spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011. 17(10):1791-1798. DOI: 10.3201/eid1710.110655
16. Jyoti S, Meera S, Pallab R. Detection of TEM and SHV genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. Indian J Med Res. 2010. 132: 332-336. [available at]
17. Hosseini S, Maleki A, Ghafourian S, Reza M, Fadavi HV, Shahmir P, Sadeghifard N. Molecular Characterization of AmpC β-Lactamases among *Klebsiella pneumoniae* isolated from Ilam and Tehran hospitals, from Iran. J Pure Appl Microbiol. 2014;8(3):2217-2220. [available at]
18. Taweeporn S, Chariya C, Kunyaluk C, Temduang L, Chaisiri W. Evaluation of different primers for detecting Meca gene by PCR in comparison with phenotypic Methods for discrimination of Methicillin-resistant *Staphylococcus aureus*. Southeast Asian J Trop Med Public Health. 2002;15(1):34-40. [available at]
19. Khorsi K, Messai Y, Hamidi M, Ammari H, Bakour R. High prevalence of multidrug-resistance in *Acinetobacter baumannii* and dissemination of carbapenemase-encoding genes blaOXA-23-like, blaOXA-24-like and blaNDM-1 in Algiers hospitals. Asian Pacific J Trop Med. 2015;8(6):438-446. DOI: 10.1016/j.apjtm.2015.05.01
20. Sana J, Mohd S, Sobia F, Anuradha S, Haris MK. Molecular characterization of genes encoding AmpC beta-lactamases in clinical isolates of *Pseudomonas* and *Acinetobacter* species. J Appl Pharmaceut Sci. 2015;5(10):048-051. DOI: 10.7324/JAPS.2015.501009
21. Mohammad SN, Sreedevi B, Chaitanya RK, Sreenivasulu D. Betalactamase antimicrobial resistance in Klebsiella and Enterobacter species isolated from healthy and diarrheic dogs in Andhra Pradesh, India. Vet World. 2017:2231-0916. [available at]
22. Kolar M, Bardon J, Chroma M, Hricova K, Stosova T, Sauer P, Koukalova D. ESBL and AmpC beta-lactamase-producing Enterobacteriaceae in poultry in the Czech Republic. Vet Med. 2010;55(3):119-124. DOI: 10.17221/165/2009-VETMED
23. Frédéric B, Catherine B, Nicole LZ. Identification of PSE and OXA βlactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. Brit Soci Antimicrobl Chemo. 2002;50:11-18. [available at]
24. José AD, Alejandra B, Juan A, Cynthia R, Ángela F, Gabriel OG. Blactamases produced by amoxicillin-clavulanate-resistant Enterobacteria isolated in Buenos Aires, Argentina: Anew blaTEM gene. Rev Argent Microbial. 2014; 46(3):210-217. DOI: 10.1016/S0325-7541(14)70075-6
25. Patrik M, Magdalena R, Milan K. Research article primer evaluation for PCR and its application for detection of carbapenemases in enterobacteriaceae Jundishapur. J Microbial. 2016; 9(1):e29314. DOI: 10.5812/jjm.29314
26. Chih-Chauan K, Meei-Fang L, Chin-Fu L, Yi-Ching H, Po-Yu L, Ching-Wen C, Zhi-Yuan S. Antimicrobial susceptibility and multiplex PCR screening of AmpC genes from isolates of *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens*. J Microbial Immunol Infect. 2010;43(3):180-187. DOI: 10.1016/S1684-1182(10)60029-1
27. Evans BA, Hamouda A, Towner KJ, Amyes SB. OXA-51-like βlactamases and their association with particular epidemic lineages of *Acinetobacter baumannii*. Clin Microbiol Infect. 2008;14(3):268-275. DOI: 10.1111/j.1469-0691.2007.01919.x
28. Tadesse E, Josephine B, Daniel A, Moses NN, Joyce N, Wondwossen AG, John SG, Appolinaire D, Ephrem E. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal Salmonella isolates from humans and animals in central Ethiopia. Antimicrobl Resist Infect Cont. 2017;10(8):189-196. DOI: 10.1186/s13756-017-0171-6
29. Nashwa MA, Maysaa EZ. Molecular study of *Acinetobacter baumannii* isolates for metallo--lactamases and extended-spectrum--lactamases genes in intensive care unit, Mansoura university hospital, Egypt. Inter J Microbiol. 2017:3925868. DOI: 10.1155/2017/3925868
30. Chimanjita P, Mangala L, Swapnil R, Kandarpa KS. Emergence of va 17 gene among vancomycin-resistant enterococci in a tertiary care hospital of North - East India. Indian J Med Res. 2016;143(3):357-361. DOI: 10.4103/0971-5916.182627
31. Mahmood MA, Essa MA. Antimicrobial activity of peptides extracted from camels' blood neutrophils against some pathogenic bacteria. Iraqi J Vet Sci. 2021;35(1):33-37. DOI: 10.33899/ijvs.2020.126239.1270

التوصيف الجزيئي والمقاومة الميكروبية للعزلات البكتيرية المختلفة في بعض منتجات الألبان

وليد خالد الخفاجى[1]، زكية أحمد علما[1] و صفاء محمد على[2]

[1]قسم النبات والميكروبيولوجى، كلية العلوم، جامعة الإسكندرية، [2]قسم بحوث الأحماض النووية، معهد الهندسة الوراثية، مدينة الأبحاث العلمية والتطبيقات التكنولوجية، الإسكندرية، مصر

الخلاصة

يعد التلوث الجرثومي للحليب ومنتجاته احدى اهم المشاكل شائعة الحدوث. في العامين الماضيين، تحدث الأمراض التي تنتقل عن طريق الغذاء نتيجة تناول الحليب ومنتجاته إلى اضطراب توزيع لبكتريا السالمونيلا انتتيريكاو الليسيريا وايشيريشيا كولاي وكمبيلاباكتر جيجونا. فكان الهدف الرئيسي من الدراسة هو عزل بكتيريا التي لها قدرة على مقاومة العديد من المضادات الحيوية التى عزلت من الحليب ومنتجات الألبان ودراسة التوصيف الجزيئي لتلك العزلات. إن ٣٠ عزلة بكتيرية من أصل ١٣١ عزلة كانت مقاومة للعديد من المضادات الحيوية. كشفت الدراسة إن البكتريا من نوع الاستافيلوكوكس هي أكثر الأنواع الموجودة بين تلك العزلات بنسبة ٤٣,٣٪ ،١٦,٧٪ من نوع الباسيلس، السالمونيلا كانت ١٣,٣٪، الإشيريشيا كولاى كانت ١٠٪، الانتيروكوكاس ٦,٧٪ و ٣,٣% للسيدوموناس، الشيغيلة كانت ٣,٣٪ والبروتيوس كان ٣,٣٪. لقد تمت الدراسات الجزيئية لوجود الجينات أو غيابها للفئة أ (TEM و CTX و BSHV) والفئة ب (VIM و IMP و KPC و NDM) والفئة ج (FOX) والفئة د (OXA-10 و OXA-24 و OXA-58). كانت جينات NDM و TEM و CITM و (OXA -10) الأكثر وفرة في العزلات البكتيرية المختارة. تشير نتائج هذه الدراسة إلى أن الجبن المصنوع من الحليب غير المبستر يمكن أن يشكل خطرا كبيرا على المستهلكين. يجب أن تخضع عمليات تصنيع منتجات الألبان إلى الرقابة الصحية لمنع مسببات الأمراض. تعتمد الحداثة في هذا العمل على فحص الجين المسؤول عن المقاومة من البكتيريا المعزولة من منتجات الألبان باستخدام تقنية البيولوجيا الجزيئية.

32. Salama EM, Saad AH, Enan GA, Suzan IY. Incidence and biocontrol of *Staphylococcus aureus* in some milk products. Second Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine; 2015. 29-35 p.
33. El-Gamal MS, El Dairouty RK, Okda AY, Sahar HS, El-Shamy SM. Incidence and interrelation of *Cronobacter sakazakii* and other foodborne bacteria in some milk products and infant formula milks in Cairo and Giza Area. World Applied Sci. J. 2013;26:1129-1141. DOI: 10.5829/idosi.wasj.2013.26.09.13542
34. Abo Zeed BA. prevalence of biological hazards in milk and some dairy products in Egyptian markets [PhD dissertation]. Cairo: Faculty of Veterinary Medicine, Cairo University; 2014.
35. Ghada ZAA, Alia MH, Soha Al-S, Magdy NA, Mohammed FS. Chemical, nutritional and microbiological evaluation of some Egyptian soft cheeses. Egyptian J Hospital Med. 2004;17:44-57. [available at]
36. Abdelrahman M, El Bagoury HS, Hadeer S. Incidence of *Escherichia coli* and *Salmonella* species with special reference to antibiotic resistant pathogenic *E. coli* isolated from locally produced cheeses in Egypt. Alexandria J Vet Sci. 2019;10:5455. DOI: 10.5455/ajvs.21944
37. Najand LM, Ghanbarpour R. A study on enteropathogenic *Escherichia coli* isolated from domestic Iranian soft cheese. Vet Arh. 2006;76:531-536. [available at]
38. Fontes CO, Silva VL, de Paiva MRB, Garcia RA, Resende JA, Ferreira-Machado AB, Diniz CG. E-negative Staphylococci isolated from soft cheese in Brazil. J Food Sci. 2013;78:594-599. DOI: 10.1111/1750-3841.12088
39. Ahmed IM. Detection of CTX-M gene in extended spectrum β-lactamases producing Enterobacteriaceae isolated from bovine milk. Iraqi J Vet Sci. 2021;35(2):397-402. DOI: 10.33899/ijvs.2020.126909.1412
40. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10:597-602. DOI: 10.1016/S1473-3099(10)70143-2
41. Sadeeq R, Tariq A, Ijaz A, Nazir AK, Bo H, Jian Gao. The growing genetic and functional diversity of extended spectrum beta-lactamases. Res Inter. 2018. DOI: 10.1155/2018/9519718