



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكرو فيلم

# بسم الله الرحمن الرحيم



**MONA MAGHRABY**



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التوثيق الإلكتروني والميكروفيلم



# شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



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# جامعة عين شمس

## التوثيق الإلكتروني والميكروفيلم

### قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



### يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



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*Cairo University*  
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# **Prevalence and Transmissibility of Antimicrobial Resistance Genes in Heat Treated Milk**

**Thesis submitted by**

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(BVSc, Cairo University, 2011; MVSc, Cairo University, 2015)

**For the degree of Ph.D.**

**(Hygiene and control of milk and its products)**

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**Abstract**

This study investigated the persistence and quantification of *bla<sub>Z</sub>*, *mecC* and *tetK* plasmid-mediated ARGs copy numbers of two staphylococcal strains in both milk and Tris - EDTA (TE) buffer over 3 weeks storage on refrigeration +4°C. During subsequent storage after pasteurization, all tested genes showed increased copy numbers. By electroporation of these genes to the *Staphylococcus aureus* RN42200 electro-competent strain, both *mecC* and *tetK* genes were still expressive and transferable. The formation of VBNC cells was estimated with viability staining and quantitative PCR of 16S rDNA copy numbers of both staphylococcal strains. On the other hand, surveying the prevalence of nine plasmid-mediated and one genomic AMR genes in 100 (50 bulk tank milk & 50 milk filters socks) samples at farm level and 152 (84 pasteurized and 68 ultra-heat-treated milk) commercial samples, results revealed that *sul2* gene was the most prevalent plasmid-mediated gene in (96%) milk filters socks, (48%) bulk tank milk, (68%) pasteurized and (43%) UHT samples; on contrary the *mecA* gene could not be detected in any sample. Moreover, currently practiced commercial pasteurization not only failed to decrease the prevalence of the *bla-TEM-B1* (43%), *tetK* (30%) and *tetA* (55%) plasmid-mediated AMR genes, but also potentially stimulates dairy microbiota to enter into a viable but non-culturable (VBNC) state. In contrast, after the sterilization treatment all the genes showed decreases in copy numbers, and viability assessment showed that UHT treatment is less to induce VBNC state. Continued research is necessary to identify bacterial species entering the VBNC state after pasteurization, assess their potential resuscitation hazard level, and shed more light on the expression and possibility of horizontal gene transfer of those plasmid-mediated AMR genes to gut microbiota.

**Keywords**

Milk pasteurization, Antimicrobial resistance genes (ARGs), Ultra High Temperature (UHT), VBNC, Scanning electron microscopy (SEM), horizontal gene transfer (HGT), Viability assay, staphylococci

## *DEDICATION*

*To my loving parents who fostered my curiosity, my sister Ayah and my brother Mohamed who believed in me even when I didn't, for their unwavering support, encouragement and unconditional love.*

*To the fascinating little bugs, that I've been working on and those I haven't yet worked on, who never failed to amaze me and fire my curiosity, to those who have taught me my greatest lessons and keep showing me how big is the knowledge yet to be discovered. Our smartest competent on Earth! this is only one chapter of our friendship and there is more to come.*

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## List of Abbreviations

<b>16S rDNA</b>	= 16S ribosomal DNA
<b>AGRF</b>	= Australian Genome Research Facility
<b>AMR</b>	= Antimicrobial Resistance
<b>ARGs</b>	= Antimicrobial Resistance Genes
<b>ATP</b>	= Adenosine Triphosphate
<b>BTM</b>	= Bulk Tank Milk
<b>CFU</b>	= Colony Forming Unit
<b>DCT</b>	= Dry Cow Treatment
<b>EMA</b>	= Ethidium Monoazide
<b>FDA</b>	= Food and Drug Administration
<b>GDP</b>	= Gross Domestic Product
<b>HGT</b>	= Horizontal Gene Transfer
<b>HTST</b>	= High Temperature Short Time
<b>IDF</b>	= International Dairy Federation
<b>IMC</b>	= Isothermal Microcalorimetry
<b>IMM</b>	= Intramammary Antimicrobials
<b>LAB</b>	= Lactic Acid Bacteria
<b>LTLT</b>	= Low Temperature Low Time
<b>MALDI-TOF</b>	= Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry
<b>MFS</b>	= Milk Filter Socks
<b>MIC</b>	= Minimum Inhibitory Concentration
<b>MRSA</b>	= Methicillin Resistance <i>Staphylococcus aureus</i>
<b>MVT</b>	= Molecular Viability Test
<b>NAS</b>	= Non-aureus Staphylococci
<b>NASBA</b>	= Nucleic Acid Sequence-Based Amplification
<b>ORF</b>	= Open Reading Frame
<b>PCR</b>	= Polymerase Chain Reaction
<b>PMA</b>	= Propidium Monoazide
<b>qPCR</b>	= Quantitative Polymerase Chain Reaction
<b>RICA</b>	= Rabbit Ileal Loop Assay
<b>RPF</b>	= Resuscitation Promoting Factors

<b>RT-PCR</b>	= Real time - Polymerase chain reaction
<b><i>SEM</i></b>	= <i>Scanning electron microscopy</i>
<b>SIP</b>	= Stable isotope probing
<b>STEC</b>	= <i>Escherichia coli</i> Shiga toxin-producing
<b>UHT</b>	= Ultra-high temperature
<b>VBNC</b>	= Viable but non culturable
<b>WGS</b>	= Whole genome sequencing
<b>WHO</b>	= World health organization

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