بسم الله الرحمن الرحيم

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شبكة المعلومات الجامعية

التوثيق الالكتروني 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

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Prevalence and Transmissibility of Antimicrobial Resistance Genes in Heat Treated Milk

Thesis submitted by

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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 blaZ*, 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 +4ºC. 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

16S rDNA = 16S ribosomal DNA

AGRF = Australian Genome Research Facility

AMR = Antimicrobial Resistance

ARGs = Antimicrobial Resistance Genes

ATP = Adenosine Triphosphate

BTM = Bulk Tank Milk

CFU = Colony Forming Unit

DCT = Dry Cow Treatment

EMA = Ethidium Monoazide

FDA = Food and Drug Administration

GDP = Gross Domestic Product

HGT = Horizontal Gene Transfer

HTST = High Temperature Short Time

IDF = International Dairy Federation

IMC = Isothermal Microcalorimetry

IMM = Intramammary Antimicrobials

LAB = Lactic Acid Bacteria

LTLT = Low Temperature Low Time

MALDI-TOF = Matrix-Assisted Laser Desorption Ionization-Time of Flight mass spectrometry

MFS = Milk Filter Socks

MIC = Minimum Inhibitory Concentration

MRSA = Methicillin Resistance *Staphylococcus aureus*

MVT = Molecular Viability Test

NAS = Non-aureus Staphylococci

NASBA = Nucleic Acid Sequence-Based Amplification

ORF = Open Reading Frame

PCR = Polymerase Chain Reaction

PMA = Propidium Monoazide

qPCR = Quantitative Polymerase Chain Reaction

RICA = Rabbit Ileal Loop Assay

RPF = Resuscitation Promoting Factors

RT-PCR = Real time - Polymerase chain reaction

SEM = *Scanning electron microscopy*

SIP = Stable isotope probing

STEC = *Escherichia coli* Shiga toxin-producing

UHT = Ultra-high temperature

VBNC = Viable but non culturable

WGS = Whole genome sequencing

WHO = World health organization

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(domestic fridge, 40C) of *Staphylococus aureus* ATCC® BAA-2312™ strain, inoculated at 106 colony forming units per millilitre-1 (CFU/mL), tested weekly.

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