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Benha University
Faculty of Veterinary Medicine
Department of Food Control



**Biofilms: Their formation and control in some food
manufacturing plants and food service establishment
in Egypt**

A Thesis Submitted to

Faculty of veterinary Medicine

Benha University

Presented By

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For

The Degree of Master in Veterinary Medicine

(Meat Hygiene)

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(2019).

Dedicated

To

*My family and every one
support me to complete
this work*

"And he raised his parents upon the throne, and they bowed to him in prostration. And he said, "O my father, this is the explanation of my vision of .before. My Lord has made it reality"

(QS 12:100).

*"Look beneath the surface; let not the several
quality of a thing or its worth escape thee"*

Marcus Aurelius Antoninus

(161 A.D)

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List of Abbreviations

ANOVA	Analysis of variance
APHA	American Public Health Association
ATCC	American Type Culture Collection
AU	Arbitrary Unit
AWD	Agar Well Diffusion
C.V	Crystal Violet
CFS	Cell Free Culture Supernatant
CFU	Colony Forming Unit
CT	Contact Time
Da.	Dalton
DGCs	diguanylate cyclases
DSMZ	German Collection of Microorganisms and Cell Cultures
ECM	Extracellular Matrix
EMB	Eosin Methylene Blue agar
EPS	Extracellular Polysaccharide
FCS	Food Contact Surfaces
GFSI	Global Food Safety Initiative
HACCP	Hazard Analysis and Critical Control Points
ICOMOS	International Council on Monuments and Sites
IDF	International Dairy Federation
ISO	International Organization for Standardization
IU	International Unit
KDa	Kilo Dalton
LAB	Lactic Acid Bacteria
MIRCEN	Microbiology Resource Center

List of Abbreviations

MPN	Most probable Number
MRS	De Man, Rogosa and Sharpe agar
MSDS	Material Safety Data Sheet
NCCLS	National Committee for Clinical Laboratory Standard
NFSA	National Food Safety Authority
OD	Optical density
PCR	Polymerase chain reaction
PDEs	Phosphodiesterase
ppm	Part Per Million
QAC	Quaternary Ammonium Compound
QS	Quorum Sensing
R%	Reduction present
R&D	Research and Development
SS	Stainless Steel
V/V	Volume per Volume
W/V	Weight per Volume
WHO	World Health Organization
XLD	Xylose Lysine Desoxycholate

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1. Introduction:

Modern food processing provides an environment for biofilm formation on surfaces due to the great complexity of processing equipment (making it difficult to adequately sanitize), mass production of products, lengthy production cycles, and the vast surface areas available for biofilm development (**Lindsay and Von Holy, 2006**).

In the meat industry, contamination of products with foodborne pathogenic bacteria is a serious public health concern and often results in product recalls with significant financial loss. As meat consumption increases around the world, so do concerns and challenges to meat hygiene and safety (**Sofos and Geornaras, 2010**). Incidence of foodborne illness in public health, there are also huge economic costs, which are estimated to exceed \$50 billion annually in the United States alone (**Scharff, 2012**).

Meat is typically subjected to bacterial contamination at some point following the slaughter of the animal and further processing with the equipment recognized to be the primary vehicle of cross-contamination throughout the meat processing chain (**Giaouris and Simões, 2018**).

In situ biofilms have been recognized in meat processing environments (**Gounadaki et al., 2008; Marouani-Gadri et al., 2009; Zhao et al., 2006**), while several studies on the bacterial attachment to meat contact surfaces and its implication for meat contamination have been conducted (**Giaouris, 2015**).

Biofilms can be defined simply and broadly as communities of microorganisms that are attached to a surface. A concerted effort to study microbial biofilms began only two 2 decades ago with the rediscovery that, in natural aquatic systems, bacteria are found predominately attached to surfaces (**O'Toole et al., 2000a**). Nearly 99% of microorganisms living on the earth live in microbial communities known as biofilms. Biofilms are formed by adhesion of bacterial cells to surfaces through an exopolymeric matrix. This matrix is important in formation and structure of the biofilm and also on the protection of the bacterial cells as it prevents antimicrobials and xenobiotics from gaining access to the cells inside the biofilm (**Adetunji and Isola, 2011**).

Thus, a unique feature of biofilms is that once these have been developed on food processing facility and equipment surfaces, they are difficult to eradicate, mainly due to their stable and extremely strong matrix. This covers the cells and contains EPS, such as bacterial derived exopolysaccharides and sugars, proteins, lipids, teichoic and nucleic acids, and other minor components. All these provide biofilms with mechanical stability mediate their strong adhesion to surfaces and form a cohesive, three-dimensional polymer network that interconnects and transiently immobilizes the enclosed cells (**Flemming and Wingender, 2010**).

Improperly cleaned surfaces promote soil build-up, and, in the presence of water, contribute to the development of bacterial biofilms which may contain pathogenic microorganisms, such as *Salmonella*, *E.coli* and *Staphylococcus aureus* (**Lindsay and von Holy, 2006**).

Biofilms are problematic in particular food industry sectors such as: poultry processing and red meat processing (**Frank, et al., 2003; Somers and Wong, 2004; and Chen, et al., 2007**).

Biofilms formed on food contact surfaces are of considerable interest in the context of food hygiene, since these may contain both spoilage and pathogenic bacteria and can result in post-processing contamination, leading to lowered shelf life of products and transmission of diseases (**Giaouris and Simões, 2018**).

There are several problems, not the least of which is product contamination, that occur from the formation of biofilm. Product contamination occurs from sloughing bacteria that are shed periodically by the film and can reattach on equipment somewhere else in the product flow or make their way into food product. Any company that has been involved in a recall, or whose product has been associated with an illness can attest to the fact that they are damaging to the business and extremely expensive (**Cramer, 2006**).

In recent decades, biofilm formation in the food industry by bacterial pathogens, such as *Salmonella* spp., pathogenic *Escherichia coli* and *Staphylococcus aureus* has attracted much attention, given that microorganisms within biofilms are protected from sanitizers, increasing the likelihood of survival and subsequent contamination of food (**Chmielewski and Frank, 2003**).

Biofilms are more resistant to antimicrobials compared to planktonic cells and this makes their elimination from food processing facilities a big challenge. Moreover, the emergence of resistant bacteria to conventional antimicrobials clearly shows that new biofilm control strategies are required **(Simões, et al., 2010)**.

Nowadays, the food hygienist and sanitarians faces a challenge in overcoming food safety problems come from formation and persistence of bacterial biofilms in food industry. As well as interest in using natural biocides and ecofriendly alternatives in food processing environments, because of the potential hazards of synthetic chemical agents both for public health and the environment. Therefore, understanding biofilms and their related issues, investigate ability of some natural biocides to control biofilm formation are steps toward controlling and solve this challenge; therefore this study was conducted to fulfill the following:

1. Isolation and identification of biofilms forming bacteria from some selected food contact surfaces in both food processing and food services establishments.
2. Confirming ability of isolated bacteria to form biofilms.
3. Study the effect of disinfectants commonly used in food processing establishments (chlorine, iodine and Quaternary Ammonium Compounds QACs) at recommended concentration at usual temperature and pH circumstances.
4. Study the effect of chitosan, nisin, chitosan/ nisin mixture and other lactobacillus bacteriocin on isolated pathogenic bacteria in both planktonic and Biofilm.