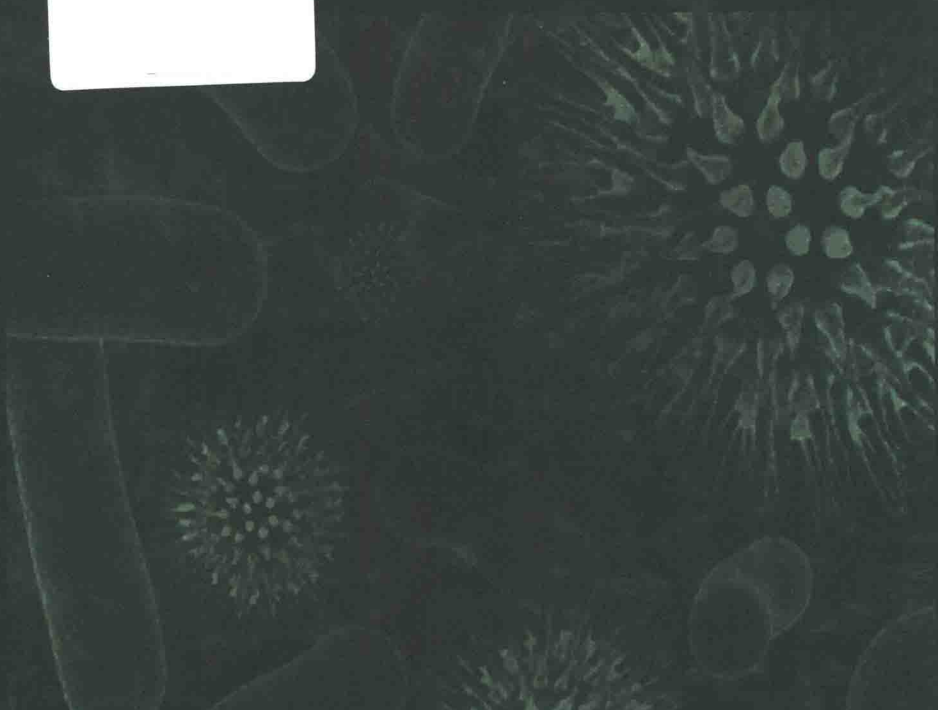
Food Associated Pathogens

Editors:
Wilhelm Tham
Marie-Louise Danielsson-Tham



CRC Press
Taylor & Francis Group
A SCIENCE PUBLISHERS BOOK

Food Associated Pathogens

Editors

Wilhelm Tham

School of Hospitality
Culinary Arts and Meal Science
Örebro University
Grythyttan, Sweden

Marie-Louise Danielsson-Tham

School of Hospitality
Culinary Arts and Meal Science
Örebro University
Grythyttan, Sweden

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business
A SCIENCE PUBLISHERS BOOK

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper

International Standard Book Number: 978-1-4665-8498-3 (Hardback)

Library of Congress Cataloging-in-Publication Data

Food associated pathogens / editors, Wilhelm Tham, Marie-Louise Danielsson-Tham.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-4665-8498-3 (hardcover : alk. paper)
I. Tham, Wilhelm, editor of compilation. II. Danielsson-Tham, Marie-Louise, 1947- editor of compilation.
[DNLM: 1. Food Microbiology. 2. Foodborne Diseases--microbiology. 3. Food Safety. QW 85]

RA601.5
664.001'579--dc23

2013028931

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

CRC Press Web site at
http://www.crcpress.com

Science Publishers Web site at
http://www.scipub.net

Food Associated Pathogens

Preface

Food borne diseases associated with microbial pathogens account for a significant share of human illnesses. Changes in both, lifestyle and eating habits increase the chances of transmission of pathogenic microorganisms in foods. Examples, of this are the consumption of raw foods, which increases the risk of food borne infections and intoxications, and refrigerated ready-to-eat foods, as some food borne pathogens are capable of being active even at refrigeration temperatures. Extending shelf life of many food items is not always safe for consumers. In addition, personal hygiene standard of food workers has worsened, leading to enhanced food borne illnesses. Food safety education needs to be reintroduced, even at the primary level. The number of people susceptible to food borne disease has increased, especially among the population over 65 years of age. Thus, food microbiologists working with food-associated pathogens need to be familiar with the type of microorganisms associated with a food product in its natural state to be able to predict the general types of microorganisms expected in a particular food product. Food microbiologists must also be familiar with the incubation periods and clinical symptoms of different food borne diseases. The effective prevention of food borne diseases requires cooperation and open-mindedness among different authorities and professionals, e.g., physicians, medical officers, veterinarians, public health officers, food producers, national surveillance institutes, scientists, and government departments.

Future food safety should be an important factor in trade, and countries with the best food safety will gain market share. Change is the way of life, and new pathogens will emerge and old pathogens will reemerge in response to these changes; therefore, food microbiologists never will be short of work.

Food Associated Pathogens examines pathogenic bacteria, viruses, protozoan parasites, moulds, and mycotoxins in food, discusses food-associated antimicrobial resistance and lessons learned from actual food borne outbreaks, and explores the clinical aspects of food borne diseases. The book is intended for postgraduate students, physicians, veterinarians, scientists, technologists, and inspectors in public health-related fields who regularly contend with the issues related to food microbiology, food borne infections and intoxications, and food safety. The chapters in the book are entirely the work of the authors, who had freedom to write the text as they wanted, but were urged, to divide the chapter into four main parts: The Microorganism, The Patient, The Risk Food and its Control and Prevention. Pathogens are presented in an alphabetical order.

Grythyttan, Sweden
June 2013

Wilhelm Tham
Marie-Louise Danielsson-Tham

Contents

CHAPTER 1

Food-associated Pathogens—Insights and Reflections

Marie-Louise Danielsson-Tham[#]

Food Microbiology

To be a food microbiologist is special. You have to be a combination of a laborer and a detective and have an understanding of complex food systems and different food preparation systems, microbes, and, of course people. In addition, if something goes wrong, you must solve all problems as rapidly as possible.

The search for bacteria in foods is slightly different to that in clinical samples: sometimes, a food sample resembles a fecal sample more than what it actually is. As a food product is never sterile, the analyses are complicated by the high levels of indigenous microflora present in some foods. These microorganisms can interfere with the isolation and identification of a specific bacterium. Foods contain infinite arrays of ingredients, including proteins, carbohydrates, fats and numerous other components that can interfere with the detection of pathogens. The structure of foods also varies between liquid, semisolid, solid, or other forms, which can interfere with consistent extraction and isolation of bacteria and obtaining a uniform food homogenate for reproductive analysis.

Various treatments used in food processing such as heat, cold, freezing, drying, and the use of additives, e.g., preservatives, can cause sublethal injury to bacterial cells. The injured or stressed cells are extremely sensitive

[#] School of Hospitality, Culinary Arts and Meal Science, Örebro University, Box 1, S-712 02 Grythyttan, Sweden.
Email: marie-louise.danielsson-tham@oru.se

to ingredients used in selective microbiological media; consequently, standard microbiological methods can miss the stressed bacteria.

The tests for some specific pathogens (e.g., *Salmonella* and *Listeria monocytogenes*) need to be highly sensitive because of the zero tolerance for these organisms in all or certain foods. The psychotropic bacteria, e.g., *Yersinia enterocolitica* and *L. monocytogenes*, may be present at very low levels immediately after processing, but on storage and distribution, these bacteria can multiply to potentially hazardous levels in the food. In addition, low infective dose pathogens such as STEC and *Campylobacter* demand special isolation procedures.

Some food poisoning is caused by toxic compounds produced by bacteria actively growing in food and not through invasion of the human body by the organism, for example staphylococcal food poisoning. Therefore, a food could be free from staphylococci and yet be involved in an outbreak of food poisoning caused by it.

Therefore, a food microbiologist needs to be familiar with the type of microorganisms associated with a food product in its natural state. With this kind of knowledge, a food microbiologist can predict general type of microorganisms expected in a particular food product at a later stage in its history and organisms not usually present in a particular food product.

The food microbiologist must also be familiar with the incubation periods and clinical symptoms for different foodborne diseases. For example, the first reported gastrointestinal outbreak of listeriosis in Sweden was preceded by many specialists initially being skeptical about listeriosis as the diagnosis due to the short incubation period and atypical symptoms such as diarrhea and vomiting.

Food as Ecosystems

Foods are ecosystems and comprise the environment and the organisms that live or thrive in them. The microenvironment may be altered during processing, e.g., oxygen is driven out of the food during cooking and diffuses back into the food so slowly that most of the products remain anaerobic.

The characteristics of the food are intrinsic factors and include pH, water activity, oxidation-reduction potential (Eh), nutrient content, antimicrobial constituents, and biological structures, e.g., shells of eggs or skins on fruits. Through determination of the intrinsic factor for a food, the types of microorganisms likely to grow in the particular food can be generally predicted.

Extrinsic factors are external to the food and include the properties of the storage environment that affects both the foods and their microorganisms. The extrinsic factors with the largest influence on microbial growth are temperature of storage, gas composition, and relative humidity of the environment.

Estimating the Burden of Foodborne Illness

Foodborne diseases associated with microbial pathogens are an important cause of human illness. However, the true incidence of foodborne illness is unknown and depends on many circumstances. To begin with, someone must suspect the illness is caused by food and then the food and adequate samples from the patient must be analyzed. A number of events need to occur for a laboratory confirmed case: the ill person must seek medical care, a specimen must be submitted, the laboratory must test for the pathogen, and the case must be reported to health authorities. A notifiable disease is required by law to be reported to government authorities. However, the nature of notifiable foodborne diseases varies from country to country. Some countries report finding of certain pathogenic bacteria, e.g., salmonella, even though the patient does not present any symptoms, other countries only report findings if the patient is hospitalized. Furthermore, not all outbreaks are investigated for every possible etiology as it is too expensive and at the time of investigation, the agent may have decreased below the detection level in the food and patient. Often food and stool samples are only analyzed routinely, thus, rarer bacteria will be undetected. If only conventional culturing is used, bacterial food poisonings caused by enterotoxins produced directly into the food, such as by, e.g., staphylococci, may be missed. Viruses still cannot be cultivated, thus, *in vitro* detection of viruses in food is problematic and as the infective dose for food-mediated viruses associated with illness is low, this further complicates their detection.

The cause of many incidents of food poisoning is obscured as leftover remains of the meals are discarded before the environmental health officer is contacted. Outbreaks associated with hospital and nursing homes are more frequently reported than outbreaks in restaurants and private homes, as sometimes food plants and restaurants deny they have had a problem with food poisoning due to fear of negative publicity and financial loss. However, most culturing methods are developed for known pathogens, not new yet unidentified, unknown pathogens; therefore, it is important to be curious about peculiar growth on agar plates!

New Eating Habits

Changes in lifestyle, social attitudes, and eating habits increase the opportunity for transmission of pathogenic bacteria in foods. The consumption of raw protein foods, such as raw fish in the form of sushi, ceviche, raw seafood, or raw meats, such as steak tartar implies significant risks for foodborne infections and intoxications. Undercooked eggs (soft boiled, soft scrambled, fried "sunny-side-up") and the use of unpasteurized eggs in food products are major factors in the incidence of *Salmonella Enteritidis*. As raw milk often harbors pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes* and Salmonella, milk and all dairy products should be pasteurized before consumption. In some countries, raw milk cheeses are stored at a minimum temperature of +2°C for at least 50 or 60 days to ensure the death of pathogenic microorganisms. However, subsequent research and documented outbreaks of foodborne illness caused by cheese have proved this storage is ineffective, particularly against *Salmonella* and *L. monocytogenes.*

Risk Groups

The number of people susceptible to foodborne disease has increased, especially among persons above 65 years. Susceptibility is increased in the rising number of people immunocompromised due to the HIV epidemic, with the increase in cancer and the use of immunosuppressive drugs, diabetes, alcoholism, malnutrition and antacids use. Pregnant women (and fetuses) are particularly susceptible to pathogens such as *L. monocytogenes* and *Toxoplasma gondi.*

New Food Processing and New Foods

Food production has changed with regard to new processes and new food products. For instance, the new generation of refrigerated foods, which are characterized by mild heat treatment, increased water activity, low salt content, low or no nitrite additive, a combination of several foods in the same product, new packaging methods, and long shelf life. *L. monocytogenes* and other foodborne pathogens are capable of growth even at refrigeration temperatures, in vacuum packages, and in controlled atmospheres. Thus, the extended shelf life of many food items can be a threat to the consumer's health.

Modified food processing has produced unusual opportunities for growth of bacteria. *Clostridium botulinum* has been identified in a variety of foods where it would never have been suspected previously. Botulism is reported to emanate from baked potatoes stored in the oven overnight, cloves of garlic immersed in oil stored at room temperature and from yoghurt with hazelnut cream.

Food reformulation is used as a strategy for producing foods for improved health; however, reformulation also changes the intrinsic physicochemical properties of the food, which may result in foods with shorter shelf lives and increased risk for the growth of pathogenic microorganisms. These foods often have higher water activity due to replacing NaCl with KCl or reducing the fat content.

Decreasing Budget—Poor Food Hygiene

During times with decreasing budgets and decreasing work force, there is no or too little space for preventive food hygiene. Inadequate cleaning procedures, lack of interest, complicated machinery and equipment increase the risk for foodborne infections and intoxications. In addition, personal hygiene has worsened, and improperly cleaned hands of food worker are increasingly responsible for foodborne illness. Cross-contamination from unclean cutting boards and knives and perishable food being left out of the refrigerator for too long are other important risk factors. The teaching of food safety needs to be reintroduced, even in primary education. Consumers, food handlers and food managers must be taught how to handle food products safely.

Analyzing Food Samples

In food microbiology, many selective culturing media are used. Some ingredients of the medium favor the growth of the targeted bacteria, other medium components inhibit the growth of competing microorganisms. However, if target organisms are injured, they may be killed by the selective component. Selective media are never entirely selective, in that, microorganisms other than the target microorganism will also grow. Thus, the identity of suspect pathogens isolated on selective media must be confirmed and the subtype determined through phenotyping and genotyping methods.

For some bacteria, e.g., Salmonella, there is zero tolerance in foods, and that means finding one Salmonella bacterium hiding among several million other bacteria. In such cases, the usual procedure is preenrichment in a nonselective medium, then enrichment in a selective medium: the pathogen can then be isolated on a selective agar. Injured bacterial cells and "viable but nonculturable" cells pose additional problems to food microbiologists, as these cells cannot form colonies and be detected, but can make people ill. Injured and "viable but non culturable" cells are also important from the aspect of food safety: if such bacterial cells are considered dead after, e.g., heat treatment, the effect of heating will be overestimated. Bacterial cells that escape detection during post-processing controls may repair during food storage and cause illness when the food is eaten.

Although a number of PCR-based methods have been developed to increase the speed of the isolation procedure, most foods contain inhibitory factors for PCR reaction. Traditional methods should never be replaced by alternative methods; therefore, molecular biological methods should be used in conjunction with conventional bacteriological methods. There will always be a need for viable bacterial cells obtained through standard cultural procedures and viable cells as bacterial strain collections will be important for the future.

Typing, Subtyping, and Characterization

The typing methods can be divided into phenotyping and genotyping methods.

Phenotypic typing comprises biotyping, serotyping, phage-typing and antimicrobial susceptibility testing. Genotyping methods characterize a nucleic acid target and include ribotyping, Multilocus Enzyme Electrophoresis (MEE), Random Amplification of Polymorphic DNA (RAPD), Pulsed Field Gel Electrophoresis (PFGE), Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA), and Multi-Locus Sequence Typing (MLST) or Multi-Virulence-Locus Sequence Typing (MVLST). In the investigation of foodborne diseases, the isolation of a bacterial species from patients and the isolation of the same species from a food product, direct suspicion towards a particular product. In an outbreak investigation, fast methods such as serotyping are useful as a first-step typing method for screening strains. However, a more discriminating method for identifying the epidemic strain is also needed; as it is preferable, the isolates from both the food and the patient are genetically identical. Typing methods can also be used when investigating routes of contamination in certain environments, for example, the spread of food borne pathogens, such as *L. monocytogenes*, in a food processing plant. Typing would clarify whether a variety of clonal types are just passing through the plant, or if the contamination is firmly established. In the case of a house flora consisting of a specific clonal type, the eradication regime may be different if new strains continuously enter the plant. Sometimes more than one clonal type of the pathogenic bacterium is present in the suspected food and/or the patient. Therefore, the isolation and typing of more than one colony from the primary culture medium is recommended.

Prevention

To address the problem of food safety, several countries have established national data collection and surveillance systems. National surveillance systems are useful for identifying trends in both the overall burden of foodborne illnesses and the changes in the frequency at which specific pathogens are identified. Surveillance systems are useful for detecting emerging pathogens and new routes or vehicles of transmission. This requires the data within the system to be correct and include samples analyzed from both patients and foods and the subtyping of a sufficient number of strains through discriminating methods. At times with decreasing budget, it could be tempting to reduce information collection and only analyze as many samples as necessary for the safety of the patients. However, this type of misdirected strict economy can have devastating consequences in the future as the improved comprehensive monitoring and surveillance systems, both intra- and inter-country and even for antibiotic drug resistance, need to be established.

Effective prevention to foodborne diseases is not possible through efforts just targeting one specific object. A complete picture of the overall situation is important, and the entire pathway should be represented from crop growing until consumption of the food product. This requires an interdisciplinary approach and collaboration between different disciplines, from the molecular biologist to the clinician. Inherent in this is cooperation and open-mindedness between different authorities and professionals, e.g., physicians, medical officers, veterinarians, public health officers, food producers, government ministers and departments.

Future food safety should be an important factor in trade and countries with the best food safety will gain new international markets. As change is the way of life, it is certain new pathogens will emerge and old pathogens will reemerge in response to change. Therefore, food microbiologists will never be short of work.

CHAPTER 2

Food Associated Antimicrobial Resistance

Annamari Heikinheimo[#]

Introduction

Throughout history, infectious diseases have been a major threat to human and animal health and a significant cause of mortality. Thus, it is not surprising that the discovery of antimicrobial drugs to treat bacterial infections has been termed as one of the greatest innovations in medicine. However, bacterial resistance to antimicrobial drugs has emerged rapidly and antimicrobial resistance has become a major public health problem globally (WHO 2011). In 2007, altogether 400,000 people in Europe suffered from infections caused by multidrug-resistant bacteria. More than 25,000 people died from these infections. The additional expenditure in terms of hospital costs and productivity losses exceeds €1.5 billion each year in Europe (ECDC and EMEA 2009). In the United States, antimicrobial-resistant infections cause $20 billion excess health care costs, $35 billion societal costs, and 8 million additional hospital days (Roberts et al. 2009).

Any kind of use of antimicrobial drugs in humans, animals or plants can promote the development of resistance. Antimicrobial resistance does not respect geographical or biological borders either. The global economy and the movement of foods, animals and humans have a massive and rapid impact over a wide area, enabling the spread of resistant bacteria.

Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, P. O. Box 66, FI-00014 University of Helsinki, Helsinki, Finland.
Email: annamari.heikinheimo@helsinki.fi

The purpose of this article is to introduce antimicrobial resistance related to the food chain, and shed light on important food-borne pathogens with elevated resistance levels.

The History of Antimicrobial Drugs

The first commercially available antimicrobial drug was prontosil (sulfanilamide), a textile dyestuff which was found to be active against streptococci in experimental study in mice (Domagk 1935). At that time, other antibacterial agents were also discovered. In 1929, Alexander Fleming reported his observations on the antimicrobial action of a fungus from the genus *Penicillium* (Fleming 1929). Howard Florey and Ernst Chain succeeded in purifying the first penicillin further, procaine penicillin G. Both discoveries of prontosil and penicillin were rewarded with Nobel Prizes in Physiology and Medicine; Gerhard Domagk for prontosil in 1939, and Ernst Chain, Alexander Fleming and Howard Florey for penicillin in 1945.

A large number of new antimicrobial compounds continued to be discovered during the following decades. Many completely synthetic drugs became available, belonging to new and effective classes of antimicrobial drugs. Today, there are only few antibacterial agents under development. Due to the rapid antimicrobial resistance development in bacteria, there is need for new antibacterial agents with new mechanism of actions. There is a particular lack of effective agents to treat infections due to multidrug-resistant gram-negative bacteria (ECDC and EMEA 2009).

Antimicrobial Drugs and their Mechanisms

Antimicrobial drugs are essential for the health and welfare of both humans and animals. They are substances that have the capacity to selectively inhibit the growth and destroy microorganisms. Antimicrobial drugs may be substances of microbial origin, or synthetically manufactured. Originally the word antibiotic refers to substance of microbial origin, although the term is commonly used for all these compounds (Giguère 2006, Guardabassi and Courvalin 2006).

Antimicrobial drugs have different mechanisms by which they affect bacteria. Depending on the mechanism, the final result is either inactivation or actual death of the bacteria. Antimicrobial drugs are termed as bactericidal or bacteriostatic, depending on whether they inactivate or actually kill the bacteria. Bactericidal drugs are those that kill the target organisms, whereas bacteriostatic drugs inhibit or delay bactericidal growth and replication. Some antimicrobial drugs can have both bacteriostatic and bactericidal effects, depending on dosage, duration of exposure and the