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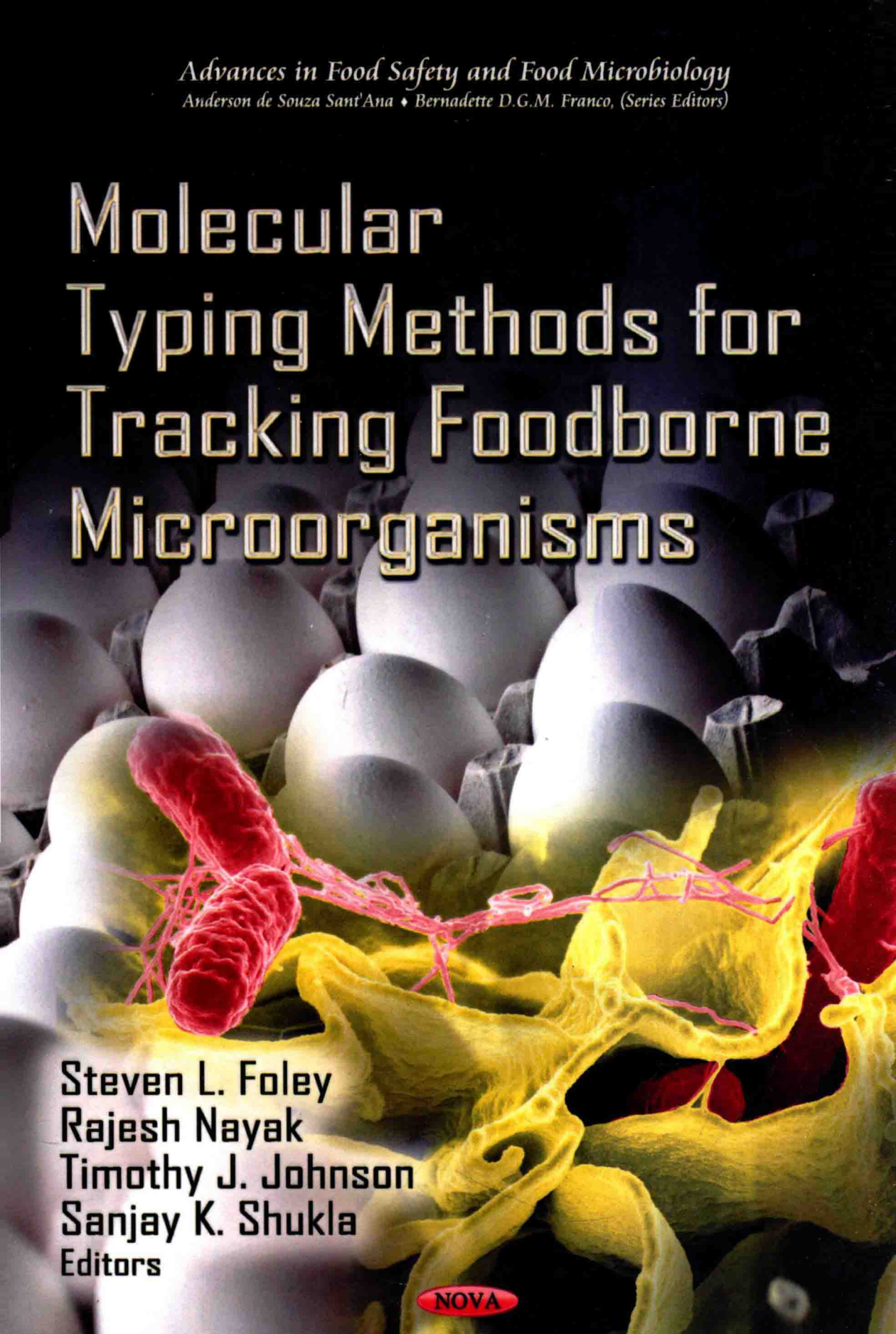
Anderson de Souza Sant'Ana ♦ Bernadette D.G.M. Franco, (Series Editors)

Molecular Typing Methods for Tracking Foodborne Microorganisms



Steven L. Foley
Rajesh Nayak
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Sanjay K. Shukla
Editors

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ADVANCES IN FOOD SAFETY AND FOOD MICROBIOLOGY

MOLECULAR TYPING METHODS FOR TRACKING FOODBORNE MICROORGANISMS

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PREFACE

Foodborne illness remains a major problem throughout the world. In the United States for example it is estimated that there are over 48 million illnesses each year associated with the consumption of contaminated foods. Of those with known etiologies, approximately 39% are associated with bacterial pathogens[1]. These bacterial infections often tend to be more severe than their viral counterparts resulting in approximately 64% of hospitalizations and deaths each year from foodborne pathogens. Thus being able to get to the source of the contamination of foods is an important endeavor to begin to develop strategies to lessen the burden of bacterial foodborne illness on the human population.

We thank Dr. Anderson de Souza Sant'Ana for the invitation to edit this book as part of the broader series on *Advances in Food Safety and Food Microbiology*. We hope that the book will be useful as a reference to students, practitioners and food safety scientists who need to gain a fundamental understanding of the different options available for molecular typing of foodborne pathogens. A goal of the book is to provide insights for scientists interested in using these molecular typing methods as part of source tracking protocols.

In the process of planning the book, we chose to break the book down into three major sections that will provide the reader with an integrated experience to understand the utility of molecular typing methods for tracking foodborne microorganisms. The first section focuses on bacterial foodborne pathogens and factors that play into their distribution and detection in the food production and processing environments. This section includes a brief introduction into the major foodborne pathogens and description of surveillance and outbreak investigation basics. The section then focuses on the genomics of the pathogens and the contribution of plasmids to microbial diversity and function. The final two chapters in the section explore the important topic of antimicrobial resistance and methods to rapidly detect the presence of bacterial pathogens in foods.

The second section will focus on the specific typing methods, providing insights into the methodology and utility of these methods for foodborne pathogens. The first chapter of the section provides an introduction both into the phenotypic methods for characterization of foodborne pathogens as well as a brief introduction the molecular methods covered in the rest of the section. We felt it important to cover the phenotypic methods because they still remain in use today and many laid the groundwork for current molecular methods. For the molecular

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[1] Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States-major pathogens. Emerg Infect Dis. 2011;17(1):7-15.

methods, pulsed field gel electrophoresis was given its own chapter due to its status as the "gold standard" method for many foodborne pathogens. Other chapters focus on some of the more commonly used techniques along with special focus on some of the more recent advances in typing methods (sequence-based methodologies, microarray, etc.).

The third section will focus on the analysis of the typing data and utility of molecular typing methods in a source tracking scheme. The first chapter examines the methods used to analyze the various types of molecular typing data, which is important for the interpretation of the results. The second chapter in the section focuses on the incorporation of molecular typing data into epidemiological investigations and the final chapter wraps up the book and hopefully provides some insights into the future of molecular typing of bacterial foodborne pathogens.

The views and information presented in the individual chapters are those of the authors and since they were invited to write the chapters based on their expertise in the subject areas. Likewise the views and editorial decisions in the book are those of the editors and do not necessarily represent those of our respective organizations, the U.S. Food and Drug Administration, Marshfield Clinic Research Foundation or the University of Minnesota. Additionally, the use of trade names in this book is for identification purposes only, and does not imply endorsement by the U.S. Food and Drug Administration or the U.S. Department of Health and Human Services.

We hope that you find this book useful and that you enjoy reading the information present herein.

Steven L. Foley
Rajesh Nayak
Timothy J. Johnson
Sanjay K. Shukla

FOREWORD

Diseases caused by foodborne pathogens are major causes of morbidity and mortality throughout the world. Estimates by the Centers for Disease Control indicate that there could be as many as 48 million cases of foodborne illnesses in the United States each year. While the incidence of disease caused by some foodborne pathogens has fallen in the past few years, the incidence of disease caused by pathogens such as *Salmonella enterica* seem to remain largely unchanged. The epidemiology of foodborne illnesses has been changing and the apparent proportion of cases associated with fresh fruits and vegetables has shown large increases. It has been estimated that viruses cause the largest number of foodborne illnesses. However, bacterial foodborne pathogens cause more severe diseases than their viral counterparts, account for the largest proportion of deaths, and many have developed resistance to the antimicrobial agents that are used to treat severe infections. Outbreak investigations have relied on the timely collection of accurate surveillance information including disease clusters, strain identification, and potential sources of contamination. Vital to any analysis of an outbreak is the use molecular technologies to characterize the cause of the infections and these technologies have been essential tools of source attribution. Molecular analyses of foodborne pathogens have been used to understand how these pathogens disseminate through the food production and processing environments and have been essential in the development of strategies to limit the exposure of consumers to foodborne pathogens. PulseNet, a pulsed field electrophoresis-based tool, has been an important tool implemented by the CDC and used in outbreak investigations. Thus, molecular subtyping methods are vital tools that are being used along with epidemiological investigations to implement successful microbial source tracking schemes that can help limit bacterial foodborne illnesses.

In the recent years there have been numerous reviews and research articles written about the use of various molecular subtyping for the characterization of bacterial foodborne pathogens. This book, *Molecular Typing Methods for Tracking Foodborne Microorganisms*, provides an integrated experience for the reader to gain a thorough understanding of the utility of the different molecular typing methods for identifying and tracking bacterial foodborne pathogens. The chapters in this book look at different subtyping methods in an integrated way and provide discussion of recent literature to bring the reader an up-to-date understanding of the current state of the field. The editors of the book have brought together an excellent group of authors with expertise in the areas of food safety and molecular subtyping that provide valuable insights for readers. This book should be a valuable

reference for those who are interested in learning the fundamentals of the different types of molecular subtyping methods, as well as gaining insights on what the future of the molecular subtyping holds.

The editors have chosen to present the book in three major sections that include "Foodborne Pathogens", "Molecular Typing Methods" and "Analysis and Utility of Molecular Typing Methods", which will provide the reader with a logical flow from understanding the pathogens through to the interpretation and application of subtyping methods. The first section provides an overview of the pathogens, including chapters related to bacterial genetics, antimicrobial resistance, and methods to detect pathogens in the food production and processing environments. The second section focuses on the specific subtyping methods and the application of these methods to the characterization of foodborne pathogens. The third section focuses on the analyses of subtyping data, the utility of typing in epidemiological investigations and a look to the future of molecular subtyping for tracking foodborne pathogens. Because of the comprehensive nature of the book, I believe that it will be a valuable reference for students, practitioners and food safety scientists who need to gain a strong understanding of the molecular typing of foodborne pathogens.

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SECTION I. THE FOODBORNE PATHOGENS

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Chapter 1

INTRODUCTION TO FOODBORNE PATHOGENS AND INFECTIONS

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ABSTRACT

Foodborne illnesses are a major public health concern worldwide. Foods of animal origin (meat, poultry and eggs), seafood, and raw and processed foods have been implicated in foodborne illnesses. In the US alone, there are over 48 million illnesses associated with foodborne pathogens, costing the US economy $10 to 83 billion annually. This chapter will highlight the characteristics of some leading agents that are involved in foodborne illnesses and the factors that are attributed to outbreaks caused by these pathogens. We address emerging foodborne pathogens and drug-resistant bacteria, and their contributions to food safety issues. Furthermore, we describe how foodborne investigations are carried out, the public health surveillance systems that monitor trends in infection rates of foodborne pathogens, and the agencies that are involved in investigating and monitoring operations. Lastly, we include some general strategies that can be used to reduce or eliminate the contamination of foods by these pathogens, thereby limiting the scope of accidental or deliberate outbreaks in human populations.

FOODBORNE ILLNESSES

Food is an important vehicle of transmitting diseases in humans. There are over 200 known microbial, physical and chemical agents that can cause food-related illnesses [1]. Foodborne pathogens are a major cause of morbidity and mortality throughout the world (http://www.who.int/foodsafety/foodborne_disease/ferg/en/index.html) [2-5]. There is limited

epidemiological information available on the global burden caused by foodborne diseases [6]. In the United States, the Centers for Disease Control and Prevention (CDC) estimates that foodborne diseases cause approximately 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths in the United States each year [7]. Of these, nearly 9.4 million illnesses, 56,000 hospitalizations, and 1,350 deaths are caused by known and identified pathogens [8]. These reported foodborne illnesses are only a small percentage of those that are actually occurring. According to the CDC, small outbreaks occurring in the US are unlikely to be reported to public health officials [9]. According to the US Department of Agriculture (USDA), foodborne illnesses cost the US economy 10 to 83 billion dollars each year [5]. The costs include loss of productivity, legal fees, medical expenses, loss of sales, loss of wages and loss of life [5, 10].

The leading bacterial species associated with illness include *Salmonella enterica, Campylobacter* spp., *Shigella* spp., *Clostridium* spp., *Escherichia coli* and *Yersinia enterocolitica* [4, 6]. In addition, viruses, such as hepatitis (A and E), Norwalk and Rotavirus [4, 11, 12], and parasites, such as *Cryptosporidium parvum*, *Entamoeba*, *Cyclospora* spp., *Giardia lambia*, *Toxoplasma gondii* and *Trichinella spiralis* [4, 13], have been implicated in food related illnesses. Foods of animal origin, particularly meat, poultry and eggs, have been associated with outbreaks [6, 14]. Other food vehicles associated with foodborne illnesses, include unpasteurized milk, other dairy products, raw fruits and vegetables, unpasteurized fruit juices and raw sprouts, fish and seafood and ready-to-eat foods (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/foodborneinfections_g.htm#riskiestfoods) [15-19] . Outbreaks of foodborne illness have been attributed to rapid population growth, shifts in demographics and lifestyles, human behavior, changes in industry and technology, changes in travel and commerce, the shift towards a global economy, microbial adaptation, breakdowns in public health infrastructure, a higher proportion of immuno-compromised individuals, changes in farming practices, lack of knowledge on food safety and handling practices, and climate change [3, 4, 20-24].

Other valuable resources describing foodborne pathogens include the FDA Bad Bug Book (http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodborne PathogensNaturalToxins/BadBugBook/default.htm), the CDC website (http://www.cdc.gov/ foodsafety/diseases) and others, so this chapter will not include an in-depth look at each individual foodborne pathogen. Table 1, however, provides an overview of the microbial agents responsible for causing foodborne illnesses.

EMERGING PATHOGENS

The ability of bacterial pathogens to adapt to and survive unfavorable environmental and processing conditions has been attributed to the diverse genetic background [22]. The mechanisms by which these pathogens evolve vis-à-vis their pathogenicity, genome rearrangement and physiology has contributed to emergence of new pathogens [5]. These emerging pathogens may exhibit greater pathogenicity and antimicrobial resistance than their existing counterparts, resulting in serious public health implications [25-29].

Table 1. A selective list of agents involved in foodborne illnesses*

Agents	Basic Information	Incubation period	Duration of disease	Nature of disease/symptoms	Selective species/ serotypes involved in foodborne illnesses	Foods associated with outbreaks	Major outbreaks (Serotype)	Treatment
Salmonella	• Gram negative, rod shaped, motile bacterium. • >2,500 serotypes identified • Wide spread in animals, particularly poultry and swine. • Also found in the environment (water, soil, raw feces, and insects) and raw meat and poultry. • Infective dose 15-20 CFU.	1-3 days	4-7 days	Nausea, diarrhea, vomiting, cramps, fever, headaches, chronic arthritis. Complications may include reactive arthritis and Reiter's syndrome	Typhimurium, Enteritidis, Newport Heidelberg, Javiana	Contaminated eggs, poultry, unpasteurized milk, raw fruits and vegetables, sprouts	Shelled eggs (Enteritidis), Raw produce (St. Paul), Cantaloupe (Litchfield), Peanut (Tennessee), Tomatoes (Typhimurium), and ice cream (Enteritidis)	Supportive care. Severe cases treated with ampicillin, gentamicin, sulfa drugs or quinolones
Campylobacter	• Gram negative, slender, curved, and motile rod. • Microaerophilic • Relatively fragile and sensitive to environmental stresses (e.g., 21% oxygen, drying, heating, disinfectants, acidic conditions) • Animal pathogen • Leading cause of diarrheal disease in the US	2-5 days	2-10 days	Nausea, vomiting, cramps, fever, headaches, chronic arthritis. Complications may include reactive arthritis and Reiter's syndrome	*C. jejuni, C. coli*	Raw and undercooked poultry, contaminated milk and water	Clams, raw goat and cow milk, drinking water	Supportive care. Severe cases treated with erythromycin or quinolones