

METHODS IN MOLECULAR BIOLOGY

Series Editor

John M. Walker

School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes:
<http://www.springer.com/series/7651>

Streptococcus pneumoniae

Methods and Protocols

Edited by

Federico Iovino

*Department of Microbiology, Tumor and Cell Biology Karolinska Institutet, Bioclinicum, Stockholm,
Sweden*

Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden



Editor

Federico Iovino
Department of Microbiology, Tumor and Cell Biology
Karolinska Institutet, Bioclinicum
Stockholm, Sweden

Department of Clinical Microbiology
Karolinska University Hospital
Stockholm, Sweden

ISSN 1064-3745

ISSN 1940-6029 (electronic)

Methods in Molecular Biology

ISBN 978-1-4939-9198-3

ISBN 978-1-4939-9199-0 (eBook)

<https://doi.org/10.1007/978-1-4939-9199-0>

Library of Congress Control Number: 2019934805

© Springer Science+Business Media, LLC, part of Springer Nature 2019

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Humana Press imprint is published by the registered company Springer Science+Business Media, LLC part of Springer Nature.

The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Aim of the Book

Abstract

The Gram-positive bacterium *Streptococcus pneumoniae*, the pneumococcus, is a leading cause of mortality and morbidity worldwide and considered a serious threat in today's public health. It is a major contributor of severe diseases such as pneumonia and bacteremia and the main etiological cause of bacterial meningitis. All these diseases are defined as invasive pneumococcal disease (IPD). Even though pneumococci can cause invasive diseases, *S. pneumoniae* is a commensal, and in fact, it normally colonizes the nasopharyngeal epithelium asymptotically. In the last decade, there have been important advances in the development of new methodologies to study the cell biology of the pneumococcus and how *S. pneumoniae* interacts with the human host. The aim of this book is to shed light into the materials and methods used to study pneumococci and IPD.

Key words: *Streptococcus pneumoniae* • Pneumococcus • Host • Molecular biology • Methods

Federico Iovino

Preface

The Gram-positive bacterium *Streptococcus pneumoniae*, the pneumococcus, is considered a serious threat in today's public health, not only because it is a major cause of serious diseases like pneumonia, bacteraemia, and meningitis but also because of the overuse and misuse of antibiotics; the cases of antibiotic resistance have increased dramatically [1–4]. In addition, introduction of the pneumococcal conjugate vaccines (PCVs) has decreased the incidence of pneumococcal meningitis caused by the 7 (PCV7) or 13 (PCV13) serotypes included in the vaccine, but the incidence of invasive pneumococcal disease caused by non-vaccine types has increased [5, 6].

Nowadays, there have been important advances in many techniques used to study the molecular biology of the pneumococcus, from the methodologies to study protein and gene expression to novel experimental setups to study invasive pneumococcal disease *in vivo*. Importantly, in the recent years, new discoveries, like the CRISPR/Cas9 system, have had a tremendous impact in biomedical research. Imaging techniques have grown tremendously in the recent years; today, the molecular mechanisms regulating the cell biology of the pneumococcus and bacterial interaction with the human host can be investigated with high-, through live-cell imaging, and super-resolution microscopy. Last but not least, epidemiological studies have become more and more comprehensive and accurate, thanks to the extensive use of whole-genome sequencing and the availability of collections from many countries and international research consortia of bacterial clinical isolates.

Materials and methods are the bridge that consent scientists to verify hypotheses, collect results, and create knowledge. The aim of this book is to shed light into all the methods, materials, equipment, and new technologies developed and used nowadays to study the cell biology of the pneumococcus, at a protein and gene level, the pneumococcal interaction with the human host, both *in vitro* and *in vivo*, and the epidemiology of IPD. Essentially, each chapter aims to describe a specific technique or application in an easy-to-follow step-by-step format for the scientific community.

*Karolinska Institutet
Stockholm, Sweden*

Federico Iovino

References

1. Laxminarayan R, Duse A, Wattal C (2013) Antibiotic resistance-the need for global solutions. *Lancet Infect Dis* 13:1057–98
2. Dockrell DH, Whyte MKB, Mitchell TJ (2012) Pneumococcal pneumonia: mechanisms of infection and resolution. *Chest* 142:482–491

3. O'Brien KL, Wolfson LJ, Watt JP (2009) Hib and pneumococcal global burden of disease study team, burden of disease caused by streptococcus pneumoniae in children younger than 5 years: global estimates. *Lancet* 374:893–902
4. van de Beek, D, de Gans J, Tunkel AR (2006) Community-acquired bacterial meningitis in adults. *N Engl J Med* 354:44–53
5. Browall S, Backhaus E, Naucler P (2014) Clinical manifestations of invasive pneumococcal disease by vaccine and non-vaccine types. *Eur Respir J* 44:1646–57
6. Galanis I, Lindstrand A, Darenberg J (2016) Effects of PCV7 and PCV13 on invasive pneumococcal disease and carriage in Stockholm, Sweden. *Eur Respir J* 47:1208–1218

Contents

Preface	vii
Contributors	xi

PART I CULTIVATION OF *STREPTOCOCCUS PNEUMONIAE* IN VITRO

- 1 Optimal Conditions for *Streptococcus pneumoniae* Culture:
In Solid and Liquid Media 3
Norma Suárez and Esther Texeira

PART II MICROSCOPY TECHNIQUES TO STUDY THE BIOLOGY OF *STREPTOCOCCUS PNEUMONIAE* AND PNEUMOCOCCAL INTERACTIONS WITH THE HOST

- 2 Electron Microscopy to Study the Fine Structure of the Pneumococcal Cell 13
Sven Hammerschmidt and Manfred Rohde
- 3 Immunofluorescent Staining and High-Resolution Microscopy
to Study the Pneumococcal Cell 35
Federico Iovino and Birgitta Henriques-Normark
- 4 Construction of Fluorescent Pneumococci for In Vivo Imaging
and Labeling of the Chromosome 41
Morten Kjos
- 5 High-Resolution and Super-Resolution Immunofluorescent
Microscopy Ex Vivo to Study Pneumococcal Interactions with the Host 53
Federico Iovino and Birgitta Henriques-Normark

PART III THE GENETICS OF *STREPTOCOCCUS PNEUMONIAE*

- 6 Natural Genetic Transformation: A Direct Route to Easy Insertion
of Chimeric Genes into the Pneumococcal Chromosome 63
*Isabelle Mortier-Barrière, Nathalie Campo, Mathieu A. Bergé,
Marc Prudhomme, and Patrice Polard*
- 7 Gene Expression Analysis in the Pneumococcus 79
*Rory A. Eutsey, Carol A. Woolford, Surya D. Aggarwal,
Rolando A. Cuevas, and N. Luisa Hiller*
- 8 Transcriptional Knockdown in Pneumococci Using CRISPR Interference 89
Morten Kjos

PART IV THE PROTEOME AND PROTEOMICS OF *STREPTOCOCCUS PNEUMONIAE*

- 9 Protein Expression Analysis by Western Blot and Protein–Protein Interactions 101
María Dolores Cima-Cabal, Fernando Vazquez, Juan R. de los Toyos, and María del Mar García-Suárez
- 10 Mass Spectrometry to Study the Bacterial Proteome from a Single Colony 113
Jianwei Zhou, Lu Zhang, Huixia Chuan, Angela Sloan, Raymond Tsang, and Keding Cheng
- 11 Bead-Based Flow-Cytometric Cell Counting of Live and Dead Bacteria 123
Fang Ou, Cushla McGoverin, Joni White, Simon Swift, and Frédérique Vanholsbeeck

PART V *STREPTOCOCCUS PNEUMONIAE*-HOST INTERACTIONS:
IN VITRO AND IN VIVO MODELS

- 12 In Vitro Adhesion, Invasion, and Transcytosis of *Streptococcus pneumoniae* with Host Cells 137
Terry Brissac and Carlos J. Orihuela
- 13 Growing and Characterizing Biofilms Formed by *Streptococcus pneumoniae* 147
Yashuan Chao, Caroline Bergenfelz, and Anders P. Hakansson
- 14 In Vivo Mouse Models to Study Pneumococcal Host Interaction and Invasive Pneumococcal Disease 173
Federico Iovino, Vicky Sender, and Birgitta Henriques-Normark
- 15 Two-Photon Intravital Imaging of Leukocytes in the Trachea During Pneumococcal Infection 183
Miguel Palomino-Segura and Santiago F. Gonzalez
- 16 IVIS Spectrum CT to Image the Progression of Pneumococcal Infections In Vivo 195
Adam Sierakowiak, Birgitta Henriques-Normark, and Federico Iovino

PART VI PUBLIC HEALTH, EPIDEMIOLOGY, AND BIOSTATISTICS

- 17 The Pneumococcus and Its Critical Role in Public Health 205
Godwin Oligbu, Norman K. Fry, and Shamez N. Ladbani
- 18 The Epidemiology and Biostatistics of Pneumococcus 215
Godwin Oligbu, Norman K. Fry, and Shamez N. Ladbani
- Index* 225

Contributors

SURYA D. AGGARWAL • *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA*

MATHIEU A. BERGÉ • *Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), Centre de Biologie Intégrative (CBI), Toulouse, France; Centre National de la Recherche Scientifique (CNRS), Université de Toulouse, Université Paul Sabatier (UPS), Toulouse, France*

CAROLINE BERGENFELZ • *Wallenberg Laboratory, Division of Experimental Infection Medicine, Department of Translational Medicine, Lund University, Malmö, Sweden*

TERRY BRISSAC • *Department of Microbiology, School of Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA*

NATHALIE CAMPO • *Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), Centre de Biologie Intégrative (CBI), Toulouse, France; Centre National de la Recherche Scientifique (CNRS), Université de Toulouse, Université Paul Sabatier (UPS), Toulouse, France*

YASHUAN CHAO • *Wallenberg Laboratory, Division of Experimental Infection Medicine, Department of Translational Medicine, Lund University, Malmö, Sweden*

KEDING CHENG • *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada; Department of Human Anatomy and Cell Sciences, College of Medicine, University of Manitoba, Winnipeg, MB, Canada*

HUIXIA CHUAN • *Henan Center for Disease Control and Prevention, Zhengzhou, Henan, People's Republic of China*

MARÍA DOLORES CIMA-CABAL • *Escuela Superior de Ingeniería y Tecnología (ESIT), Universidad Internacional de La Rioja (UNIR), Logroño, Spain*

ROLANDO A. CUEVAS • *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA*

JUAN R. DE LOS TOYOS • *Área de Inmunología, Facultad de Medicina y Ciencias de la Salud, Universidad de Oviedo, Oviedo, Spain*

MARÍA DEL MAR GARCÍA-SUÁREZ • *Escuela Superior de Ingeniería y Tecnología (ESIT), Universidad Internacional de La Rioja (UNIR), Logroño, Spain*

RORY A. EUTSEY • *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA*

NORMAN K. FRY • *Immunisation and Countermeasures Division, National Infection Service, Public Health England, London, UK; Respiratory and Vaccine Preventable Bacterial Reference Unit (RVPBRU), National Infection Service Laboratories, Public Health England, London, UK*

SANTIAGO F. GONZALEZ • *Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland*

ANDERS P. HAKANSSON • *Wallenberg Laboratory, Division of Experimental Infection Medicine, Department of Translational Medicine, Lund University, Malmö, Sweden*

SVEN HAMMERSCHMIDT • *Department of Molecular Genetics and Infection Biology, Interfaculty Institute for Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany*

- BIRGITTA HENRIQUES-NORMARK • *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Bioclinicum, Stockholm, Sweden; Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden; Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Lee Kong Chian School of Medicine (LKC), Nanyang Technological University (NTU), Singapore, Singapore*
- N. LUISA HILLER • *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA*
- FEDERICO IOVINO • *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Bioclinicum, Stockholm, Sweden; Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden*
- MORTEN KJOS • *Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway*
- SHAMEZ N. LADHANI • *Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St. George's, University of London, London, UK; Immunisation and Countermeasures Division, National Infection Service, Public Health England, London, UK*
- CUSHLA McGOVERIN • *Department of Physics, The University of Auckland, Auckland, New Zealand; The Dodd-Walls Centre for Photonic and Quantum Technologies, Auckland, New Zealand*
- ISABELLE MORTIER-BARRIÈRE • *Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), Centre de Biologie Intégrative (CBI), Toulouse, France; Centre National de la Recherche Scientifique (CNRS), Université de Toulouse, Université Paul Sabatier (UPS), Toulouse, France*
- GODWIN OLIGBU • *Paediatric Infectious Diseases Research Group, Institute for Infection and Immunity, St. George's, University of London, London, UK; Immunisation and Countermeasures Division, National Infection Service, Public Health England, London, UK*
- CARLOS J. ORIHUELA • *Department of Microbiology, School of Medicine, The University of Alabama at Birmingham, Birmingham, AL, USA*
- FANG OU • *Department of Physics, The University of Auckland, Auckland, New Zealand; The Dodd-Walls Centre for Photonic and Quantum Technologies, Auckland, New Zealand*
- MIGUEL PALOMINO-SEGURA • *Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland; Graduate School of Cellular and Molecular Sciences, Faculty of Medicine, University of Bern, Bern, Switzerland*
- PATRICE POLARD • *Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), Centre de Biologie Intégrative (CBI), Toulouse, France; Centre National de la Recherche Scientifique (CNRS), Université de Toulouse, Université Paul Sabatier (UPS), Toulouse, France*
- MARC PRUDHOMME • *Laboratoire de Microbiologie et Génétique Moléculaires (LMGM), Centre de Biologie Intégrative (CBI), Toulouse, France; Centre National de la Recherche Scientifique (CNRS), Université de Toulouse, Université Paul Sabatier (UPS), Toulouse, France*
- MANFRED ROHDE • *Central Facility for Microscopy, HZI—Helmholtz Centre for Infection Research, Braunschweig, Germany*
- VICKY SENDER • *Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Bioclinicum, Stockholm, Sweden*
- ADAM SIERAKOWIAK • *Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden*

ANGELA SLOAN • *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada*

NORMA SUÁREZ • *Department of Biotechnology, Faculty of Medicine, Institute of Hygiene, University of the Republic, Montevideo, Uruguay*

SIMON SWIFT • *School of Medical Sciences, The University of Auckland, Auckland, New Zealand*

ESTHER TEXEIRA • *Department of Biotechnology, Faculty of Medicine, Institute of Hygiene, University of the Republic, Montevideo, Uruguay*

RAYMOND TSANG • *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada*

FRÉDÉRIQUE VANHOLSBEECK • *Department of Physics, The University of Auckland, Auckland, New Zealand; The Dodd-Walls Centre for Photonic and Quantum Technologies, Auckland, New Zealand*

FERNANDO VAZQUEZ • *Departamento de Microbiología, Hospital Universitario Central de Asturias & Fundación para la Investigación y la Innovación Biosanitaria del Principado de Asturias (FINBA), Oviedo, Spain; Departamento de Biología Funcional & Ophthalmology, Vision Sciences and Advanced Therapies Research Group, Instituto Universitario Fernández-Vega, Universidad de Oviedo, Oviedo, Spain*

JONI WHITE • *The Dodd-Walls Centre for Photonic and Quantum Technologies, Auckland, New Zealand; School of Medical Sciences, The University of Auckland, Auckland, New Zealand*

CAROL A. WOOLFORD • *Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA*

LU ZHANG • *Henan Center for Disease Control and Prevention, Zhengzhou, Henan, People's Republic of China*

JIANWEI ZHOU • *National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada*

About the Editor



FEDERICO IOVINO has studied Biological Sciences, bachelor's and master's, at the University of Pavia in Italy. After his undergraduate studies, he moved to the University Medical Center of Groningen, the Netherlands, to perform his PhD in Medical Microbiology under the supervision of Prof. Jan Maarten van Dijl and Prof. Grietje Molema, studying how *Streptococcus pneumoniae* interacts with endothelial cells causing invasive pneumococcal disease, in particular meningitis. After his PhD graduation in November 2013, he immediately started his postdoc in December 2013 at the Karolinska Institutet in the laboratory of Prof. Birgitta Henriques-Normark where he continued his studies on pneumococcal meningitis. Federico's postdoc studies have been published in leading international journals like the *Journal of Clinical Investigation*, the *Journal of Experimental Medicine*, the *Journal of Infectious Diseases*, and *Trends in Microbiology*.

Federico Iovino is currently Assistant Professor at the Karolinska Institutet. His team conducts research focused on studying how bacterial pathogens after translocation across the blood-brain barrier interact with the different cell types of the brain (astrocytes, pericytes, neurons and microglia).