

## EDITORIAL

# Research on the biology of the pneumococcus and pneumococcal diseases: the number one cause of death in children worldwide.

Investigación sobre la biología del neumococo y las enfermedades neumocócicas: una de las principales causas de muerte en niños en todo el mundo.

Pesquisa sobre a biologia de doenças causadas pelo pneumococo e pneumocócicas: a causa numero um de óbitos de crianças ao redor do mundo.

Recherche sur la biologie des pneumocoques et des maladies à pneumocoques: la première cause de décès chez les enfants dans le monde.

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*Streptococcus pneumoniae* has been for decades the number one bacterial killer of children in the world. Although vaccination with pneumococcal conjugate vaccines [PCV7, PCV10, and PCV13 (children) or PPSV23(adults)] has helped decrease the burden of pneumococcal disease (PD), mortality remains high. The introduction of pneumococcal vaccines has also created a niche for vaccine-escape clones. Moreover, the rise of multidrug resistant clones around the world has also posed a serious threat in recent years. Rapid and accurate identification of the pneumococcus in patients suspected of having PD is a high priority as rapid identification of the etiology will lead to better outcomes and thus may help in reducing mortality associated with PD. Efforts have been made for the last ten years in my laboratory to get insights into the biology of the pneumococcus and pneumococcal genetics in order to develop knowledge and tools to assist in decreasing the burden of disease. In my laboratory, a translation science laboratory, we have undertaken or participated in basic science studies, epidemiological studies around the world, clinical trials of new potential antibiotics, and we have spent several years developing and validating molecular technology to identify the pneumococcus and its 90+ serotypes.

### **Streptococcus pneumoniae: a commensal and a pathogen**

The Gram-positive bacterium *Streptococcus pneumoniae* (the pneumococcus) is a common asymptomatic resident of the human upper airways including the nasopharynx and oropharynx, but it is also a primary etiologic agent in pneumonia, otitis media, meningitis, and sepsis. Pneumococcal pneumonia is the leading cause of childhood death in developing countries, killing around one million children every year, while also causing a significant burden of morbidity, mortality, and healthcare costs in

developed countries such as the United States. Colonization of the nasopharynx, while not an immediate cause of pneumococcal disease, is a necessary step along the path to pneumococcal disease. Pneumococcal colonization has been a primary focus of the investigations in my laboratory at Emory University, which will be detailed in the subsequent sections.

### **Studies of colonization and serotype replacement.**

Despite taking the lives of many children every year, the pneumococcus is carried in the nasopharynx by ~90% of children<sup>1</sup>. For example, our 2009 study in Peru demonstrated a very high carriage prevalence in healthy children. Approximately half of the isolated strains belonged to a serotype targeted by the PCV7 vaccine and therefore the most likely to produce PD<sup>2</sup>. PCV7 was introduced late in 2009 in Peru and in follow-up studies two years later (2011) we demonstrated that carriage prevalence had remained similarly high, although we observed serotype replacement, i.e. a significant decrease of serotypes targeted by the vaccine, which were replaced by vaccine-escape serotypes<sup>3</sup>. Whether this change in the serotypes that children carry after the introduction of PCV7 has changed the burden of disease remains to be elucidated. In any case, carriage of potential human pathogens poses a risk of disease. We recently described, in a study conducted in Tanzania, a strong association between lower respiratory tract infection, including clinical pneumonia, and carriage of *S. pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*<sup>4</sup>. Pneumococcal serotypes were not investigated.

In Mexico, the pneumococcal vaccine PCV7 was introduced by the national immunization program in 2008, and by 2013, it was replaced by a vaccine with extended coverage: PCV13.

Whereas the burden of disease in Mexico, mirroring similar trends around the world, has decreased post introduction of pneumococcal vaccines, to the best of my knowledge, studies of colonization in the country have not yet been conducted. This is an important piece of information given that colonization is a required step towards the development of pneumococcal disease.

#### **Development of technology to identify and serotype pneumococcal strains.**

Five years ago, we were fortunate enough to be invited by the Bill and Melinda Gates Foundation in the USA, and the Murdoch Children Research Institute in Australia, to participate in a global consortium aiming to develop new molecular methods for the identification and serotyping of pneumococcal strains<sup>5</sup>. We developed more than 70 single-plex quantitative PCR reactions that identify and quantify the bacterial load of most pneumococcal serotypes known to date<sup>6</sup>. These reactions represent a significant breakthrough since, prior to our studies, available molecular reactions only targeted ~25% of pneumococcal strains. All of these single-plex reactions were recently multiplexed into a high-throughput platform known as a Taqman array card (TAC) which in a single reaction detects most pneumococcal serotypes with high sensitivity and specificity<sup>7</sup>. The TAC technology, along with other technologies developed by the consortium, will be implemented during the years to come to monitor the burden of PD as well as the efficacy of pneumococcal vaccines.

#### **Nasopharyngeal colonization.**

Compelling evidence from my laboratory and others has demonstrated that the pneumococcus colonizes the nasopharynx forming bacterial aggregates, which, for simplicity in this editorial, I will call biofilms. Nasopharyngeal biofilms allow the pneumococcus to persist in the human population. Controlling pneumococcal colonization may be a way to reduce the burden of disease, and so biofilm research has been a focus of basic science studies in my laboratory. We developed a life-like model that simulates the human upper and lower airways to investigate production of colonizing biofilms<sup>8</sup>. The last few years, we have learned that pneumococcal biofilms are controlled by quorum sensing, small molecules that activates other mechanisms of virulence<sup>9</sup>, including a potent toxin known as pneumolysin or Ply<sup>10</sup>. Thanks to a grant funded by the National Institutes of Health (NIH) of the USA, we are now focusing on investigating population dynamics of bacterial consortiums in the human nasopharynx, including recombination leading to the appearance of new serotypes and the emergence of new antibiotic resistant strains. These studies are now beginning to provide explanations for observations we have made in epidemiological studies, including the negative association for carriage of the

pneumococcus and *Staphylococcus aureus* in the human nasopharynx. On this regard, we recently demonstrated that pneumococcus kills *Staphylococcus aureus* strains by physical contact<sup>11</sup>. Besides explaining why, they do not get along together in the human upper airways, our data has opened up a new area of investigation as *S. aureus* strains are known to be important nosocomial pathogens. Having new tools to eradicate strains of *S. aureus* is a priority given the high burden of antibiotic resistance clones isolated in clinical studies.

#### **From the nasopharynx to cause disease.**

Despite being such a successful pathogen, and for reasons we do not yet understand, epidemiological studies have demonstrated that the pneumococcus persists in the nasopharynx of colonized children for only ~60 days, whereas the adult is colonized for ~30 days<sup>1,12</sup>. On entering the nasopharynx, and during its residence there, the pneumococcus shares this anatomical and physiological niche with an array of other viral and bacterial inhabitants. From the nasopharynx, and by a mechanism under active investigation, the pneumococcus migrates to the ear epithelium to cause otitis media, or it goes down into the lungs causing pneumonia. Invasive pneumonia is a severe form of pneumococcal pneumonia that becomes bacteremic. Pneumococcal meningitis is another lethal presentation of pneumococcal disease that can be caused by invasion of the meninges (the membranes covering the brain and spinal cord) by bacteria in circulation in septicemic patients, or bacteria translocated directly from the infected ear to the meninges. Understanding how pneumococcus migrates through the lung epithelium to the circulation is a current hot spot in my laboratory. We hypothesize that if we identify the molecular mechanism, i.e., targets on lung cells utilized by pneumococcus to get inside the cells and move into the bloodstream, we should be able to propose new therapeutic approaches aiming to avoid lethal invasive disease.

#### **Remarks**

Pneumococcal disease remains a serious threat to humans, especially for children and the elderly, and the burden of disease is particularly high in developing countries, including Mexico. As we develop vaccines and new antibiotics for prevention and therapeutics aimed to control PD, these bacteria evolve to successfully persist in the human population and, perhaps involuntarily, cause damage leading to high mortality rates. We in the field, from different fronts, are combining efforts to identify the best ways to control pneumococcal infections.

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