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Original Research

Characterization of Bioactive Secondary Metabolites Produced by *Streptococcus pneumoniae*, Mechanism of Colonization, and New Vaccine Strategies

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Abstract:

Small chemicals called metabolites are involved in metabolic activities that are critical for the growth, maintenance, and function of cells. Metabolites typically have concentrations spanning multiple orders of magnitude and a molecular weight ranging from 50 to 1500 Da. Many environmental factors affect metabolites, and the metabolome is very dynamic and time-dependent. Researching the chemical substances that Streptococcus pneumoniae produces that have real biological activity was the goal of this laboratory investigation. Among the many illnesses that the Gram-positive streptococcus pneumoniae (S. pneumoniae, pneumococcus) bacterium can induce are otitis media, nasosinusitis, pneumonia, bacteremia, and meningitis. Pneumococcal conjugate vaccines (PCVs) were created and extensively used globally to control and prevent pneumococcus infections. Infections caused by PCV serotypes were reduced with the introduction of PCVs, however vaccine efficacy was compromised by serotype substitutions. Many medicines, such as penicillin, macrolides, fluoroquinolones, and sulfamethoxazole-trimethoprim, have been able to overcome the resistance that S. pneumoniae has evolved. As a result, antibiotic-resistant infections can impact the management of associated illnesses. Newly discovered pathways have been molecularly characterised, and genetic functional investigations have been updated.

Keywords: Streptococcus pneumoniae, Secondary metabolites,

Introduction:

Many different types of metabolites are released into the environment on a regular basis. Primary metabolites, like nutrients from meals, are broken down by the digestive tract and other organs. Secondary metabolites, including medicines, flavourings, and recreational drugs, are inhaled or excreted by the body. No one technique can capture and analyse the full metabolome simultaneously because metabolites are chemically so diverse in terms of polarity, charge, pKa, solubility, volatility, stability, and reactivity [1]. Therefore, numerous extraction techniques were devised with the purpose of identifying and quantifying particular types of metabolites. Gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis (CE) online connected to a mass spectrometer (MS), and nuclear magnetic

resonance (NMR) spectroscopy are the most used methods for unravelling the metabolome. The detection of all metabolites in a biological sample is an ambitious objective due to the contextual and chemical variety [2]. Identification remains a difficulty because of the metabolome's complexity and chemical diversity, with metabolite concentrations spanning a wide range of magnitudes. Not only are there many distinct kinds of devices, each with its own unique way of working and collecting data, but there are also many different kinds of data processing programmes. Different fragmentation methods, including collision induced dissociation (CID), higher energy collision dissociation (HCD), electron transfer dissociation (ETD), and pulsed-Q dissociation (PQD) [3-6], as well as collision energy, resolution, targeted, and non-targeted LC-MS instrument settings, can result in varying fragment species and intensities. Therefore, using or creating spectral databases that are particular to metabolites for the purpose of compound identification is a challenging undertaking. An instrument-specific MRM approach for a collection of preselected metabolites is one of the best, although time-consuming, solutions [7, 8].

Alonso and colleagues provide a comprehensive overview of analytical methods and necessary advancements in non-targeted metabolomics. A METLIN search for the exemplary parent mass of 136 Da yields 131 isobaric, distinct metabolites, a few of which share nearly identical fragment spectra due to their extremely similar structures. The outcome is a prioritised list derived from similarity scores, and in order to confirm the identification, cutoff values are required [9–11]. How many false-positives are actually included and whether this is enough are both up for debate. While there is a concerted effort to enhance spectral databases, an extensive collection of reference metabolite spectra is still needed for the creation of reliable automatic identification algorithms. One of the most important processes is target identification, hence in silico target identification approaches like CSNAP (Chemical Similarity Network Analysis Pulldown) use chemical similarity database searches. There are a number of approaches to interpreting and deciphering unknown peaks, but long-term validation criteria were lacking [12–15]. In 2005, the Metabolomics Standards Initiative (MSI) came up with a set of criteria and minimal requirements for validating metabolite identification. This was done to facilitate the effective use, sharing, and reuse of data. Metabolomics data and metadata are being developed via the "COordination of Standards in MetabOlomicS" (COSMOS) effort, which is also creating strong data infrastructures.

Streptococcus pneumoniae:

Invasive illnesses like pneumonia, sepsis, and meningitis are caused by the streptococcus pneumoniae bacteria. Disease has a disproportionate impact on the youngest and oldest populations in both industrialised and developing nations. The global spread of pneumococcal resistance to penicillin and other medicines has made pneumococcal infection treatment more challenging. Asymptomatic colonisation occurs before pneumococcal illness, and it is more common in children. When it comes to invasive diseases caused by strains of the vaccine type, the present seven-valent conjugate vaccination works wonders [16, 17]. Nevertheless, there is a significant concern regarding the coverage of vaccines and the potential for disease-causing serotype replacement in the near future. Consequently, it is critical to find novel vaccine candidates that protect against a wider variety of pneumococcal strains. Several vaccines involving surface-associated proteins are presently being studied. A further crucial question is whether the goal should be to eradicate nasopharyngeal colonisation in order to prevent pneumococcal disease or to prevent bacterial invasion while keeping colonisation mostly untouched in order to avoid replacement colonisation and disease [18-21]. In order to demonstrate the significance of pneumococcal colonisation in regard to pneumococcal disease and disease prevention, we will go over the epidemiology and mechanism of colonisation, the intricacy of relations within and between species, and the results of various strategies for preventing pneumococcal colonisation. In both industrialised and developing nations, Streptococcus pneumoniae is a leading cause of invasive diseases and infections of the respiratory system. Patients with immunodeficiencies, young children, and the elderly are at increased risk for pneumococcal

infections, which can lead to meningitis, sepsis, and pneumonia.1 One million children under the age of five succumb to pneumonia and other invasive infections every year. Pneumococcal infections cause 40,000 deaths each year in the United States.2 There is a shockingly high case-fatality rate of community-acquired pneumococcal meningitis (20% in more developed nations and 50% in less developed countries). Permanent consequences, including as hearing loss, neurological abnormalities, and cognitive impairment, affect 30–60% of survivors as they mature.3 Pneumococcal infection protection is achieved through opsonin-dependent phagocytosis. The classical complement system is activated by antibody-initiated complement-dependent opsonisation [22-25], which is believed to be the primary immunological mechanism that protects the host from pneumococcal infections.4 Neutrophils or phagocytic cells from the liver, spleen, or lung interact with complement and type-specific antibodies (IgA, IgM, IgG) to initiate the clearance process. Cirrhosis of the liver and functional or anatomical asplenia both put people at high risk for severe pneumococcal infection. Another risk factor for pneumococcal infection is a congenital deficiency in either immunoglobulin or complement. S pneumoniae is a member of the oesophageal commensal flora. In the nasopharyngeal niche, they coexist with Staphylococcus aureus, Moraxella cattarrhalis, Haemophilus influenzae, Neisseria meningitidis, and a number of hemolytic streptococci. Pneumococcal colonisation often causes no symptoms at all, but it has the potential to develop into respiratory or even systemic illness (figure 1). Pneumococcal illness cannot develop unless the homologous strain has colonised the nasopharynx before [26-28]. It is also thought that pneumococcal carriage is a major vector for the horizontal transmission of this disease in the general population. In close quarters, including in jails, daycares, and hospitals, pneumococcal strains can spread more easily horizontally [30]. Pneumococcal colonisation and crowding index are both highest in children, suggesting that this age group is primarily responsible for the horizontal transmission of pneumococcal strains in the population. Pneumococcal illness prevention efforts should include measures to reduce the likelihood of nasopharyngeal colonisation, particularly in younger patients. Since nasopharyngeal colonisation is crucial to pneumococcal illness and transmission, this review focuses on the various aspects of nasopharyngeal colonisation in children. In order to shed light on the transmission path of pneumococcal illness, we will go over what is currently known about the process of colonisation, the causes and prevalence of pneumococcal carriage, and the present state of vaccination-based colonisation prevention.

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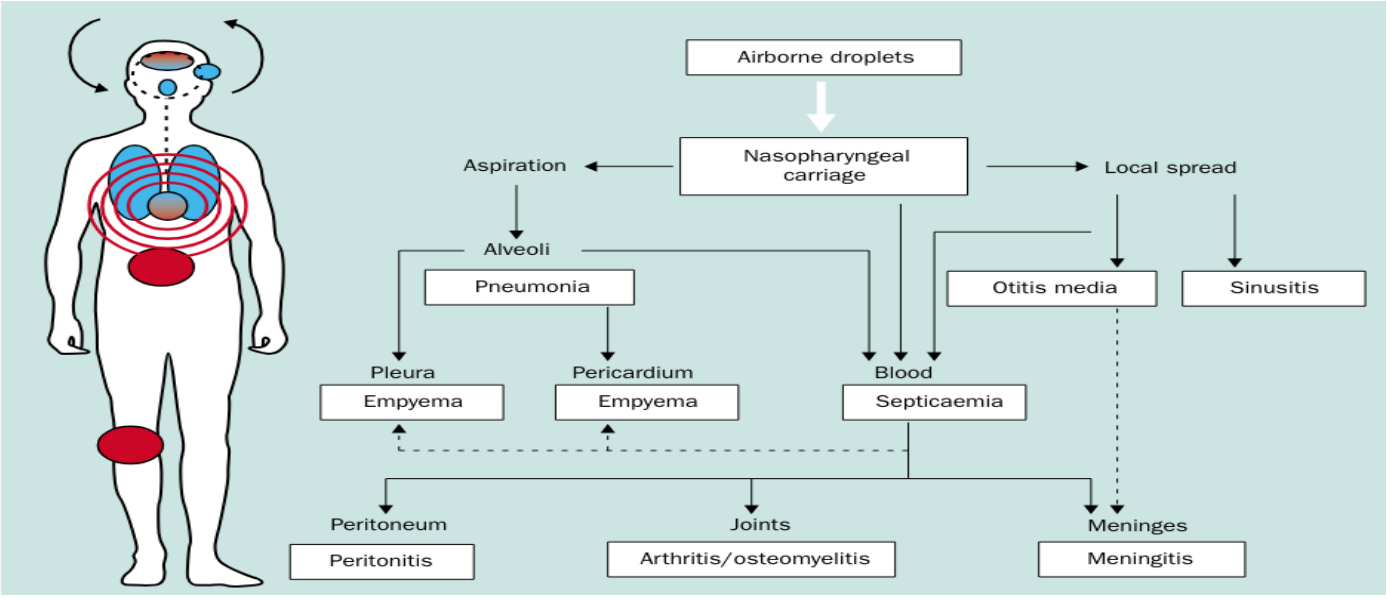

Figure 1. A mechanism by which S. pneumoniae infection becomes pathogenic. Derived from the second source. Blue represents organs infected by the airborne route and red represents organs infected through the hemorrhogenic route.

The dynamics of colonisation of the nasal passages:

Numerous bacterial species find a suitable habitat in the upper respiratory tract. The first few months of a child's life are crucial for the establishment of the nasopharyngeal flora.7,18 Many different kinds of microbes can colonise the nasopharyngeal niche, such as S. pneumoniae, H. influenzae, and M. catarrhalis. These infections are likely to colonise every person at some point throughout their lives. While asymptomatic carrying is the norm, illness might develop after colonisation in rare instances.19,20 After infections colonise an area, they often spread to nearby people by horizontal dissemination, which in turn causes the disease to spread across the community. Acquired and carried bacterium rates are reported differently for different age groups, geographic areas, genetic backgrounds, and socioeconomic situations.11, 23-26. When it comes to controlling the movement of infections in the upper respiratory tract, the local immune response plays a crucial regulatory role [31–33]. A rapid local immune response to the pathogen will eradicate colonisation and prevent recolonization, while a weak mucosal immune response can cause persistent and recurring colonisation and thus infection.28, 29 As a rule, mucosal immunity begins to develop at 6 months of age, much before systemic immunity does.After S. pneumoniae colonisation, children's saliva contains 28 IgG and secretory IgA antibodies that target capsular polysaccharides and surface-associated proteins. There is a constant ebb and flow of colonising species and serotypes during nasopharyngeal colonisation. Additionally, it is believed that interspecies competition influences the diversity of the nasopharyngeal flora [34, 35]. First, it's crucial to maintain a balance between native plants and invasive species. Colonisation by S. pneumoniae, H. influenzae, S. aureus, and M. catarrhalis is inhibited by the indigenous flora, which includes -haemolytic streptococci. Ghaffar and colleagues28 demonstrated the significance of this inhibitory function by discovering an antibiotic-alterable competitive balance among -haemolytic streptococci, S pneumoniae, and H influenzae. While S pneumoniae, H influenzae, and M catarrhalis are the most common bacteria in upper respiratory tract infections, there is evidence that viridans streptococci have an inverse relationship with these other bacteria [36, 37]. There is a competitive interaction between the many pathogenic species. Pericone et al.35 demonstrated a favourable correlation between Neisseria meningitidis and Streptococcus pneumoniae in in vitro investigations. Meningococcal catalase likely mediated the enhanced growth of S pneumoniae observed in meningococcal cultures. Pneumococci or pneumococcal culture supernatant, on the other hand, inhibited meningococcal growth. The presence of pneumococcal peroxide was the reason given by the researchers for the latter impact. Even when co-cultured with H influenzae and M catarrhalis, S pneumoniae exhibited this inhibitory effect. In addition, S. pneumoniae can impede S. aureus growth; pneumococcal hydrogen peroxide has also been linked to this impact [38-40]. Competition between Staphylococcus aureus and Staphylococcus pneumoniae significantly contributes to the age-related dynamics of nasopharyngeal colonisation in children, as demonstrated in a cross-sectional carriage study involving 3200 children.38 Regev-Yochay and colleagues have validated our results.39 We also discovered that while the immune system matures and pneumococcal colonisation declines with age, the carriage rate of S. aureus increases, going from 10% in the first few years of life to 50% at the age of 10. Environmental variables, including smoking and overcrowding, also impact the nasopharyngeal niche's composition [41, 42]. Evidence of rivalry amongst pneumococcal serotypes is scant. As an example, Lipsitch and colleagues investigated the possibility of interstrain competition among pneumococcal strains using a mouse model of intranasal carriage of pneumococci.

How colonialism occurs:

A polysaccharide capsule covers the outer surface of pneumococcal bacteria. So far, about a hundred distinct capsular serotypes have been identified, demonstrating the great heterogeneity of capsular polysaccharides.5 Pneumococcal protection from

phagocytosis is provided by the polysaccharide capsule, which is the most significant virulence component of pneumococci. Lower expression levels allow more antibodies and complement to bind to the pneumococcal surface, leading to an enhanced immune response and subsequent clearance [43–45]. Immunogenicity is high for capsular polysaccharides. By stimulating opsonophagocytosis, antibodies against them provide protection against infections caused by the homologous serotype. Although the capsule's antigenicity is type-specific, cross-reactions can still happen due to shared polysaccharides. The cell wall, a polysaccharide and teichoic acid layer that lies beneath the capsule, anchors surface proteins that are connected with the cell wall. Pneumococcal infection triggers a severe inflammatory response, which the cell wall causes by activating the complement cascade, cytokine release, and the inflow of inflammatory cells.43 The surrounding polysaccharide capsule is thought to shield the cell wall from the host response. S pneumoniae adheres to the respiratory tract's epithelial lining in order to colonise [46-48]. When pneumococcal bacteria adhere to N-acetyl-glycosamine, a carbohydrate found on cell surfaces, they infect non-inflamed, resting epithelium and cause asymptomatic colonisation. Pneumococcal surface adhesin A (PsaA; figure 2) and other cell wall-associated surface proteins mediate stickiness to these sugars. The hydrophobic and electrostatic surface properties of pneumococci are aided by their surface proteins, which may also aid adhesion to host cells through non-specific physicochemical interactions [49]. Symptomatic illness is typically not observed after colonisation. Local production of inflammatory factors including interleukin 1 and tumour necrosis factor, as observed in viral infections, is necessary for the conversion of asymptomatic colonisation to invasive illness.[50] The receptor types and numbers on target endothelium and epithelial cells are altered by this inflammatory cascade. The affinity of pneumococcal cell-wall choline for the platelet activating factor receptor is higher than those of the other upregulated receptors. Bacterial invasion occurs when this receptor is bound, which causes pneumococci to internalise and enhances their transcellular motility via the respiratory epithelium and vascular endothelium [51]. Moreover, cytokine-activated human cells display an enhanced affinity for immobilised sialic acid and lacto-N-neotetraose, as demonstrated by one of the cell-surface proteins, choline-binding protein A (CbpA).48 CbpA enhances migration across the mucosal barrier by directly interacting with the polymeric Ig receptor.49 It is still not known how pneumococcus evades endocytosis-mediated death. The number 52. Weiser and colleagues have recently clarified the role of IgA1 protease. They found that when human IgA was present, pneumococci adhered more strongly to the cells lining the lungs. Pneumococcal cell-wall choline is believed to be physically closer to the platelet-activating factor receptor and undergo a change in surface charge as a consequence of opsonising IgA cleavage by IgA1 protease, which is believed to be the cause of this action. Furthermore, CbpA disrupts the host immune response by binding to the secretory IgA component and interacting with the complement system. Neuroradidase is a further pneumococcal enzyme that helps in colonisation by reducing mucus viscosity by cleaving N-acetylneuraminic acid from mucin. It is believed that the host epithelial cells' N-acetyl-glycosamine receptors are exposed when neuraminidase cleaves glycolipids, glycoproteins, and oligosaccharides [52]. The enhanced adherence of pneumococci observed during viral infections may be due, in part, to the neuraminidase activity of viruses like influenza and parainfluenza. Distinct strains may have different colonisation and invasion capacities because to changes in the makeup, expression, or exposure of surface-associated proteins. Research highlighting the complexity of this process has found reversible phenotypic variation within pneumococcal strains and its involvement in host interaction. Variants in the transparent phase adhere better than those in the opaque phase. In this phenotypic variant, carbohydrate-containing cell-wall components and certain proteins on the cell surface are overexpressed, while capsule polysaccharides are underexpressed.56 and 58 There has been a surge of interest in surface-

associated proteins as possible vaccine candidates due to our growing understanding of the mechanics of colonisation [53]. While pneumolysin and pneumococcal surface protein A (PspA) are surface-associated proteins that protect against systemic illnesses, PsaA and CbpA show promise as potential anti-colonization agents. Combining proteins with different functions in bacterial virulence could, in theory, provide superior protection against S pneumoniae colonisation and infection.

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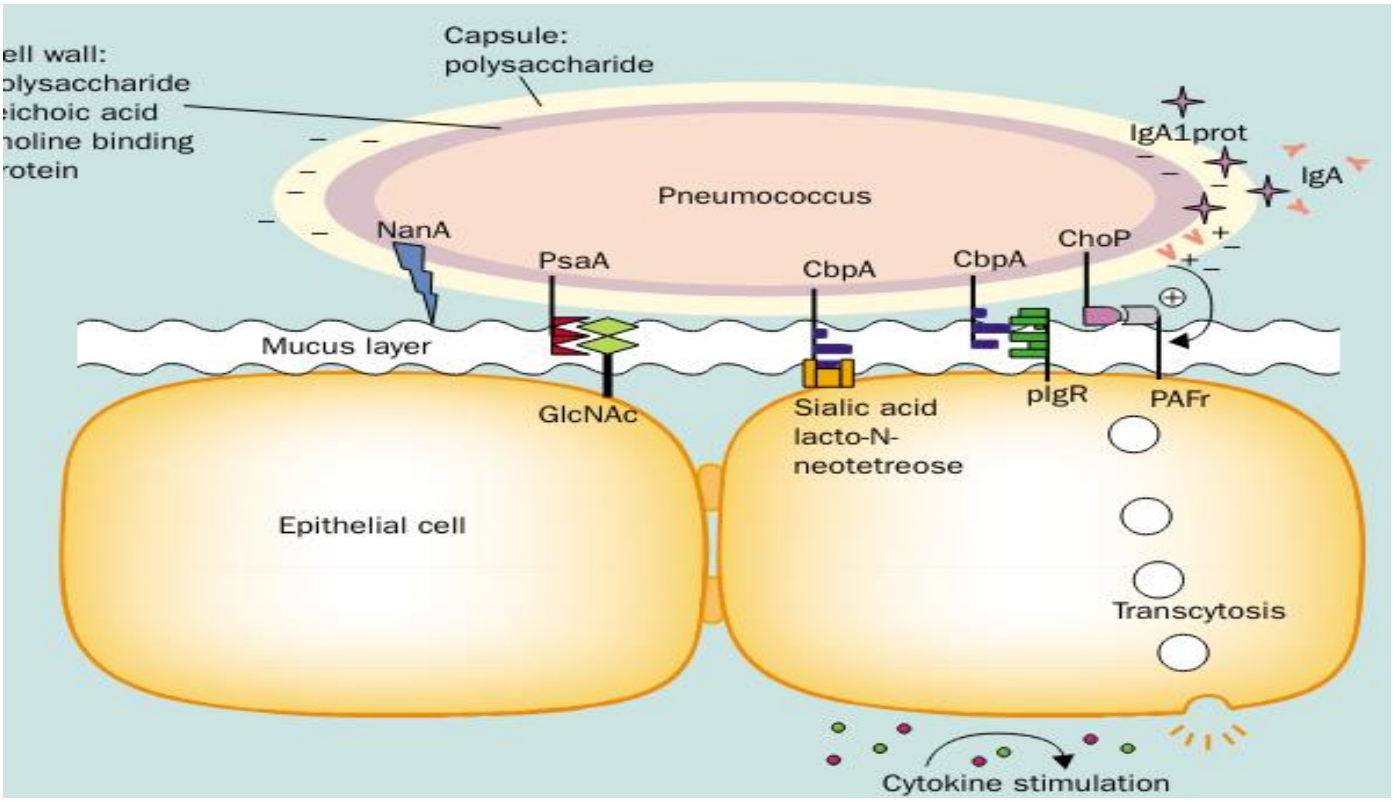


Figure 2. Pathway of Streptococcus pneumoniae in relation to epithelial cells. In order to facilitate interactions between epithelial cells and pneumococcal surface-associated proteins like PsaA, neuraminidase (NanA) reduces mucus viscosity and reveals N-acetyl-glycosamine (GlcNAc) receptors. The platelet-activating factor receptors (PAFr) are upregulated by host epithelial cells in reaction to cytokine stimulation. Because of the phosphocholine in its cell wall, the pneumococcus has a higher affinity for PAFr. Additionally, CbpA, another choline-binding protein, enhances transcytosis by binding directly to the polymeric Ig receptor (pIgR) and exhibiting heightened affinity for immobilised sialic acid and lacto-N-neotreatose. The opsonising IgA is cleaved by the pneumococcal IgA1 protease, which changes the surface charge to zero and brings ChoP closer to the PAFr.

Childhood pneumococcal colonization:

Age is the primary factor determining whether or not S pneumoniae colonises the nasal passages of youngsters. We studied a big group of healthy children and teenagers (ranging in age from 1 to 19) to determine the age-dependent carriage rate.38 Pneumococcal colonisation peaked at 55% at 3 years of age. After the age of 10, the prevalence continued to fall steadily until it reached a stable 8%. While few research on colonisation have followed their subjects into adulthood, those that have found a similar downward trend. In contrast, after birth, the nasopharyngeal niche begins to fill up. Hence, pneumococcal carriage is more common in children younger than two years old [55]. The prevalence of nasopharyngeal carriage in children between the ages of 2 and 24 months

ranged from 13% for those younger than 6 months to 43% for those older than 19 months, according to a study conducted in Finland. In cases of respiratory infections, the proportion rose to 22–45%, lending credence to the idea that adherence is higher during (viral) illnesses. It appears that risk variables also dictate the frequency of pneumococcal carriage in the healthy population. Ethnicity, population density, environmental conditions, and socioeconomic status are independent predictors of nasopharyngeal colonisation. Family size (especially the number of elder siblings), income, smoking (both passive and active), and antibiotic use in the past several years are all environmental and socioeconomic risk factors [56-58]. Colonisation and the dissemination of pneumococcal strains are both accelerated by overcrowding. Nursery visits are linked to substantially higher colonisation rates in young children. One study in the Netherlands found that compared to children cared for at home, those who went to daycare centres had a 1•6 relative risk of pneumococcal colonisation of the nasopharynx. Furthermore, consistent with earlier findings, that investigation also found elevated genetic grouping among pneumococcal isolates.From 86 to 88 This data lends credence to the idea that certain pneumococcal strains may be more easily disseminated horizontally among nursery workers [59]. These results are in line with what Raymond and colleagues79 found; they found that up to 82% of newborns living in orphanages colonised. The study's findings of high levels of genetic similarity among pneumococcal isolates point to their rapid horizontal transmission. People of African American, Apache, Navajo, and Alaska Native descent are more likely to contract pneumococcal colonisation and invasive diseases.89 Among children between the ages of 24 and 35 months, the risk of invasive pneumococcal illnesses is 64.7 cases per 100,000, compared to 116.4 cases per 100,000 among Black Americans and 73-227 cases per 100,000 among native Americans in the United States.89 The US Advisory Committee on Immunisation Practices (ACIP) has recommended pneumococcal vaccination for all age groups within the Native American population due to the elevated risk of invasive illness.1 The risk of pneumococcal infection is so high for children attending day-care facilities that it is advised that they have a seven-valent pneumococcal conjugate vaccine (Prevnar, Wyeth, USA) that covers the most common serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. The use of antibiotics in recent years has also contributed to an increase in pneumococcal colonisation, particularly among bacteria that show resistance to these drugs [60]. It is widely believed that the transmission of antibiotic-resistant pneumococcal strains in the population is caused by the selection of these bacteria at the nasopharynx. Consequently, clones that are resistant to many drugs have already begun to proliferate globally. Pneumococcal disease risk groups do not necessarily exhibit higher colonisation rates than the overall population. Colonisation rates are comparable in healthy children and children with sickle cell disease and HIV. This resemblance arises from the underlying immunological disease, which is associated with a compromised response to or clearance mechanism for pneumococci following invasion, rather than a defect or increased challenge of the main defensive mechanism against pneumococcal invasion. The CD4-positive T cell count, which is critical for an adequate antipolysaccharide response, is reduced in HIV-positive children. Splenic function, which is involved in direct phagocytosis and the start of the antipolysaccharide response, is impaired in children with sickle-cell disease. Nevertheless, these individuals still possess a functional primary mucosal barrier, which includes the mucosal immune response. Colonisation rates can vary globally, although they are typically higher in some contexts, such as in cases of respiratory tract infections or otitis media, and among certain populations, such nursery workers. Furthermore, it is more evident in healthy youngsters than in risk groups, but generally speaking, colonisation rates are higher when nasopharyngeal samples are acquired via the oropharynx rather than the transnasal method. We recommend the transnasal technique to the nasopharynx for future studies.

Novel approaches to vaccine design:

Using proteins linked with the pneumococcal surface is at the centre of new vaccine methods. Several benefits can be gained using this method. First, developing nations should be able to afford to produce protein vaccines because of how cheap they are predicted to be. Additionally, it is anticipated that a vaccination based on proteins will provide protection to individuals of all ages, even those less than two years old. Lastly, widespread and serotype-independent protection can be anticipated when vaccine components consist of highly conserved proteins or protein epitopes. The function of the vaccine's proteins, however, will determine the level and kind of protection. By talking about the best possible protein vaccine candidates, we can show this impact in action. In order to decrease the efficacy of the complement-receptor-mediated pathways of clearance, PspA, a member of the family of structurally related cholinebinding surface proteins, can obstruct recruitment of the alternative pathway, which in turn interferes with complement fixation [61–63]. This mechanism implies that PspA plays a major role in the upkeep of invasive pneumococcal illness, which is especially crucial once bacterial invasion has taken place. Animal studies including active PspA vaccination have shown some protection against invasive infections, mucosal illness, and nasopharyngeal carriage, but only to a limited degree. Humans in the first phase of a vaccination experiment with a single recombinant PspA variant protected mice when exposed intraperitoneally to pneumococci because the trial induced broadly cross-reactive antibodies to heterologous PspA molecules. The metal-binding lipoprotein PsaA is another possible option; it is believed to be involved in the transfer of manganese into pneumococci as part of an ABC transporter complex. In asymptomatic colonisation, this protein plays a key role. While the initial PsaA vaccination trials demonstrated strong protection against colonisation, the results for invasive infections were only moderate. Oral immunisation of mice with PsaA encapsulated in microalginate microspheres provided substantial protection against oral challenge-induced colonisation, pneumonia, and septicaemia, as demonstrated by Seo and colleagues 135. According to these results, PsaA immunisation protects against colonisation and invasive diseases at the same time. Nevertheless, conflicting findings have been observed in clinical investigations regarding the association between PsaA antibodies and the likelihood of pneumococcal acute otitis media. For children older than nine months, Rapola and colleagues found that higher titres of anti-PsaA were associated with a decreased risk of pneumococcal acute otitis media; however, for younger children, the risk was found to be higher with higher antiPsaA concentration. These results raise the possibility of an age-related difference in the origin of the antibody response as well as in the extent to which antibodies defend against PsaA. Having more pneumococcal encounters in the past, whether through colonisation or infection, may explain why some people have a higher anti-PsaA titre than others. Instead of a reduced infection risk, it may account for the correlation with a predisposition to pneumococcal acute otitis media. Pneumolysin is a protein with a cholinebinding domain and several activities, such as complement fixation and phagocyte function suppression, that may disrupt host immunity and inflammatory responses. Because it stops the bronchus' ciliary function, it plays a significant role in the development of lung infections.138 total Pneumolysin knock-out mutagenesis has revealed a function in infection, colonisation, and virulence.positions 139–141 Despite its limited effectiveness against invasive pneumococci, pneumolysin has been characterised by multiple study groups as having protective effects on mice [64]. When PspA and pneumolysin are administered together, they provide animals with enhanced defence against invasive diseases. Colonisation and otitis media are both prevented in animals when PsaA and PspA are combined. Therefore, different combinations of vaccine components can be utilised for different targets. We still need to find out which combination of proteins is best for vaccinations. There have also been investigations into alternate immunisation methods. Research has shown that nasal or oral

vaccination is just as effective as systemic injection. Not only that, but Lynch and colleagues discovered that, unlike intramuscular administration, intranasal administration of a conjugate vaccination including interleukin 12 protected against both invasive illness and nasal carriage. The latter impact is achieved by eliciting strong mucosal IgA responses. Because they are less intrusive, mucosal methods of administration are ideal. Additionally, children already receive several vaccines intramuscularly as part of community vaccination initiatives. Because their mucosal immune responses are still functioning properly, children with HIV/AIDS are also likely to remain protected, unlike with pneumococcal conjugate vaccines and polysaccharide vaccinations, even as the disease progresses.

Extracellular Molecules:

All of an organism's biological reactions put together make up its metabolism. Typically, metabolites are tiny molecules that serve as metabolic intermediates or end products. A. Kossel first used the term "secondary" in 1891 to describe metabolites that are not essential to an organism's survival, in contrast to the primary metabolites that are present in all cells that may divide. Although they are byproducts of primary metabolism, secondary metabolites are not structural components of the living thing. Contrary to primary metabolite [66], its absence does not immediately end an organism's life, but it significantly reduces the organism's chances of survival. Phylogenetically disadvantaged species exhibit its presence and synthesis [67].

Since many of the intermediates in primary metabolism overlap with the intermediates of secondary metabolites, the distinction between primary and secondary metabolites is unclear. Despite their status as a primary metabolite byproduct, amino acids are also unquestionably a secondary metabolite. While it's true that sterols are essential components of numerous cellular structures, this is not necessarily the case. Primary and secondary metabolism follow a same metabolic pathway, as shown by the mosaic character of intermediates [86-69].

To prevent the primary metabolic process from becoming inactive due to an excess of carbon and nitrogen, secondary metabolites provide a buffer zone. Metabolic breakdown of secondary metabolites allows the stored carbon and nitrogen to return to primary metabolites upon demand. The processes of primary and secondary metabolism are constantly changing and intricately balanced, depending on factors such as the cell's or body's internal and external environments, as well as its own growth, tissue differentiation, and development.

Production of Bacterial Secondary Metabolites

Metabolism is a continuous and collective biochemical activity that happens in all living things, whether they have one cell or many. Catabolism and anabolism are the two main categories into which biochemical processes fall. Metabolites are the by-products of these pathways that are utilised to create intermediates and substrates for other metabolic pathways.

Metabolites have multiple biological features that are important in agriculture, nutrition, and pharmaceuticals. Primary metabolites are defined as molecules that are involved in specific metabolic processes, while secondary metabolites are defined as those that are not. Amino acids, pyruvate, citric acid [70], and lactic acid are examples of primary metabolites that provide energy for the biochemical and physiological processes carried out by live cells. Secondary metabolites, on the other hand, aren't required for cell proliferation but instead help the organism stay alive in tough situations.

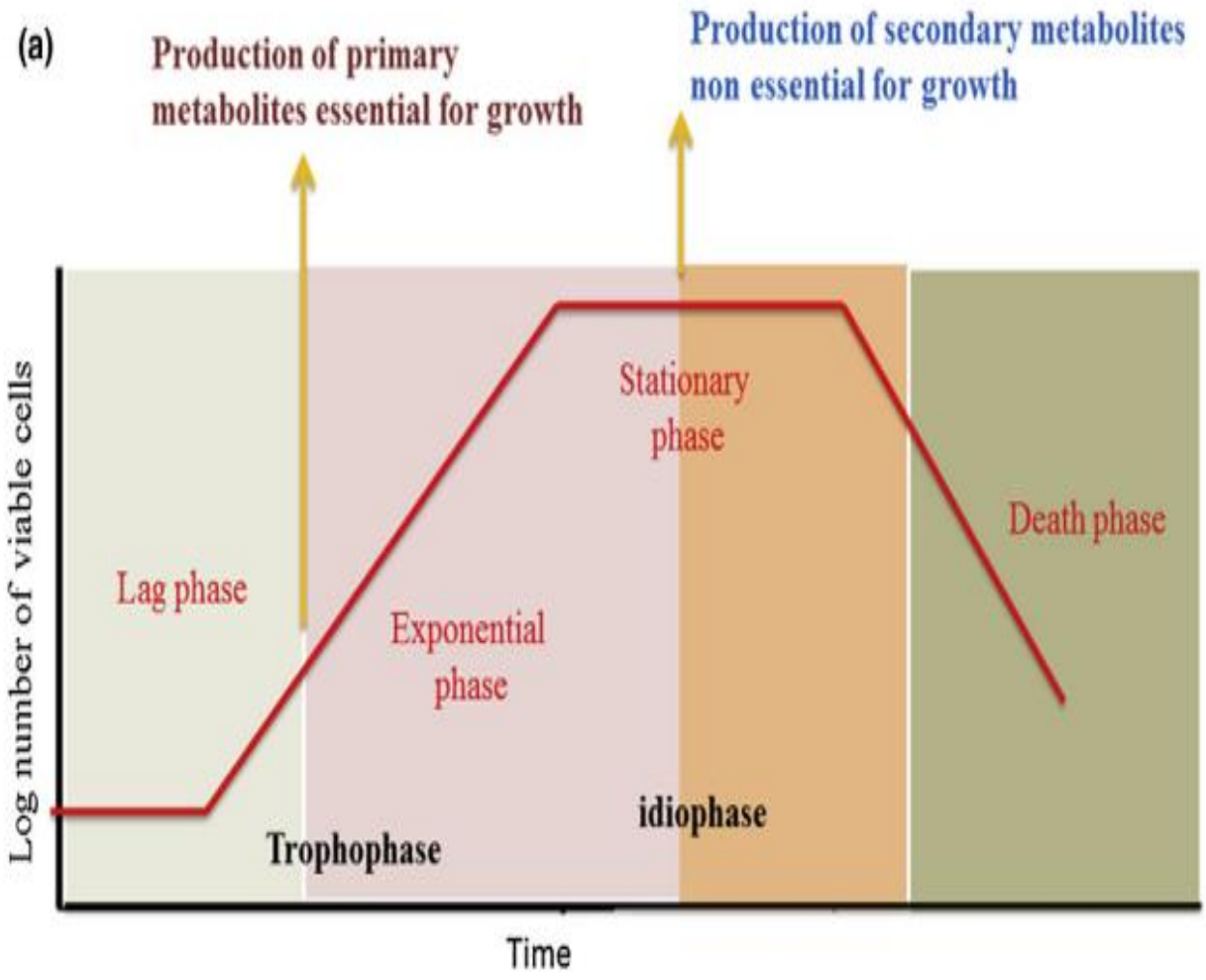
Table 1 shows that the primary focus of this chapter is on bacterial secondary metabolite synthesis. During their late and stationary phases of growth, the bacteria that produce secondary metabolites create these complex and bioactive compounds (Figure 1). Nutrient depletion, environmental stress, and restricted growth circumstances set off the creation of secondary metabolites. Bacteria, fungus, plants, and marine creatures are common sources of the secondary metabolites. In addition to antibacterial, toxic, metal-transporting, sex hormone, pigment, anticancer, pesticide, immunomodulating, immunosuppressant, receptor

agonist, and antagonist metabolites, these microbes can produce a wide variety of additional metabolites [71–74].

An enzyme or a set of enzymes catalyses a reaction in a secondary metabolic pathway. Secondary metabolites are synthesised from intermediate or end-products of primary metabolic pathways through their systematic metabolic routes.

Table 1. Analysed biochemically and physiologically primary and secondary metabolites.

Primary metabolites	Secondary metabolites
Small molecules	Small molecules
Produces few intermediates or end-products	Produces array of molecules
End-products are building blocks for macromolecules.	Synthesize new compounds
Essential for growth and cell viability	Not vital for the cell growth
Known physiological function	Analysis of physiological function is difficult
Composed of simple chemical structure	Products of complex unusual chemical structure
End-products are used for Coenzyme synthesis	End-products are used an antibacterial agent
Production occurs at log phase	Production occurs at late and dormant phase
Primary metabolites are used in food and feed industry	Secondary metabolites are used in food, cosmetic, agricultural and farming industry
Provides the energy for cellular activities	Protects the organisms under various harsh environment



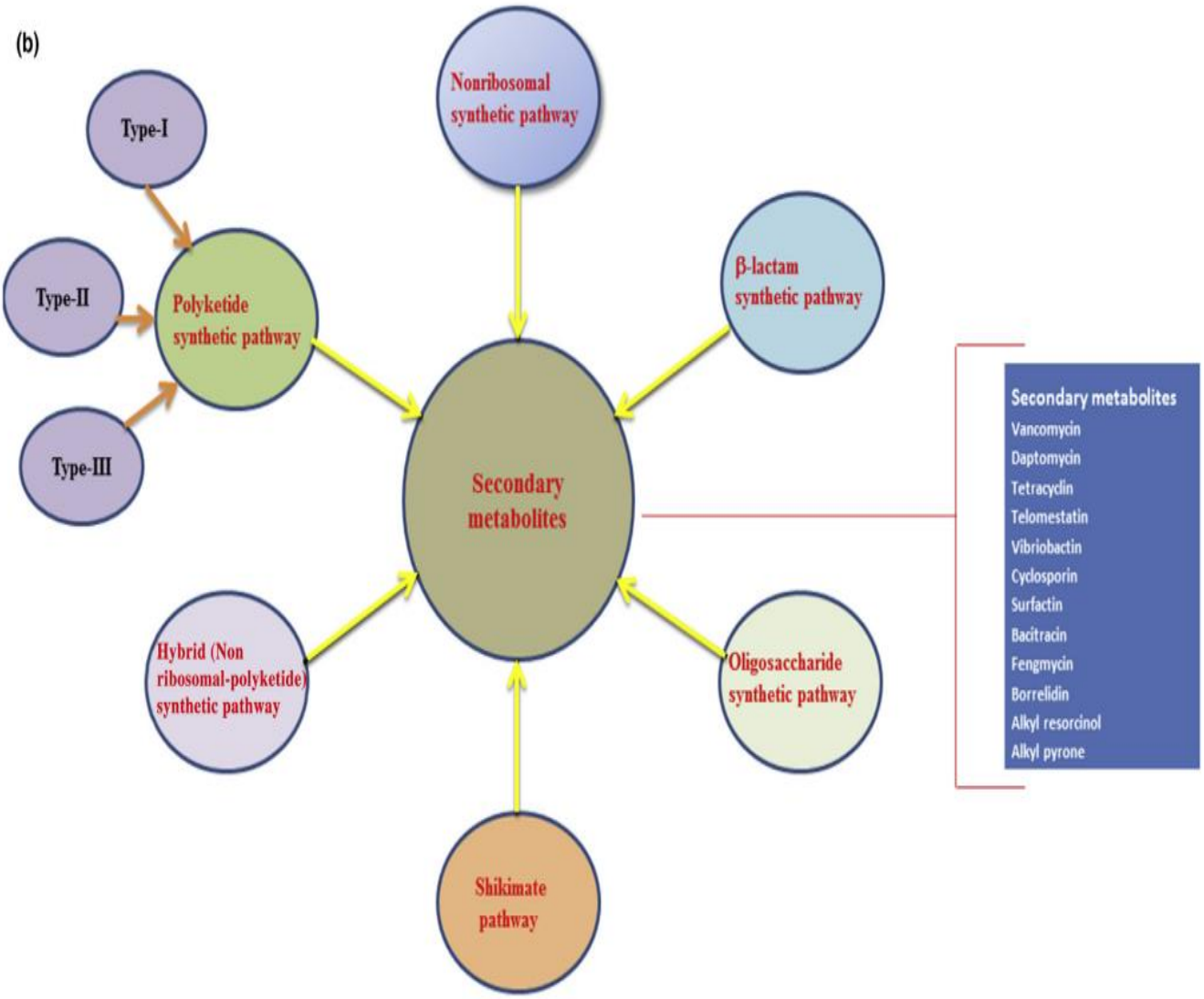


Figure 3. (a) Different stages of bacterial development and metabolite synthesis. During the late lag phase and the middle of the exponential phase is when most of the primary metabolites are produced. During the persistent phase, which follows the stationary phase, secondary metabolites are produced. (b) Various pathways responsible for the assembly of secondary metabolites.

Metabolites produced by microbes and their characteristics:

• The idea and method of synthesising natural fermentation products can be effectively used to increase its impact on the medical, agricultural, food, and environmental sectors.

• The metabolite can be used as a building block to create a desired product, which can then be further modified through chemical or biological processes.
• The development of new compounds can be sped up by creating analogues or templates that use secondary metabolites as lead molecules.

Analysing Biological Data:

Translating changes in metabolite concentration in bodily fluids to organ biochemistry and (molecular) physiological interpretation is still an area where a lot of knowledge is lacking [74, 75]. The limited amount of data that is now available is often specialised to a single species, organ, or bodily fluid, and it is not easy to transfer this data to another. That isn't being considered by software tools like route or enrichment analysis. If it is discovered that metabolite X from a different pathway is lowered, what does it actually mean? A

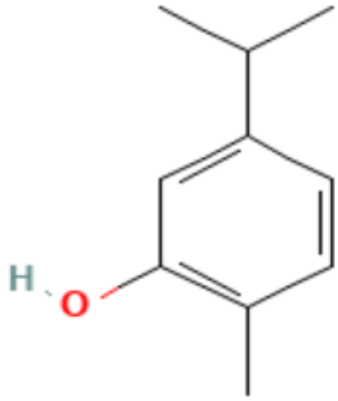
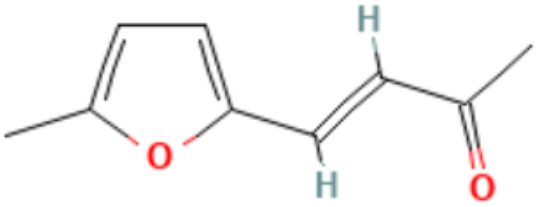
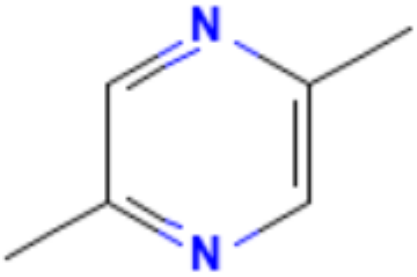
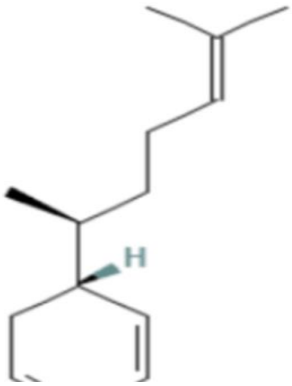
drop in metabolite X concentration may result from either an increase in the activity of enzymes further down the pathway or a decrease in the activity of the enzyme itself. In order to maintain cellular homeostasis, enzyme activity can be modulated by their specific products through steric inhibition, feedback inhibition, or activation. This approach sidesteps major metabolic shifts that could harm the cell. Metabolites are involved in numerous pathways and are either produced by or used as a substrate by a wide variety of enzymes and metabolic processes. As a result, identifying the exact route or enzyme responsible for a changed metabolite can be quite challenging. However, the data on the pathways may already provide the right response, or at least a clue, to a biological query. By associating changes in metabolite abundances with certain pathways, one can obtain mechanistic details about the process being studied. Combining metabolic profiling results with genomic, proteomic, clinical, and environmental information can help find biomarkers that might not have been found using focused investigations alone [75, 76].

One benefit of metabolome profiles is their ability to detect changes on a worldwide scale as well as undiscovered chemicals (shotgun) (shotgun and targeted). Because many metabolites come from several sources, confirmation requires targeted approaches, such as isotope-labeled MFA or enzyme knockdown. Metabolomics researchers are always developing new bioinformatics tools to help with things like analysing profiling data and answering other crucial problems in the area. Comprehensive metabolomic data processing, visualisation, and interpretation, including complex statistical computations, is made possible by MetaboAnalyst (www.metaboanalyst.ca) [77, 78]. At the system level, metabolic profile data can be seen and understood biologically with the help of metabolic pathway enrichment analysis (MPEA). Using a ranked query compound list, the tool determines if metabolites that are part of certain preset pathways are at the beginning or end of the list. When combined with expression and metabolite data, IMPaLA [80] allows for pathway enrichment and over-representation analysis. This demonstrates the significance of metabolomics; yet, many of these programmes receive financing for a limited time before being discontinued or becoming obsolete due to permanent changes in file formats and accompanying software. On top of that, the majority of programmes have their own unique requirements for raw or input data, which are often not compatible with one another. In order to identify unique and specific metabolic traits that are indicative of particular clinical disorders, illnesses, or cancers, biomarker research is fueled by the use of new technology, protocols, and software tools. Incredibly, the moment of clinical breakthrough has not yet arrived. However, there have been reports of metabolomic important features that are predictive of disorders such as schizophrenia, depression, diabetes, cardiovascular disease, diabetes mellitus type 2, and malignancies of the liver, ovaries, and breasts [81]. There is enormous and ever-changing variance in metabolic level across individuals, tissues, and time points. In contrast to the highly variable metabolites, the states of the genome, epigenome, transcriptome, and proteome are far more stable. The goal of research into biomarkers is to identify indicators of cancer at an early stage so that patients can receive the most effective treatment and have the best chance of survival. No novel biomarkers have been approved in recent years. The existing approach to identifying individual disease biomarkers is hindered by the high and dynamic variations of metabolites [82–85]. We should likely look for classes of metabolites or pathways that are differentially regulated for more reliable biomarkers in the future, since every disease alters not just one metabolite but entire metabolic networks. An integrative strategy that considers several subdisciplines of omics is the most effective way to achieve this [65–68]. Revealing, assessing, and tracking molecular patterns—which reflect disease-perturbed networks—will, therefore, require reliable multi-data analyses.

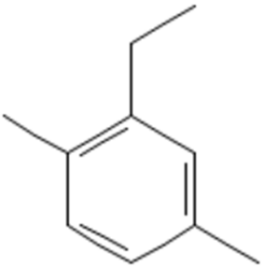
Table 1. Secondary bioactive compounds identified by gas chromatography-mass spectrometry.

Compound	Formula	M.W
Carvacrol	$C_{10}H_{14}O$	150.22 g/mol
1-(5(Methyl-2-furanyl)-1-buten-3-one	$C_9H_{10}O_2$	150.17 g/mol
beta.Sesquiphellandrene	$C_{15}H_{24}$	204.35 g/mol
Pentasiloxane, dodecamethyl	$C_{12}H_{36}O_4Si_5$	384.84 g/mol
Pyrazine, 2,5-dimethyl-	$C_6H_8N_2$	108.14 g/mol
Benzene, 2-ethyl-1,4-dimethyl	$C_{10}H_{14}$	134.22 g/mol
tumerone	$C_{15}H_{22}O$	218.33 g/mol
AR- tumerone	$C_{15}H_{20}O$	216.32 g/mol
ALPHA- tumerone	$C_{15}H_{22}O$	218.33 g/mol
6-Aza-5,7,12,14-tetrathiapentacene	$C_{17}H_9NS_4$	355.5 g/mol
β-HIMACHALENOXIDE	$C_{15}H_{24}$	204.35 g/mol
3-Ethyl-o-xylene	C10H14	134.22 g/mol
Trimethylphenylsilane	$C_9H_{14}Si$	150.29 g/mo
1,3-Hexadiene, 2,5-dimethyl-	C_8H_{14}	110.20 g/mol
o-Mercaptoaniline	C_6H_7NS	125.19 g/mol
Benzonitrile, 4-amino-	$C_7H_6N_2$	118.14 g/mol
Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4 g/mol
3-Decen-5-one	$C_{10}H_{18}O$	154.25 g/mol
Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.4 g/mol
Methyl linoleate	$C_{19}H_{34}O_2$	294.5 g/mol
Gentisic acid	$C_7H_6O_4$	154.12 g/mol
Shogaol	$C_{17}H_{24}O_3$	276.4 g/mol
Isooctyl phthalate	$C_{24}H_{38}O_4$	390.6 g/mol
2,6,10,-Pentamethyl-2,6, 18-eicosapentaene	$C_{25}H_{42}$	342.6 g/mol
Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	533.1 g/mol
N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31 g/mol

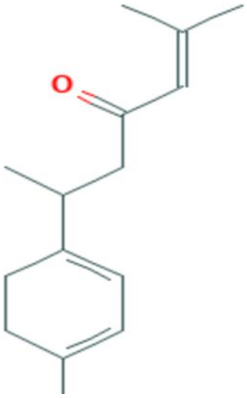
Table 2. Structures of metabolites produced by *Streptococcus pyogenes*

Compound	Structure
Carvacrol	
1-(5(Methyl-2-furanyl)-1-buten-3-one	
Pyrazine, 2,5-dimethyl-	
beta.Sesquiphellandrene	

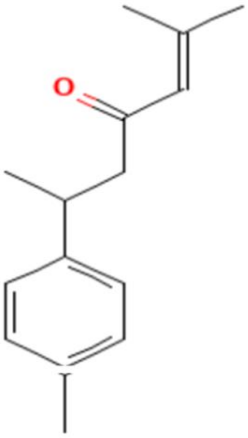
Benzene, 2-ethyl-1,4-dimethyl



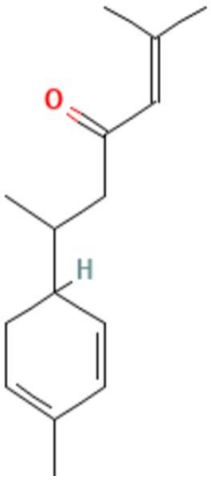
TUMERONE



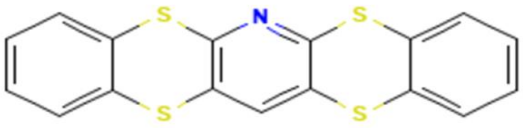
AR-TUMERONE



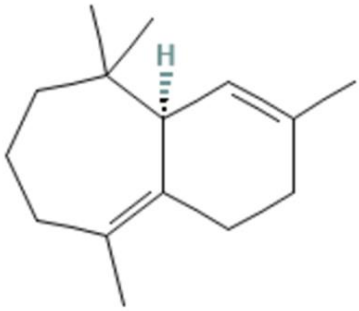
ALPHA.-



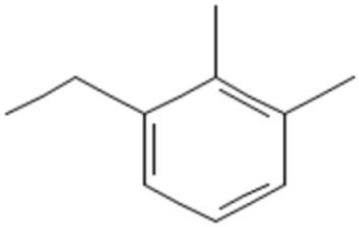
6-Aza-5,7,12,14-tetrathiapentacene



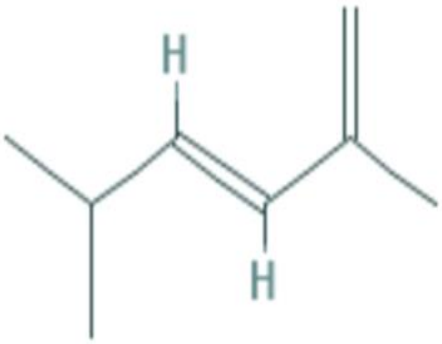
β-HIMACHALENOXIDE



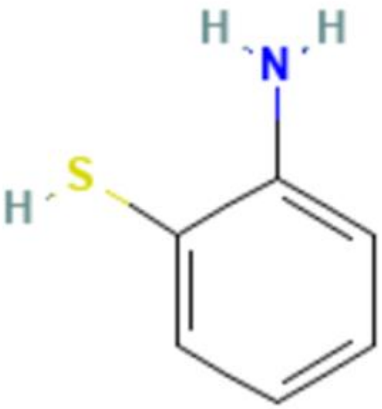
3-Ethyl-o-xylene



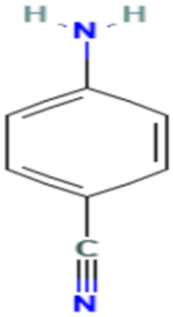
1,3-Hexadiene, 2,5-dimethyl-



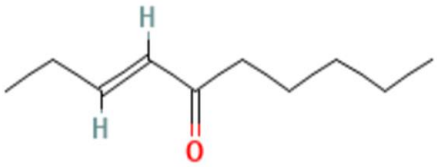
o-Mercaptoaniline



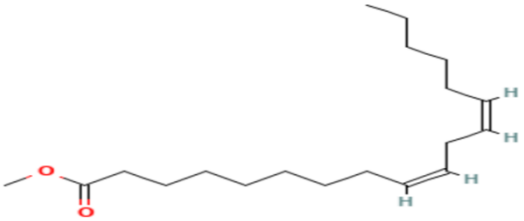
Benzonitrile, 4-amino-



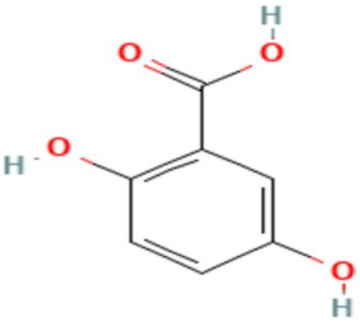
3-Decen-5-one



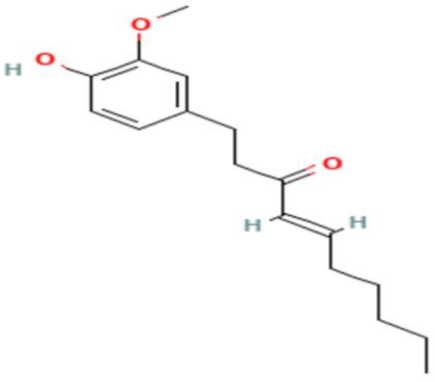
Methyl linoleate



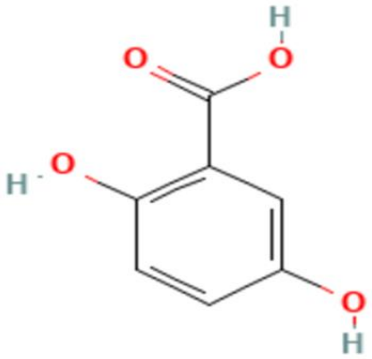
Gentisic acid



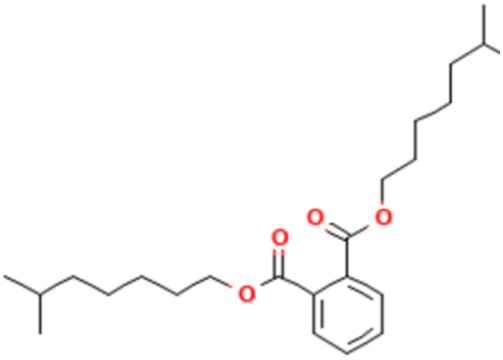
Shogaol



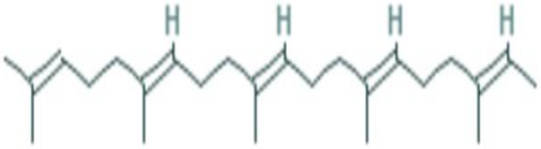
Gentisic acid



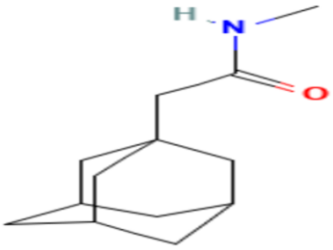
Isooctyl phthalate



2,6,10,14,18-Pentamethyl-eicosapentaene



N-Methyl-1-adamantaneacetamide



Conclusion:

Outbreaks can be caused by Streptococcus pneumoniae, which can cause numerous infectious disorders. Updating previous epidemic evaluations, identifying control methods, and commenting on transmission are the goals of this review. Infections caused by S. pneumoniae can present in a variety of ways. There is enough proof that infection with S. pneumoniae is not necessary for illness to occur. Upholding high vaccination rates and revaccination in accordance with US CDC/ACIP recommendations is likely to be beneficial in preventing the early outbreaks. Interventions aimed at preventing the spread of infection, such as vaccination and droplet precautions, should be prioritised once an outbreak has occurred. The CDC does not currently advise using prophylactic antibiotics for exposed persons due to concerns

about the development of antibiotic resistance. Interventions aimed targeting the co-circulating infections may reduce pneumococcal transmission in cases where two or more pathogens are present in the bloodstream at the same time. Curiously, the bacteria responsible for conjunctival pneumococcal outbreaks do not produce a capsule, meaning that the pneumococcal vaccines that are now available do not protect against them. There is still a lot to learn about S. pneumoniae, even though it was found more than a century ago.

References:

1. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 1997; 46: 1–24.
2. Obaro S, Adegbola R. The pneumococcus: carriage, disease and conjugate vaccines. J Med Microbiol 2002; 51: 98–104.
3. Koedel U, Scheld WM, Pfister HW. Pathogenesis and pathophysiology of pneumococcal meningitis. Lancet Infect Dis 2002; 2: 721–36.
4. Paton JC, Andrew PW, Boulnois GJ, Mitchell TJ. Molecular analysis of the pathogenicity of Streptococcus pneumoniae: the role of pneumococcal proteins. Annu Rev Microbiol 1993; 47: 89–115.
5. Bruyn GA, Zegers BJ, van Furth R. Mechanisms of host defense against infection with Streptococcus pneumoniae. Clin Infect Dis 1992; 14: 251–62.
6. Gray BM, Converse GM 3rd, Dillon HC Jr. Epidemiologic studies of Streptococcus pneumoniae in infants: acquisition, carriage, and infection during the first 24 months of life. J Infect Dis 1980; 142: 923–33.
7. Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. J Infect Dis 1997; 175: 1440–45.
8. Hoge CW, Reichler MR, Dominguez EA, et al. An epidemic of pneumococcal disease in an overcrowded, inadequately ventilated jail. N Engl J Med 1994; 331: 643–48.
9. Kristinsson KG. Epidemiology of penicillin resistant pneumococci in Iceland. Microb Drug Resist 1995; 1: 121–25.
10. Munoz R, Coffey TJ, Daniels M, et al. Intercontinental spread of a multiresistant clone of serotype 23F Streptococcus pneumoniae. J Infect Dis 1991; 164: 302–06.
11. Principi N, Marchisio P, Schito GC, Mannelli S. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Ascanius Project Collaborative Group. Pediatr Infect Dis J 1999; 18: 517–23.
12. de Galan BE, van Tilburg PM, Sluijter M, et al. Hospital-related outbreak of infection with multidrug-resistant Streptococcus pneumoniae in The Netherlands. J Hosp Infect 1999; 42: 185–92.
13. Mandigers CM, Diepersloot RJ, Dessens M, Mol SJ, van Klingeren B. A hospital outbreak of penicillinresistant pneumococci in The Netherlands. Eur Respir J 1994; 7: 1635–39. 14 Millar MR, Brown NM, Tobin GW, Murphy PJ, Windsor AC, Speller DC. Outbreak of infection with penicillin-resistant Streptococcus pneumoniae in a hospital for the elderly. J Hosp Infect 1994; 27: 99–104.
14. Reichler MR, Rakovsky J, Slacikova M, et al. Spread of multidrug-resistant Streptococcus pneumoniae among hospitalized children in Slovakia. J Infect Dis 1996; 173: 374–79. #
15. Shi ZY, Enright MC, Wilkinson P, Griffiths D, Spratt BG. Identification of three major clones of multiply antibiotic-resistant Streptococcus pneumoniae in Taiwanese hospitals by multilocus sequence typing. J Clin Microbiol 1998; 36: 3514–19. 17
16. Leiberman A, Dagan R, Leibovitz E, Yagupsky P, Fliss DM. The bacteriology of the nasopharynx in childhood. Int J Pediatr

Otorhinolaryngol 1999; 49 (suppl 1): S151–53.
17. Faden H, Duffy L, Williams A, Krystofik DA, Wolf J. Epidemiology of nasopharyngeal colonization with nontypeable Haemophilus influenzae in the first two years of life. Acta Otolaryngol Suppl 1996; 523: 128–29.
18. Kyaw MH, Christie P, Jones IG, Campbell H. The changing epidemiology of bacterial meningitis and invasive non-meningitic bacterial disease in scotland during the period 1983–99. Scand J Infect Dis 2002; 34: 289–98.
19. Nouwen JL, van Belkum A, Verbrugh HA. Determinants of Staphylococcus aureus nasal carriage. Neth J Med 2001; 59: 126–33.
20. Givon-Lavi N, Fraser D, Porat N, Dagan R. Spread of Streptococcus pneumoniae and antibiotic-resistant S. pneumoniae from day-care center attendees to their younger siblings. J Infect Dis 2002; 186: 1608–14.
21. Faden H, Stanievich J, Brodsky L, Bernstein J, Ogra PL. Changes in nasopharyngeal flora during otitis media of childhood. Pediatr Infect Dis J 1990; 9: 623–66.
22. Bogaert D, Engelen MN, Timmers-Reker AJ, et al. Pneumococcal carriage in children in The Netherlands: a molecular epidemiological study. J Clin Microbiol 2001; 39: 3316–20.
23. El Ahmer OR, Essery SD, Saadi AT, et al. The effect of cigarette smoke on adherence of respiratory pathogens to buccal epithelial cells. FEMS Immunol Med Microbiol 1999; 23: 27–36.
24. Bakir M, Yagci A, Ulger N, Akbenlioglu C, Ilki A, Soyletir G. Asymtomatic carriage of Neisseria meningitidis and Neisseria lactamica in relation to Streptococcus pneumoniae and Haemophilus influenzae colonization in healthy children: apropos of 1400 children sampled. Eur J Epidemiol 2001; 17: 1015–18.
25. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997; 10: 505–20.
26. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J Antimicrob Chemother 2002; 50 (suppl C): 59–74.
27. Ghaffar F, Friedland IR, McCracken GH, Jr. Dynamics of nasopharyngeal colonization by Streptococcus pneumoniae. Pediatr Infect Dis J 1999; 18: 638–46.
28. Harabuchi Y, Faden H, Yamanaka N, Duffy L, Wolf J, Krystofik D. Nasopharyngeal colonization with nontypeable Haemophilus influenzae and recurrent otitis media. J Infect Dis 1994; 170: 862–66.
29. Simell B, Korkeila M, Pursiainen H, Kilpi TM, Kayhty H. Pneumococcal carriage and otitis media induce salivary antibodies to pneumococcal surface adhesin a, pneumolysin, and pneumococcal surface protein a in children. J Infect Dis 2001; 183: 887–96.
30. Simell B, Kilpi TM, Kayhty H. Pneumococcal carriage and otitis media induce salivary antibodies to pneumococcal capsular polysaccharides in children. J Infect Dis 2002; 186: 1106–14.
31. Uehara Y, Kikuchi K, Nakamura T, et al. Inhibition of methicillin-resistant Staphylococcus aureus colonization of oral cavities in newborns by viridans group streptococci. Clin Infect Dis 2001; 32: 1399–407.
32. Tano K, Grahn-Hakansson E, Holm SE, Hellstrom S. Inhibition of OM pathogens by alpha-hemolytic streptococci from healthy children, children with SOM and children with rAOM. Int J Pediatr Otorhinolaryngol 2000; 56: 185–90.
33. Ghaffar F, Muniz LS, Katz K, et al. Effects of large dosages of amoxicillin/clavulanate or azithromycin on nasopharyngeal

carriage of Streptococcus pneumoniae, Haemophilus influenzae, nonpneumococcal alphahemolytic streptococci, and Staphylococcus aureus in children with acute otitis media. Clin Infect Dis 2002; 34: 1301–09.

34. Pericone CD, Overweg K, Hermans PW, Weiser JN. Inhibitory and bactericidal effects of hydrogen peroxide production by Streptococcus pneumoniae on other inhabitants of the upper respiratory tract. Infect Immun 2000; 68: 3990–97.
35. Mc Leod JW, Gordon J. Production of hydrogen peroxide by bacteria. Biochem J 1922; 16: 499–506.
36. Dahiya RS, Speck ML. Hydrogen peroxide formation by lactobacilli and its effect on Staphylococcus aureus. J Dairy Sci 1968; 51: 1068–72.
37. Bogaert D, Koppen S, Boelens H, et al. Epidemiology and determinants of nasopharyngeal carriage of bacterial pathogens in healthy Dutch children. Program and abstracts of the 21st Annual Meeting of the European Society for Paediatric Infectious Diseases; Giardini Naxos; April 9–11, 2003.
38. Regev-Yochay G, Dagan R, Raz M, et al. Is nasopharyngeal carriage of Streptococcus pneumoniae protective against carriage of Staphylococcus aureus? 43rd ICAAC; Chicago; Sept 14–17, 2003. Abstr G-2048.
39. Lipsitch M, Dykes JK, Johnson SE, et al. Competition among Streptococcus pneumoniae for intranasal colonization in a mouse model. Vaccine 2000; 18: 2895–901. 41 Watson DA, Musher DM. A brief history of the pneumococcus in biomedical research. Semin Respir Infect 1999; 14: 198–208.
40. Magee AD, Yother J. Requirement for capsule in colonization by Streptococcus pneumoniae. Infect Immun 2001; 69: 3755–61.
41. Bruyn GA, van Furth R. Pneumococcal polysaccharide vaccines: indications, efficacy and recommendations. Eur J Clin Microbiol Infect Dis 1991; 10: 897–910.
42. Swiatlo E, Champlin FR, Holman SC, Wilson WW, Watt JM. Contribution of choline-binding proteins to cell surface properties of Streptococcus pneumoniae. Infect Immun 2002; 70: 412–15.
43. Tuomanen EI. The biology of pneumococcal infection. Pediatr Res 1997; 42: 253–58. 46 Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. Streptococcus pneumoniae anchor to activated human cells by the receptor for platelet-activating factor. Nature 1995; 377: 435–38.
44. McCullers JA, Rehg JE. Lethal synergism between influenza virus and Streptococcus pneumoniae: characterization of a mouse model and the role of platelet-activating factor receptor. J Infect Dis 2002; 186: 341–50.
45. Rosenow C, Ryan P, Weiser JN, et al. Contribution of novel choline-binding proteins to adherence, colonization and immunogenicity of Streptococcus pneumoniae. Mol Microbiol 1997; 25: 819–29.
46. Balachandran P, Brooks-Walter A, Virolainen-Julkunen A, Hollingshead SK, Briles DE. Role of pneumococcal surface protein C in nasopharyngeal carriage and pneumonia and its ability to elicit protection against carriage of Streptococcus pneumoniae. Infect Immun 2002; 70: 2526–34.
47. Tuomanen EI. Pathogenesis of pneumococcal inflammation: otitis media. Vaccine 2000; 19 (suppl 1): S38–40. 51 Ring A, Weiser JN, Tuomanen EI. Pneumococcal trafficking across the blood-brain barrier: molecular analysis of a novel bidirectional pathway. J Clin Invest 1998; 102: 347–60.
48. Weiser JN, Bae D, Fasching C, Scamurra RW, Ratner AJ, Janoff EN. Antibody-enhanced pneumococcal adherence

requires IgA1 protease. Proc Natl Acad Sci USA 2003; 100: 4215–20.

49. Hammerschmidt S, Talay SR, Brandtzaeg P, Chhatwal GS. SpsA, a novel pneumococcal surface protein with specific binding to secretory immunoglobulin A and secretory component. Mol Microbiol 1997; 25: 1113–24.
50. Tong HH, Blue LE, James MA, DeMaria TF. Evaluation of the virulence of a Streptococcus pneumoniae neuraminidase-deficient mutant in nasopharyngeal colonization and development of otitis media in the chinchilla model. Infect Immun 2000; 68: 921–24.
51. McCullers JA, Tuomanen EI. Molecular pathogenesis of pneumococcal pneumonia. Front Biosci 2001; 6: D877–89.
52. Weiser JN, Markiewicz Z, Tuomanen EI, Wani JH. Relationship between phase variation in colony morphology, intrastrain variation in cell wall physiology, and nasopharyngeal colonization by Streptococcus pneumoniae. Infect Immun 1996; 64: 2240–45.
53. Weiser JN, Kapoor M. Effect of intrastrain variation in the amount of capsular polysaccharide on genetic transformation of Streptococcus pneumoniae: implications for virulence studies of encapsulated strains. Infect Immun 1999; 67: 3690–92.
54. Kim JO, Weiser JN. Association of intrastrain phase variation in quantity of capsular polysaccharide and teichoic acid with the virulence of Streptococcus pneumoniae. J Infect Dis 1998; 177: 368–77.
55. Briles DE, Hollingshead S, Brooks-Walter A, et al. The potential to use PspA and other pneumococcal proteins to elicit protection against pneumococcal infection. Vaccine 2000; 18: 1707–11.
56. Muhlemann K, Matter HC, Tauber MG, Bodmer T. Nationwide surveillance of nasopharyngeal Streptococcus pneumoniae isolates from children with respiratory infection, Switzerland, 1998–1999. J Infect Dis 2003; 187: 589–96.
57. Daw NC, Wilimas JA, Wang WC, et al. Nasopharyngeal carriage of penicillin-resistant Streptococcus pneumoniae in children with sickle cell disease. Pediatrics 1997; 99: E7.
58. Norris CF, Mahannah SR, Smith-Whitley K, Ohene-Frempong K, McGowan KL. Pneumococcal colonization in children with sickle cell disease. J Pediatr 1996; 129: 821–27.
59. Coles CL, Kanungo R, Rahmathullah L, et al. Pneumococcal nasopharyngeal colonization in young South Indian infants. Pediatr Infect Dis J 2001; 20: 289–95.
60. Syrogiannopoulos GA, Grivea IN, Davies TA, Katopodis GD, Appelbaum PC, Beratis NG. Antimicrobial use and colonization with erythromycin-resistant Streptococcus pneumoniae in Greece during the first 2 years of life. Clin Infect Dis 2000; 31: 887–93.
61. Jebaraj R, Cherian T, Raghupathy P, et al. Nasopharyngeal colonization of infants in southern India with Streptococcus pneumoniae. Epidemiol Infect 1999; 123: 383–88.
62. Syrjanen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK. Nasopharyngeal carriage of Streptococcus pneumoniae in Finnish children younger than 2 years old. J Infect Dis 2001; 184: 451–59.
63. Soewignjo S, Gessner BD, Sutanto A, et al. Streptococcus pneumoniae nasopharyngeal carriage prevalence, serotype distribution, and resistance patterns among children on Lombok Island, Indonesia. Clin Infect Dis 2001; 32: 1039–43.
64. Rusen ID, Fraser-Roberts L, Slaney L, et al. Nasopharyngeal pneumococcal colonization among Kenyan children: antibiotic resistance, strain types and associations with human immunodeficiency virus type 1 infection. Pediatr Infect Dis J 1997; 16: 656–62.

65. Chiou CC, Liu YC, Huang TS, et al. Extremely high prevalence of nasopharyngeal carriage of penicillinresistant Streptococcus pneumoniae among children in Kaohsiung, Taiwan. J Clin Microbiol 1998; 36: 1933–37.
66. Polack FP, Flayhart DC, Zahurak ML, Dick JD, Willoughby RE. Colonization by Streptococcus penumoniae in human immunodeficiency virusinfected children. Pediatr Infect Dis J 2000; 19: 608–12.
67. Parry CM, Diep TS, Wain J, et al. Nasal carriage in Vietnamese children of Streptococcus pneumoniae resistant to multiple antimicrobial agents. Antimicrob Agents Chemother 2000; 44: 484–88.
68. Vives M, Garcia ME, Saenz P, et al. Nasopharyngeal colonization in Costa Rican children during the first year of life. Pediatr Infect Dis J 1997; 16: 852–58.
69. Berkovitch M, Bulkowstein M, Zhovtis D, et al. Colonization rate of bacteria in the throat of healthy infants. Int J Pediatr Otorhinolaryngol 2002; 63: 19–24.
70. Marchisio P, Claut L, Rognoni A, et al. Differences in nasopharyngeal bacterial flora in children with nonsevere recurrent acute otitis media and chronic otitis media with effusion: implications for management. Pediatr Infect Dis J 2003; 22: 262–68.
71. Marchisio P, Esposito S, Schito GC, Marchese A, Cavagna R, Principi N. Nasopharyngeal carriage of Streptococcus pneumoniae in healthy children: implications for the use of heptavalent pneumococcal conjugate vaccine. Emerg Infect Dis 2002; 8: 479–84.
72. Dejsirilert S, Overweg K, Sluijter M, et al. Nasopharyngeal carriage of penicillin-resistant Streptococcus pneumoniae among children with acute respiratory tract infections in Thailand: a molecular epidemiological survey. J Clin Microbiol 1999; 37: 1832–38.
73. Dagan R, Leibovitz E, Greenberg D, Yagupsky P, Fliss DM, Leiberman A. Dynamics of pneumococcal nasopharyngeal colonization during the first days of antibiotic treatment in pediatric patients. Pediatr Infect Dis J 1998; 17: 880–85.
74. Veenhoven R, Bogaert D, Uiterwaal C, et al. Effect of pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media. Lancet 2003; 361: 2189–95.
75. Raymond J, Le Thomas I, Moulin F, Commeau A, Gendrel D, Berche P. Sequential colonization by Streptococcus pneumoniae of healthy children living in an orphanage. J Infect Dis 2000; 181: 1983–88.
76. Leibovitz E, Dragomir C, Sfartz S, et al. Nasopharyngeal carriage of multidrug-resistant Streptococcus pneumoniae in institutionalized HIVinfected and HIV-negative children in northeastern Romania. Int J Infect Dis 1999; 3: 211–15.
77. Lee HJ, Park JY, Jang SH, Kim JH, Kim EC, Choi KW. High incidence of resistance to multiple antimicrobials in clinical isolates of Streptococcus pneumoniae from a university hospital in Korea. Clin Infect Dis 1995; 20: 826–35.
78. Chiu SS, Ho PL, Chow FK, Yuen KY, Lau YL. Nasopharyngeal carriage of antimicrobial-resistant Streptococcus pneumoniae among young children attending 79 kindergartens and day care centers in Hong Kong. Antimicrob Agents Chemother 2001; 45: 2765–70.
79. Petrosillo N, Pantosti A, Bordi E, et al. Prevalence, determinants, and molecular epidemiology of Streptococcus pneumoniae isolates colonizing the nasopharynx of healthy children in Rome. Eur J Clin Microbiol Infect Dis 2002; 21: 181–88.
80. Fujimori I, Hisamatsu K, Kikushima K, Goto R, Murakami Y, Yamada T. The nasopharyngeal bacterial flora in children

with otitis media with effusion. Eur Arch Otorhinolaryngol 1996; 253: 260–63.

81. Dunais B, Pradier C, Carsenti H, et al. Influence of child care on nasopharyngeal carriage of Streptococcus pneumoniae and Haemophilus influenzae. Pediatr Infect Dis J 2003; 22: 589–92.
82. Yagupsky P, Porat N, Fraser D, et al. Acquisition, carriage, and transmission of pneumococci with decreased antibiotic susceptibility in young children attending a day care facility in southern Israel. J Infect Dis 1998; 177: 1003–12.
83. Givon-Lavi N, Dagan R, Fraser D, Yagupsky P, Porat N. Marked differences in pneumococcal carriage and resistance patterns between day care centers located within a small area. Clin Infect Dis 1999; 29: 1274–80.
84. Sa-Leao R, Tomasz A, Sanches IS, et al. Carriage of internationally spread clones of Streptococcus pneumoniae with unusual drug resistance patterns in children attending day care centers in Lisbon, Portugal. J Infect Dis 2000; 182: 1153–60.
85. Pelton SI, Dagan R, Gaines BM, et al. Pneumococcal conjugate vaccines: proceedings from an Interactive Symposium at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy. Vaccine 2003; 21: 1562–71