9 Bacteria, Viruses and Fungi in Healthy and Diseased Paranasal Sinuses

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Introduction

Technological advancements in the field of microbiology have led to significant progress in our understanding of the role of bacteria, viruses and fungi in healthy and diseased paranasal sinuses. It is now known that the sinonasal tract is not sterile and that the microbes colonising the mucosa are not necessarily pathological. The sinonasal microbiota, which consists of the entire collection of microbes, including bacteria, viruses, fungi and archaea, existing within the sinuses has multiple functions, including maintaining mucosal health and effective local immune responses.

This chapter will cover: (1) the role of microbes in health and various sinonasal conditions and the relationship between the microbiota and antimicrobial treatments; (2) the various laboratory techniques utilised to investigate microbes (including culture, fluorescence in situ hybridisation and sequencing approaches); (3) current limitations and areas of controversy in the literature, particularly with regard to culture and sequencing studies of the sinonasal microbiota.

Bacteria, Viruses and Fungi in Healthy Paranasal Sinuses

Microbes begin to colonise the sinonasal mucosa from birth. The diversity of the bacterial community increases during the first 3 years of life and in adulthood becomes individualised and relatively stable over time [1]. Culture techniques have most frequently detected members from the genus *Staphylococcus*, *Corynebacterium* species and *Propionibacterium acnes* [2–4]. Sequencing approaches have similarly seen a high prevalence of *Staphylococcus* sp., *Corynebacterium* sp. and *Propionibacterium* sp. [5, 6]. These findings are summarised in Table 9.1.

The nasal metagenome (the collective genomic representation of the many organisms existing in a community) suggests that there is a set of core functional genes present in all individuals that code metabolic processes, transport systems and biosynthesis [6]. The stability of the bacterial community is achieved by key central bacteria, such as *Propionibacterium* sp., that connect many parts of this network [7]. Both culture and sequencing methods report low abundances of members from the genera (*Fusobacteria*, *Bacteroidetes*), potential pathogens (*Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Moraxella catarrhalis*) and anaerobes [6–8].

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Fungi are ubiquitous in our environment and fungal communities have also been detected in healthy sinonasal samples, dominated by the genus *Malassezia*, suggesting that they have a commensal role in the sinus microbiome [9, 10]. Furthermore, a variety of viruses and archaea (prokaryote organisms that are obligate anaerobes) have been found in healthy sinonasal samples without causing disease. The roles of these less-common microbes in the healthy microbiome are yet to be determined [11, 12].

Staphylococcus aureus, a bacteria that can cause a wide variety of illnesses, is persistently carried by 20% of the population and transiently carried by 60% [13]. While persistent *S. aureus* carriage in the anterior nares is a risk factor for infection, the mechanism of the transition from a commensal to a pathogenic bacteria is unknown. One hypothesis suggests that when the mucosal barrier is breached by a pathogen, a self-limited host immune response is generated. The mucosa interacts with the host immune system to act as a barrier against pathogens. Type 1 immune responses target viruses, type 2 immune responses target parasites and type 3 immune responses target extracellular bacteria and fungi. These responses result in the elimination of the pathogen and encourage restoration of the mucosal barrier.

Bacteria, Viruses and Fungi in Diseased Paranasal Sinuses

Culture and sequencing studies investigating the various phenotypes of sinusitis have shown that there are several potential pathogenic mechanisms that can be implicated in each of these groups. The most prevalent microbes detected from these studies are summarised in Table 9.1. The role of these microbes and the relationship between sinusitis and antimicrobial treatments will be discussed in this section.

Acute Rhinosinusitis

Acute rhinosinusitis (ARS) is a condition characterised by the sudden onset of sinonasal symptoms for less than 12 weeks. It can be subclassed into viral ARS, bacterial ARS and recurrent acute rhinosinusitis (RARS).

Table 9.1 The most prevalent bacteria, viruses and fungi detected using culture and sequencing approaches

	Culture	Sequencing
Health	Genus: *Staphylococcus*, *Corynebacterium* Species: *P. acnes*, *Staph. aureus* [2–4]	Genus: *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Malassezia* [5, 6]
Acute rhinosinusitis	Genus: *Pneumococcus* Species: *S. pneumoniae*, *H. influenza*, *M. catarrhalis* [14, 15]	Species: *Rhinovirus*
Chronic rhinosinusitis	Genus: *Corynebacterium*, *Streptococcus*, *Prevotella*, *Porphyromonas*, *Peptostreptococcus*, *Fusobacterium*, *Candida*, *Aspergillus*, *Penicillium*, *Cladosporium* Species: *Staph. aureus*, *Staph. epidermidis*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *S. pneumoniae*, *Haemophilus influenza* [16]	Genus: *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Pseudomonas*, *Haemophilus*, *Achromobacter*, *Candida*, *Aspergillus*, *Penicillium*, *Malassezia* Subfamily: *Orthocoronavirinae (Coronavirus)* Species: *Pseudomonas aeruginosa*, *Haem. influenzae*, *Staph. aureus*, *Corynebacterium neoformans*, *Rhinovirus* [7, 17–19]
Odontogenic sinusitis	Genus: *Streptococcus*, *Staphylococcus*, *Prevotella* Species: *H. influenzae* [20]	
Fungal rhinosinusitis		Genus: *Aspergillus*, *Mucor*, *Rhizomucor* [21]
Cystic fibrosis	Genus: *Pseudomonas*, *Burkholderia* Species: *Pseud. aeruginosa*, *Staph. aureus* [22]	Genus: *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Burkholderia* [22–24]
Primary ciliary dyskinesia	Species: *H. influenza*, *S. pneumoniae*, *M. catarrhalis*, *P. aeruginosa* [25]	

Viral ARS: The symptoms of ARS last fewer than 10 days. Studies have shown that viruses damage and enter the nasal epithelium, initiating host inflammatory responses leading to ARS [11]. One hypothesis is that this process may occur by the degradation of the epithelial barrier by reactive oxygen species stimulated during viral replication. Rhinoviruses are the predominant virus implicated in ARS. There is no beneficial evidence for the prescribing of antibiotics in ARS.

Acute Bacterial Rhinosinusitis (ABRS): It is defined as ARS that does not improve within 10 days of onset or ARS that worsens within 10 days after an initial improvement. Viral upper respiratory tract infection with subsequent bacterial superinfection has been suggested as a contributing factor in a proportion of these cases. Viral-induced mucosal injury may lead to translocation and overgrowth of pathogenic bacteria [26]. Commonly cultured pathogens from the sinuses of patients with bacterial ARS include *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* [14]. Penicillin-resistant *pneumococcus*, ampicillin-resistant *H. influenzae* and *M. catarrhalis* occur to a lesser extent, but are also commonly cultured [15]. In uncomplicated cases, the benefits of antibiotics are uncertain and these should only be considered if symptoms fail to resolve or worsen after a period of watchful waiting. Antibiotics can cause significant adverse effects that include gastrointestinal complaints, growing bacterial resistance and anaphylaxis. Accordingly, careful patient selection is needed.

Recurrent Acute Rhinosinusitis (RARS): This condition is characterised by four or more episodes of ARS per year with symptom-free intervals. Pathogens cultured from nasal swabs are similar to those seen for ABRS (*Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*). However, these bacteria may have a higher degree of antimicrobial resistance [14, 27]. Patients with immunodeficiency have a predisposition to developing RARS. Given the absence of studies specifically investigating antibiotic use in RARS, the criteria for antibiotic use in ARS may be adopted for this diagnosis [28].

Chronic Rhinosinusitis

Chronic rhinosinusitis (CRS) is a complex condition in which several phenotypes and endotypes have been described. However, the role of microbes in most cases of CRS remains unclear. Defining the role of bacteria, viruses and fungi in CRS, as well as the implications for appropriate antimicrobial treatment, requires careful consideration.

Bacteria that are frequently cultured from nasal swabs of patients with CRS include *Staphylococcus aureus*, *Corynebacterium* species, *Streptococcus* species, *Staphylococcus epidermidis* and *Propionibacterium acnes* [16]. It has been found that patients with more severe CRS disease, based on imaging, are more likely to culture pathogenic bacteria [29]. Sequencing studies also suggest that CRS patients have an altered microbiome with more pathogenic microbes [12, 19]. In CRS, these dysbiotic microbial communities possibly interact with a compromised mucosal barrier and host immune responses. If the damage to the mucosal barrier caused by pathogens fails to resolve, this can lead to chronic inflammation of the mucosa and tissue remodelling. The following section will discuss these potential disease mechanisms in more detail.

Single Pathogen Hypotheses

Specific pathogens, such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, are frequently cultured from the middle meatus of patients with CRS. These pathogens, in particular *Staphylococcus aureus* and its superantigens, have been proposed as potential key aetiologic agents in CRS. Staphylococcal enterotoxins are superantigens that stimulate a polyclonal activation of T cells resulting in an increased cytokine release. These enterotoxins likely act as a disease modifier by amplifying the inflammatory response in CRS; their presence has been associated both with asthma and recalcitrance after surgery [30, 31].

Studies have demonstrated an increased detection rate of serum-specific IgE to *S. aureus* enterotoxin in CRS with nasal polyps (CRSwNP),

but there are limited data to support the role of superantigens in CRS without nasal polyps (CRSsNP). In CRSwNP, specific IgE to *S. aureus* has been associated with eosinophilic and type 2 inflammation [31, 32].

Staphylococcus aureus has also been detected within the epithelium and the interstitium in sinus mucosa, and these intraepithelial and interstitial bacteria may possibly act as a reservoir of pathogenic microbes in CRS [33, 34].

More recently, instead of a single pathogen dominating all CRS microbial communities, CRS patients have been found to cluster into sub-groups, with each sub-group dominated by either *Staphylococcaceae*, *Streptococcaceae*, *Pseudomonadaceae*, or *Corynebacteriaceae*. This variation of microbial community composition may contribute to CRS disease heterogeneity [17].

Biofilms

A biofilm is a community of bacteria or fungi surrounded by an extracellular matrix that provides increased protection to the resident microbes in several ways. They are formed by planktonic bacteria that communicate their density status to other bacteria via quorum sensing molecules. Once the microbes are present in an appropriate concentration, these molecules encourage them to begin forming a biofilm [35]. There is a high prevalence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms in CRS, and it has been hypothesised that these contribute to CRS pathogenesis [36]. However, biofilms can also be found in control patients without CRS, although usually in much less dense formations [37, 38].

Biofilms may cause recurrent infections by the release of pathogenic microbes that stimulate a host immune response and also by the release of superantigens by *Staphylococcus aureus* biofilms [39]. The biofilm provides its residents with effective protection against host immune responses by phagocytosis and complement binding. Microbes within biofilms also undergo phenotypic changes to require less oxygen and nutrients. This slows down cell growth, which contributes to the likelihood of antibiotic resistance because almost all antimicrobials are more effective at killing rapidly dividing cells [40]. Sinonasal biofilms have been associated with recalcitrant CRS, an increased need for surgical intervention and worse outcomes after functional endoscopic sinus surgery (FESS) [41, 42]. As conventional culture techniques enrich the fastest-growing microorganisms, accurate identification of biofilm-forming pathogens requires sensitive histopathological methods such as fluorescent in situ hybridisation. Biofilms are typically resistant to standard antibiotics but potential biofilm-specific systemic and topical therapies are under investigation.

Microbial Dysbiosis

Studies utilising sophisticated gene-targeted and meta-omic sequencing approaches have suggested that CRS is caused by disturbances in the overall bacterial community composition and function rather than by a consistent single causative pathogen. These dysbiotic imbalanced microbial communities, otherwise known as microbial dysbiosis, interfere with the colonisation of healthy microbes and contribute to provoking host immune responses [7, 17, 24] (Fig. 9.1).

The CRS microbiome is both less diverse and stable than that seen in healthy controls, and it also has a higher total bacterial load [43–45]. CRS patients tend to have an increased relative abundance of opportunistic pathogens (such as members from the genera *Corynebacterium*, *Streptococcus* and *Staphylococcus*) and anaerobes [7, 18], which may contribute to recalcitrant CRS. Specific pathogens involved in dysbiosis may include *P. aeruginosa*, *H. influenzae* and *S. aureus* [18, 19]. Furthermore, the CRS microbiome tends to have fewer commensal bacteria, such as *Actinobacteria* sp., *Propionibacteria* sp., *Corynebacterium* sp. and *Acinetobacter johnsonii*. Key commensal bacteria may have a role in suppressing pathogenic species and therefore the loss of these communities could potentially result in pathogen outgrowth [46].

CRS patients with asthma are more likely to exhibit dysbiosis. Smoking, purulent secretions and aspirin sensitivity have also been associated

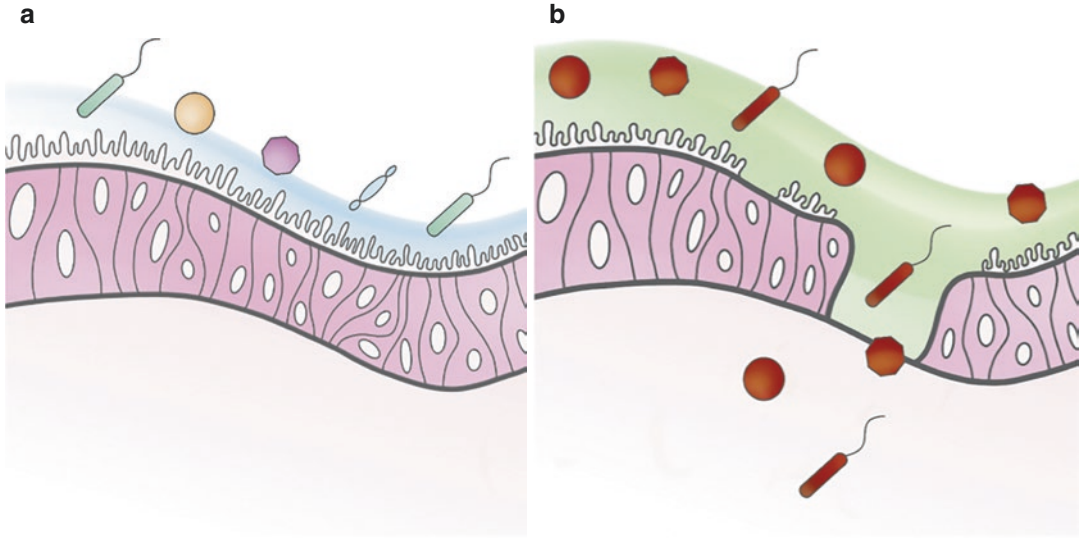

Fig. 9.1 The microbial dysbiosis theory in chronic rhinosinusitis. (**a**) Healthy mucosa with an intact mucosal barrier. The microbiota is diverse with a network of key commensal microbes. (**b**) Diseased mucosa with epithelial damage and increased mucus. The microbiota is less diverse, with an increased proportion of pathogenic microbes and loss of commensal microbes

with shifts in the sinonasal microbiome [24, 47]. Antibiotics may disrupt the commensal microbiome by decreasing bacterial diversity and increasing the relative abundance of antibiotic-resistant microbes, leading to ongoing disease [24, 48]. Furthermore, FESS has been shown to result in changes to the bacterial community composition in the sinuses, with an increased relative abundance of *Staphylococcal* species [49, 50].

Overall, the evidence is varied, and investigations into the causal relationships between microbial dysbiosis and host immunity in CRS patients are ongoing. Novel research topics in this area include:

- the identification of CRS subtypes based on their bacterial community composition profiles,
- co-culture studies that show how microbial community composition can influence the co-occurrence of certain bacteria through niche-specific competition, and
- the role of the interactions between microbe co-occurrence patterns and an altered immune response in CRS [17, 47].

Fungi

Fungal spores are ubiquitous in our environment and can be detected in both CRS and healthy sinuses. One recent study has demonstrated fungi in the maxillary sinus of over 80% of CRSwNP patients, compared with only 20% of controls [51]. Therefore, some researchers have suggested that fungi have a possible role in CRS [51–53]. Fungi have been reported to stimulate a type 2 immune response, although studies demonstrating a direct link between fungi and CRS are lacking [51–53].

The most frequently identified fungi from the sinuses of CRS and control subjects using polymerase chain reaction (PCR) and culture include members from the genera *Aspergillus*, *Cladosporium* and *Candida* [54, 55]. Only a handful of studies have performed amplicon sequencing to investigate the community composition of fungi in the sinuses. The most prevalent fungi identified include *Cryptococcus neoformans*, *Aspergillus* species and *Malassezia* species; however, results are inconsistent between studies [56, 57].

Viruses

The pathogenic role of viruses in CRS is unknown. Studies suggest higher rates of viruses in the sinuses of CRS patients compared with controls and peak viral isolation occurs in winter and spring [11, 58, 59]. Rhinovirus and coronavirus species are the most frequently isolated in CRS, although respiratory syncytial viruses, bocavirus, adenoviruses, human metapneumovirus and influenza viruses have also been detected in sinusitis [58, 59]. In vitro studies investigating CRS-derived nasal epithelial cells suggest that rhinoviruses decrease host immune responses [60, 61]. However, whether viral infections play an aetiological role in CRS or only lead to acute exacerbations of CRS (AECRS) is yet to be established. The literature has so far been inconsistent, which may be explained by seasonal fluctuations of respiratory viruses and differences in study sample collection and laboratory measures.

Acute Exacerbation of Chronic Rhinosinusitis (AECRS)

Bacterial infections probably contribute to AECRS, although there is little good evidence to support this. It has been hypothesised that impaired mucociliary clearance, evident in a subgroup of patients with chronic inflammatory mucosal changes, leads to prolonged contact with microbes [62]. Cultured organisms in AECRS included *Prevotella* sp., *Porphyromonas* sp., *Peptostreptococcus* sp., *Fusobacterium* sp., *S. pneumoniae* and *H. influenzae* [63]. Microbial dysbiosis may also elicit a host inflammatory response, and there is evidence that rhinovirus infections can drive eosinophilic inflammation. Short courses of antibiotics are often prescribed for AECRS. However, the evidence supporting the efficacy of these courses is not strong.

Odontogenic Sinusitis

Odontogenic sinusitis has been associated with the overgrowth of oral microbes into the sinuses, which tend to be more anaerobic than typical sinonasal pathogens. Common bacteria include *H. influenzae* and members of the genera *Streptococcus*, *Staphylococcus* and *Prevotella* [20].

Fungal Rhinosinusitis

Fungal spores are ubiquitous and are being inhaled into the nasal cavity continuously. While the species vary according to the locality, most fungal sinusitis cases are caused by dematiaceous fungi or *Aspergillus* spp. Manifestations of fungal sinusitis include fungal ball, invasive fungal rhinosinusitis and allergic fungal rhinosinusitis. *Aspergillus* and *Zygomycetes* (*Mucor*, *Rhizomucor*) are the genera of fungi most commonly associated with tissue invasion in invasive fungal rhinosinusitis [21]. First-line antifungal treatments for acute invasive fungal rhinosinusitis include systemic azoles (voriconazole and isavuconazole) for *Aspergillus* and amphotericin for *Zygomycetes* [64].

Cystic Fibrosis

Cystic fibrosis leads to highly viscous secretions and impaired mucociliary clearance, resulting in both sinus and lung infections. Bacteria cultured from these sites (such as genera *Pseudomonas* and *Burkholderia*) have a high degree of concordance, suggesting that the sinuses may act as a reservoir for bacterial transmission to the lower respiratory tract. CRS patients with cystic fibrosis have a higher bacterial load and are almost completely dominated by one bacterial species [23, 24]. This may well reflect the high number of powerful, broad-spectrum antibiotics administered to these patients.

Primary Ciliary Dyskinesia

Patients with primary cilia dyskinesia have a predisposition to bacterial infections, including *H. influenza*, *S. pneumoniae*, *M. catarrhalis* and *P. aeruginosa*. Influenza, pneumococcal and RSV vaccines, as well as standard vaccinations and prompt antibiotic therapy for respiratory tract infections, have been recommended [25]. Antibiotic therapy, sinus rinses and surgery may decrease pathogenic sinus bacteria, improve symptoms, reduce lung infections and improve quality of life [25, 65].

Technology

Culture

Culture methods have been used for more than a century to detect pathogenic and commensal microbes. This technique requires specific growth media and conditions depending on the microbe targeted [2] (Fig. 9.2). It remains the most common method for detecting specific pathogens, for example, *P. aeruginosa* in cystic fibrosis [66]. However, only a limited variety of microbes will grow on a specific culture medium. Therefore, culture methods tend to underestimate the diversity of the sinonasal microbial community. Culture studies in both healthy controls and patients with CRS detect approximately 3–9 microbes per subject [2]. One significant advantage of culture techniques is that they enable fast and accurate in vitro determination of antibiotic sensitivity of the isolated pathogen. Furthermore, culture remains the primary method for detecting pathogenic bacteria in clinical settings and much of our understanding of the microbiology of CRS is based on these techniques.

The following sections will discuss modern culture-independent, or molecular, approaches. These methods do not require the in vitro growth of microbes but rather detect the genes of the microbes present. These techniques have revealed the complexity of the sinonasal microbial community.

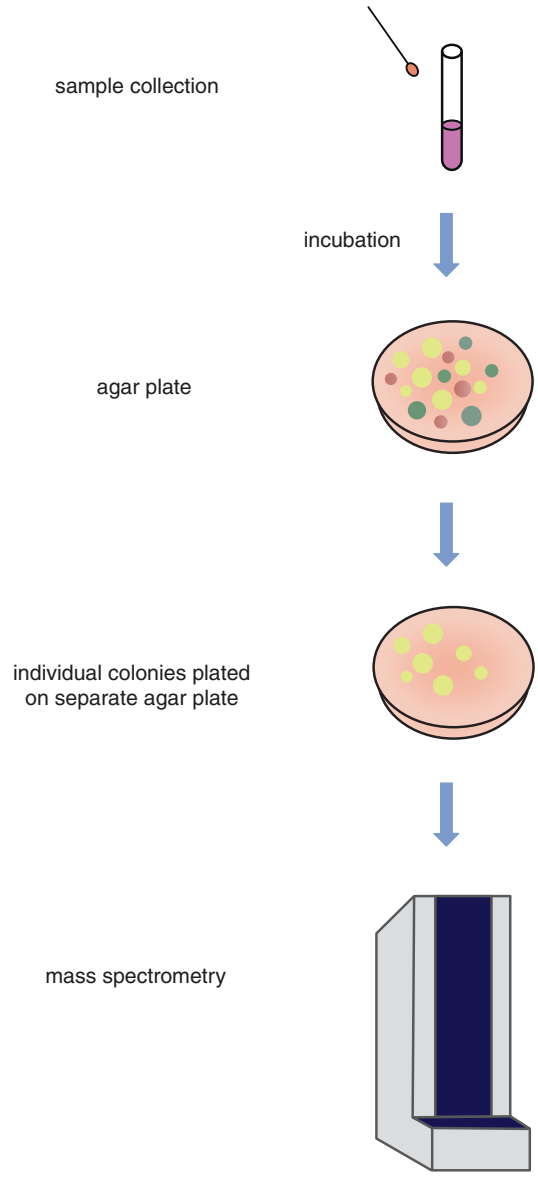

Fig. 9.2 Culture. Collected samples are placed onto agar plates, which are then incubated to promote microbial growth. Individual colonies that are morphologically or phenotypically different are plated again on separate agar plates. These microbes are then identified through MALDI-TOF (matrix-assisted laser desorption/ionisation-time of flight) mass spectrometry. Sanger sequencing can also be used to identify these individual colonies

Immunohistochemistry

Immunohistochemistry can be used to localise species-specific microbial molecules with labelled antibodies on tissue sections, which can then be visualised using microscopy. Multiple antigen–antibody labels can be used in a sample giving spatial and structural information. For example, bacteria can be seen on the surface of the epithelium (planktonic), within the epithelium (intraepithelial) or deep to the epithelium (intramucosal) (Figs. 9.3 and 9.4).

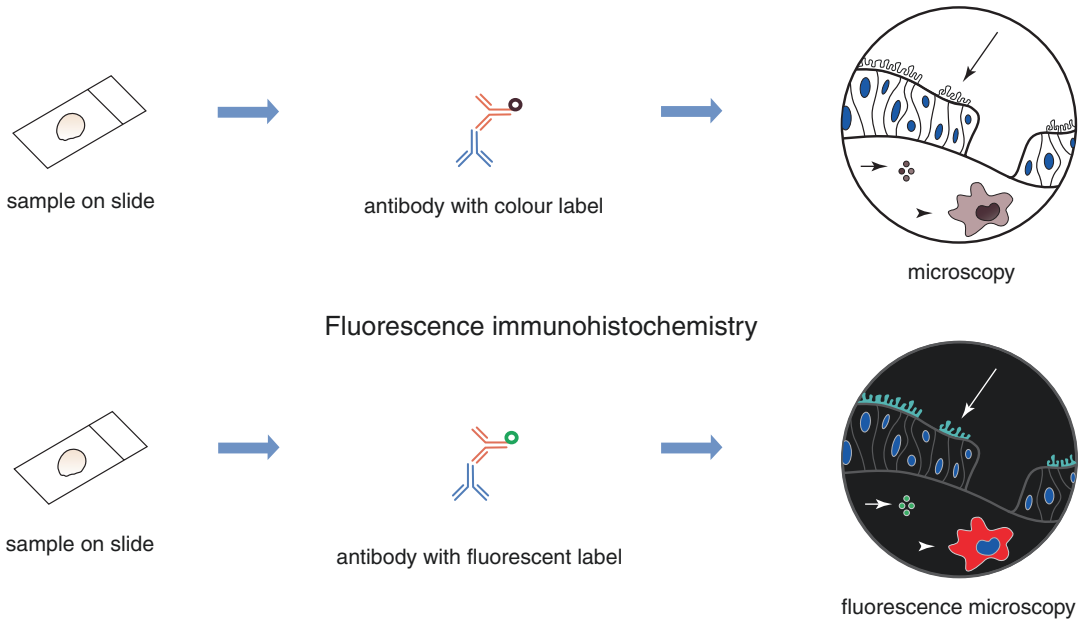

Fig. 9.3 Immunohistochemistry. Tissue sections on a slide are labelled with antibodies attached to a colour or fluorescent label. These are then visualised using microscopy. Multiple structures can be targeted, allowing the simultaneous labelling of microbes (short arrow), immune cells (arrowhead) and anatomical features such as cilia (long arrow)

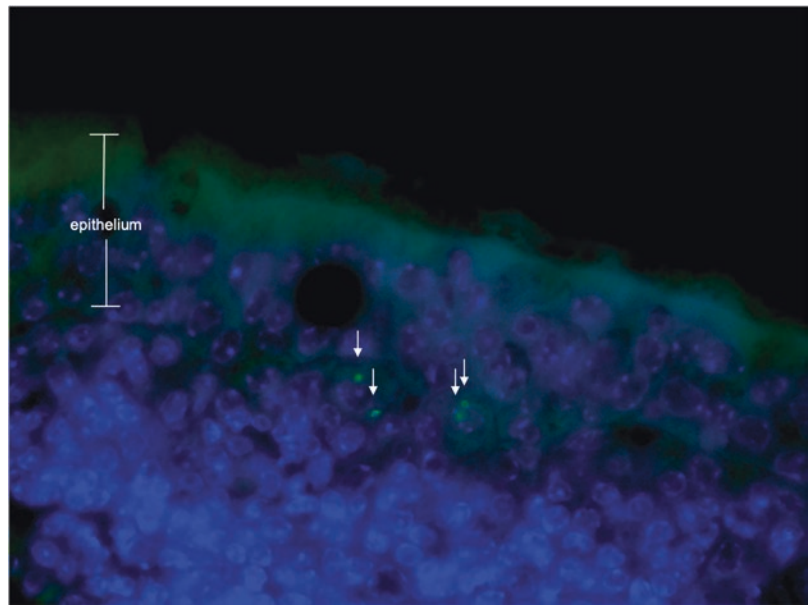

Fig. 9.4 Mouse sinus mucosa fluorescence immunohistochemistry demonstrating *S. aureus* antibody (arrows) and DAPI (4,6-diamidino-2-phenylindole) nucleic acid stain. Magnification: ×100. Unpublished image

Fluorescence In Situ Hybridisation

Fluorescence in situ hybridisation (FISH) utilises targeted probes attached to fluorescent dye molecules to identify individual microbial cells (Fig. 9.5). Classically, FISH utilised ribosomal RNA probes but modern techniques have targeted messenger RNA, plasmids and single-copy

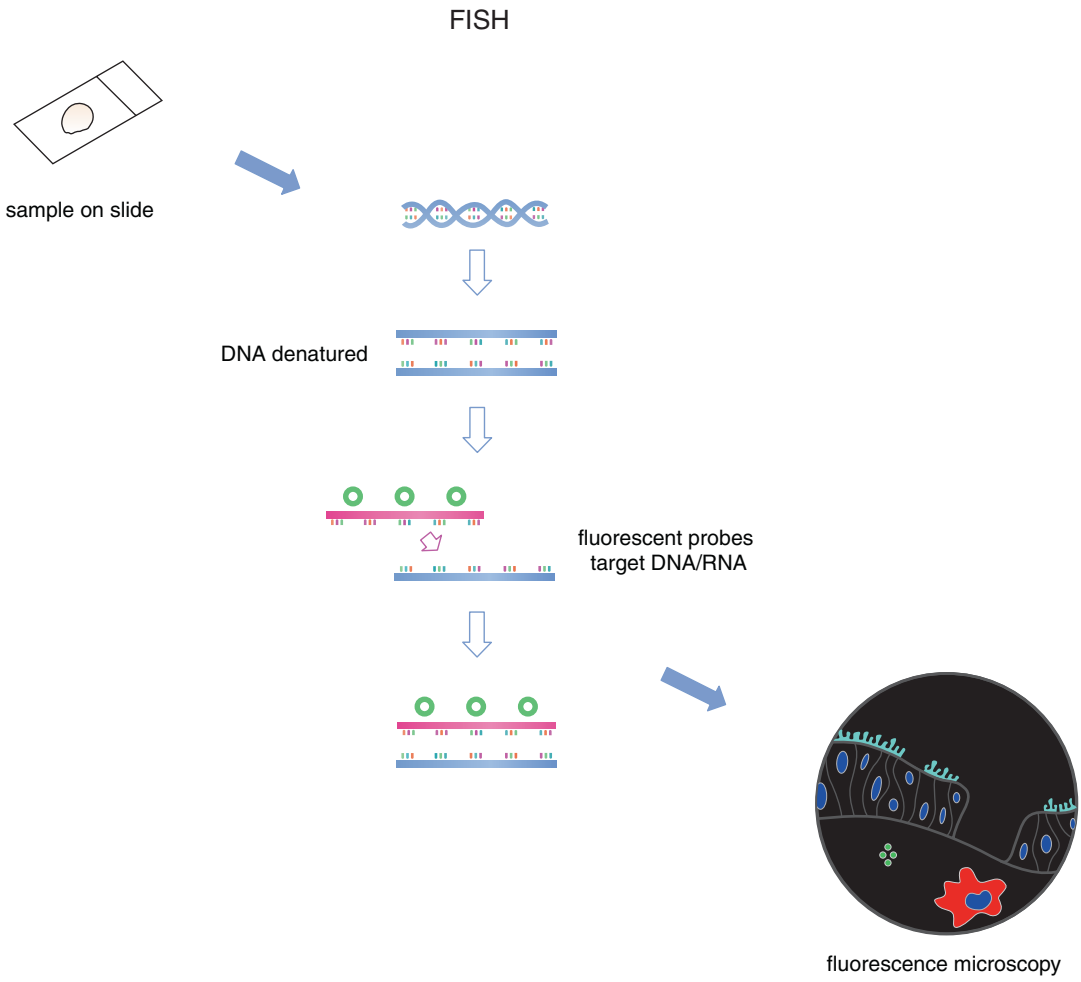

Fig. 9.5 Fluorescence in situ hybridisation. DNA within cells on the slide are denatured. Labelled probe (circles) hybridises to targeted DNA/RNA regions on the sample. These fluorescent probes are then visualised using fluorescence microscopy

genes. FISH probes can target all species (e.g. eubacterial, eufungal) or specific species. FISH allows the localisation and enumeration of these targets via either fluorescence microscopy or flow cytometry.

Amplicon Sequencing

Sequencing approaches amplify genes from the extracted genomic DNA of samples (swabs, tissue, mucus) using PCR. The amplified products are purified and then sequenced. The raw sequence reads are matched against known sequences in databases to provide a microbial profile for the sample (Fig. 9.6). This method allows the identification of potentially all of the microbes present within a sample. Gene-targeted sequencing looks at specific microbial gene sequences. The bacterial 16S rRNA gene, which is present in all bacteria, is the most common target used in sinonasal studies and can detect an average of 30 bacterial taxa (a taxonomic group of any rank, such as species, genus or phylum) per subject [7]. Fungi have also been investigated using a number of genes targets (18S rRNA and internal transcribed spacer regions), which similarly can encompass all fungal species. Unlike

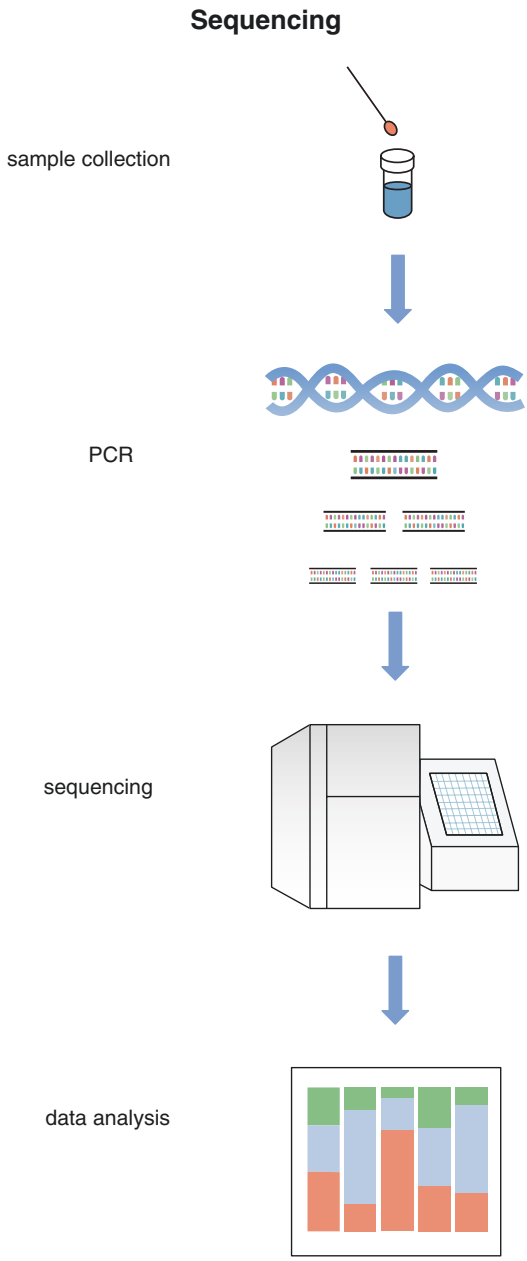

Fig. 9.6 Gene-targeted sequencing. Collected samples undergo PCR amplification. Amplicon sequencing determines the order of nucleotides in DNA. These sequences are then matched to a database to identify the microbes. Data analysis can include taxa plots, which allow comparisons of the microbiota between samples (each column represents a sample and each colour represents a microbial species)

bacteria and fungi, viruses do not have a universal gene target and so different targets are required to detect specific viruses. Consequently, novel viruses or viruses not included in a designed panel of targets cannot be detected. The presence of viruses in the sinonasal tract is therefore likely to be underreported. A weakness of the bacterial 16S rRNA gene-targeted approach is a limited resolution (the ability to resolve strains within a species), although this will improve with technological advances in this field [67].

In contrast to gene-targeted approaches for species identification, meta-omics can detect the total genetic composition or function from the organisms within a sample (whole genome sequencing). It can focus on DNA (metagenomics), RNA (metatranscriptomics) and proteins involved in cellular functions (metaproteomics). These techniques are able to simultaneously provide information on microbial community composition and function. Metagenomic approaches also allow the simultaneous detection of a wide variety of viruses.

Longitudinal gene-targeted and meta-omic studies that collect samples over multiple time points have enabled investigation into how the sinonasal microbiome changes over time. These studies have shown that the microbiota is reasonably stable over time in healthy controls and that this stability is achieved by certain commensal bacteria [7]. Contrastingly, in microbial dysbiosis, there is temporal volatility in microbial composition. This instability is also significantly affected by variables such as asthma, smoking, antibiotics and surgery [24, 49, 50]. However, these methods are resource-intensive, expensive and not easily standardised across studies. For these reasons, their clinical applications are limited. Nevertheless, as this technology improves, it will enable the sinonasal metagenome to be investigated with increasing accuracy and efficiency.

Summary of Areas of Controversy or Uncertainty

Bacteria, viruses and fungi colonise the sinonasal mucosa and have various roles and functions in healthy and disease states. With the development of sequencing technologies for investigating the microbiota, we now understand that culture tech-

niques vastly underestimate the diversity of these complex microbial communities. However, sequencing methods also have their limitations. Current evidence in the literature can often be inconsistent due to non-standardised methods and small sample sizes, reflecting the resource-intensive nature of these modern laboratory approaches.

It has been suggested that a core part of the healthy sinonasal microbiome codes metabolic processes, transport systems and biosynthesis. Furthermore, the stability of these communities is thought to be achieved by key central bacteria that connect many parts of the network [6]. Studies utilising sequencing approaches have also hypothesised that CRS is caused by microbial dysbiosis rather than a consistent single causative pathogen. These theories are not necessarily mutually exclusive. Instead, microbial dysbiosis arguably better reflects the evidence that disruption and instability of the microbiota as a whole occur in CRS. Even when single pathogens or biofilms are implicated in a patient's disease pathogenesis, these likely reflect microbial community composition shifts, with a decrease in key healthy microbes. Novel research in this field has focused on identifying CRS subtypes based on their microbiota, co-culture studies that demonstrate niche-specific competition between certain bacteria and the interactions between microbes and immune dysfunction in CRS [17, 47, 68, 69]. However, further longitudinal studies that assess the long-term stability of the microbiota rather than a single time point are required.

Key Learning Points

- The healthy sinonasal mucosa is colonised by bacteria, viruses and fungi from birth.
- The sinonasal microbiota has been investigated using traditional culture and modern sequencing approaches.
- Sequencing approaches have led to novel hypotheses on the role of the microbiota in health and various diseases.
- The current understanding of the role of pathogenic microbes in CRS is incomplete and limited by the resource-intensive nature of these methods and data from cross-sectional studies.

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