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REVIEW ARTICLE

# Molecular Methods for the Detection and Analysis of Sinonasal Microbiota: A Systematic Review

## Molekularne metody wykrywania i analizy składu drobnoustrojów zatok przynosowych – przegląd piśmiennictwa

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Article history: Received: 28.08.2025 Accepted: 26.11.2025 Published: 27.11.2025

**ABSTRACT:**

**Introduction:** Traditional culture-based diagnostic methods detect only a fraction of the microorganisms inhabiting the paranasal sinuses. This limitation is due to non-culturable organisms, biofilms, and complex microbial interactions. Molecular methods based on genetic material analysis offer enhanced insight into the sinonasal microbiome and are increasingly applied in chronic rhinosinusitis (CRS) research.

**Aim:** The aim of this paper is to present the techniques used to analyze the microbiome of the paranasal sinuses.

**Material and method:** We conducted a literature review in PubMed, Scopus, and Web of Science using the terms "microbiome" and "sinusitis" with the "humans" filter. We included 123 original articles (March 2013 – August 2025) reporting the method of sample collection, DNA extraction, sequencing platform, and bioinformatic analysis.

**Results:** The most common sampling method was swabbing (69.2%), either alone or combined with biopsies or aspirates. In 65% of studies, samples were collected endoscopically. Sampling sites included the middle nasal meatus (43.3%), ethmoid cells (20%), and maxillary sinus (18.3%), with less frequent sampling from the nasopharynx, frontal, and sphenoid sinuses. Samples were most often stored at –80°C (76%) or –20°C (16%), frequently in stabilizing solutions like RNAlater (51.4%). DNA extraction methods employed commercial kits in 87.8% of cases and included mechanical (bead-beating), enzymatic (e.g., proteinase K), and chemical (phenol-chloroform) protocols. Sequencing was most commonly performed on Illumina platforms (77.1%), followed by 454/Roche pyrosequencing (19.3%) and Ion Torrent (2.4%), typically targeting the V3–V4 regions of the *16S rRNA* gene. Bioinformatic analysis was conducted with QIIME (36%), USEARCH, Mothur, and DADA2. The SILVA database (56.3%) was the most frequently used reference taxonomy.

**Discussion:** Molecular techniques analyzing genetic material reveal microorganisms missed by traditional culture, but do not determine their metabolic activity. The reliability of results depends on the sampling technique. The middle meatus, the most sampled site, is potentially representative of the sinuses. Endoscopic-guided collection reduces contamination risk. While swabs, biopsies, and aspirates yield comparable data, biopsies may offer greater microbial diversity. Proper sample storage and temperature are essential for preserving DNA integrity. DNA extraction protocols should align with microbial cell wall properties. Illumina's dominance allows data comparability but limits species-level resolution. SILVA-based classification ensures high-quality taxonomic assignment, though methodological variability across studies remains a concern.

**Conclusions:** Molecular methods provide valuable insights into the sinonasal microbiome. However, standardization of sample collection, storage, and processing is necessary for consistent results and meaningful clinical application.

**KEYWORDS:** bioinformatics, chronic sinusitis, DNA sequence analysis, molecular diagnostic techniques, paranasal sinuses / microbiology

**STRESZCZENIE:**

**Wstęp:** Tradycyjne hodowle bakteryjne wykrywają jedynie część mikroorganizmów bytujących w zatokach przynosowych. Ograniczenia te wynikają m.in. z obecności drobnoustrojów niehodowlanych, form biofilmowych i złożonych interakcji metabolicznych. Metody molekularne, oparte na analizie materiału genetycznego, umożliwiają dokładniejszą ocenę mikrobiomu i są coraz częściej stosowane w badaniach przewlekłego zapalenia zatok przynosowych.

**Cel:** Celem tej pracy było przedstawienie stosowanych metod analizy mikrobiomu zatok przynosowych.

**Materiał i metoda:** Przeprowadzono przegląd literatury z baz PubMed, Scopus i Web of Science, z wykorzystaniem słów kluczowych „microbiome" i „sinusitis" oraz filtru „humans". Uwzględniono 123 oryginalne prace opublikowane od marca 2013 do

sierpnia 2025 r. Kryteria włączenia obejmowały opisy: sposobu pobrania próbki, metody ekstrakcji DNA, użytej platformy sekwencjonującej oraz bioinformatycznej analizy danych.

**Wyniki:** Najczęściej stosowaną metodą pobrania materiału był wymaz (69,2%), samodzielnie lub w połączeniu z biopsją bądź aspiratem. W 65% prac materiał pobierano endoskopowo. Miejsca pobrań obejmowały głównie przewód nosowy środkowy (43,3%), komórki sitowe (20%) i zatokę szczękową (18,3%). Rzadziej analizowano nosogardło, zatoki czołowe i klinowe. W większości przypadków materiał przechowywano w –80°C (76%) lub –20°C (16%), często z użyciem płynów stabilizujących, takich jak RNAlater (51,4%). W 87,8% przypadków ekstrakcję DNA przeprowadzano przy użyciu zestawów komercyjnych; dominowały metody mechaniczne (bead-beating), enzymatyczne (np. proteinaza K) i chemiczne (fenol-chloroform). W 77,1% prac wykorzystano platformę Illumina, w 19,3% pyrosekwencjonowanie 454/Roche, a w 2,4% – Ion Torrent. Najczęściej sekwencjonowano regiony V3–V4 genu *16S rRNA*. Analizę danych przeprowadzano głównie za pomocą QIIME (36%), USEARCH, Mothur i DADA2. Najczęściej używaną bazą odniesienia była SILVA (56,3%).

**Dyskusja:** Zastosowanie metod molekularnych analizujących materiał genetyczny pozwala na identyfikację bakterii niewykrywalnych w tradycyjnych posiewach, jednak nie umożliwia określenia ich aktywności metabolicznej. Jakość i wiarygodność wyników zależą od wielu czynników, m.in. sposobu pobrania próbki. Przewód nosowy środkowy, jako najczęstsze miejsce pobrań, może być reprezentatywny dla zatok przynosowych. Pobranie materiału pod kontrolą endoskopu zmniejsza ryzyko kontaminacji. Różne techniki pobrań – wymazy, biopsje, aspiraty – mają zbliżoną skuteczność, choć biopsje mogą dawać większe bogactwo gatunkowe. Sposób przechowywania próbek i temperatura mają kluczowe znaczenie dla zachowania integralności DNA. Metody ekstrakcji powinny być dostosowane do typu ściany komórkowej badanych mikroorganizmów. Dominacja platformy Illumina umożliwia porównywalność wyników, jednak ogranicza rozdzielczość gatunkową. Analiza oparta na bazie SILVA pozwala na wysokiej jakości klasyfikację taksonomiczną, choć brak standaryzacji protokołów może prowadzić do heterogeniczności wyników.

**Wnioski:** Metody molekularne są nieocenionym narzędziem w badaniach mikrobiomu zatok przynosowych, jednak wymagają dalszej standaryzacji protokołów pobierania, przechowywania i analizy materiału. Ich wdrożenie do rutynowej diagnostyki wymaga ujednolicenia procedur i lepszej interpretacji klinicznej danych mikrobiomowych.

**SŁOWA KLUCZOWE:** analiza sekwencji DNA, bioinformatyka, metody molekularne, przewlekłe zapalenie zatok, zatoki przynosowe/mikrobiologia

## ABBREVIATIONS

**CRS** – chronic rhinosinusitis
**DNA** – deoxyribonucleic acid
**OTUs** – operational taxonomic units
**RNA** – ribonucleic acid

## INTRODUCTION

Diagnostic methods making use of bacterial and fungal cultures are an important part of the diagnosis of inflammatory conditions, including chronic sinusitis (CRS) [1, 2]. However, more than 70% of bacterial species inhabiting the human body cannot be cultured on standard media due to the specific environmental conditions necessary for their growth [3, 4]. These include the unique media required to grow a particular microorganism, symbiotic relationships between microorganisms, and the characteristics of the patient's immune system. In addition, biofilm-forming bacteria can be difficult to detect due to reduced metabolic activity and phenotypic transformation [5].

As a result, 10–45% of classic bacterial cultures fail to produce bacterial growth despite the identification of microbial colonies during biofilm analysis [6], revealing the fragmentary nature of our knowledge of the microbiota, i.e., the total microorganisms inhabiting a given habitat, as acquired from traditional cultures. This knowledge gap is one of the drivers of the current rapid development of molecular diagnostics tools analyzing the genetic material in microbiome samples collected from specific locations.

## AIM

The aim of this paper is to present the techniques used to analyze the microbiome of the paranasal sinuses.

## MATERIAL AND METHOD

The paper is a review of the literature on microbiological methods used in the analysis of sinus microbiota. The authors summarized relevant literature data as available from the Medline, Pubmed, Scopus, and Web of Science databases. The analysis covered the period from March 2013 through August 2025 (Tab. I.). The query was carried out using the keywords "microbiome" and "sinusitis" with "humans" used as the search filter. In the next stage, original papers including the descriptions of study methodology, material collection and extraction methods, sequencing technique, and the bioinformatic analysis method were extracted from the set of records retrieved from the databases. Articles presenting isolated deficiencies with regard to the descriptions of studied elements were also included in the review.

## RESULTS

### Sampling methods

#### *Types of material to be collected*

The most common method for the sampling of microbiota was swabbing, which had been used in 85 studies (69.1%) (Tab. II.). In

35.7% of papers, a swab was the only source of study material; a biopsy of sinonasal mucosa was performed in addition to swabbing in 30.1% of papers, while mucus aspiration was performed as the additional sampling method in 3.3% of papers. The less frequent techniques consisted of a mucus aspiration alone (9.8%), mucosal tissue biopsy (7.3%), brush swab (7.3%), or nasal lavage (2.4%). Five papers did not specify the nature of the collected material, referring to it simply as the specimen.

### *Swab insertion techniques*

In 82 papers (65%), samples were collected endoscopically. In 2 papers, the endoscope was inserted into the nasal cavity following previous dilatation of the nasal vestibule using an ear speculum. In 33.3% of the papers, the method of visualization during sampling was not described in detail.

### *Sampling locations*

The exact location where the swab or specimen was taken from the nasal cavity and/or paranasal sinuses and/or nasopharynx was reported in 73.2% of the papers (Fig. 1.). The most frequent sampling sites included the middle nasal meatus (43.3%), ethmoid cells (20%), and maxillary sinus (18.3%). Relatively few studies dealt with the microbiome collected from the frontal sinus (1.6%), sphenoid sinus (3.3%), or nasopharynx (4.9%). In 32.5% of the studies, the material was obtained from 2 or more collection sites.

### *Swab collection technique*

Four of the 123 publications specified the number of swab rotations during material collection (a 10-fold rotation in 1 article and a 5-fold rotation in 3 articles).

### *Swab handling*

Following the collection, the material was most frequently stored for transport purposes in sterile containers on ice (in 85.9% of the articles describing the transport of the material).

In the remaining 14% of the publications, the material was frozen in liquid nitrogen immediately after collection. In some of the studies, the collected swabs or sections were transported in special fluids. The most common medium was RNAlater (Ambion Inc.,TX, USA), a liquid that stabilizes RNA and DNA molecules (51.4% of articles describing the use of transport fluids). In 37.1% of papers, saline or other transport or lysis fluids such as ESwab™ liquid transport media (Copan Diagnostic, Murietta, CA, USA), RPE buffer (Qiagen, Hilden, NRW, Germany) Triton 9100 (Triton Systems of Delaware, Long Beach, MS, USA), were used. In the remaining publications, liquids were mentioned without the name being specified.

In 40% of the analyzed articles, the temperature of –80°C was maintained during the transportation of the material. In 27% of the publications, the sample temperature of –20°C was maintained for 1–7 days. In another 27% of the publications, the material was stored over the initial few days in the dedicated liquids

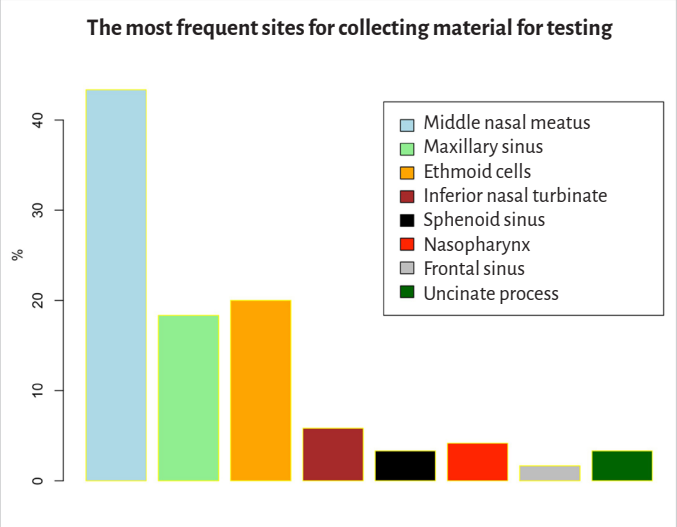


**Fig. 1.** Relative frequency of sites used for the collection of material for further bioinformatic analysis. The bar colors are also explained in the legend. Light blue – middle nasal meatus, light green – maxillary sinus, orange – ethmoid cells, brown – inferior nasal turbinate, black – sphenoid sinus, red – nasopharynx, gray – frontal sinus, dark green – uncinate process.

at 4°C. In single cases, transport temperatures of 5°C, 8°C, and –90°C were reported.

The storage temperature of the material was maintained at –80°C in 76% of the papers providing this information; in another 16%, the storage temperature was –20°C.

The time between material collection and microbiological analysis was reported in only 7% of the papers. Most frequently, it amounted to about 2 hours (in 45% of articles), 4 hours (10%), or 24 hours (20%).

### *Collection conditions*

The material was mainly collected in the operating room setting (in 81% of the articles providing relevant information). In 19% of the studies, samples were taken in an outpatient setting.

### *DNA extraction*

The DNA extraction method was described in 93 of the 123 analyzed articles. Dedicated kits were used for extraction in 81% of the studies. Physical methods, such as centrifugation and the use of microspheres, were mentioned in 39%, chemical methods, such as the phenol-chloroform method, were mentioned in 10%, and enzymatic methods were mentioned in 7% of the records.

### *Sequencing*

In 76.7% of cases, the sequencing was performed using the Illumina platform (Illumina, San Diego, CA, USA); in another 18.6% – by means of 454/Roche GS-FLX pyrosequencing (Roche Applied Sciences, Branford, CT, USA); and in 2.3% – using the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific, Waltham, MA, USA).

**Tab. I.** Articles on sinus microbiota retrieved from the database query using the "microbiome" and "sinusitis" keywords and the "humans" filter.

| DATABASE | NUMBER OF ARTICLES MEETING THE ARTICLE SEARCH CRITERIA |
|---|---|
| PubMed | **321** |
| Scopus | 33 |
| Web of Science | 31 |
| Total after removing duplicate records | 278 |
| Total including only original papers making use of molecular techniques [7–101]. | 123 |

The "Database" column lists the queried databases. The "Number of articles meeting the article search criteria" column shows the number of articles that meet the article search criteria. The penultimate row of the table contains information on the number of articles included in the analysis after removing duplicate records, while the last row contains the number of original articles.

### *Bioinformatic analysis*

A wide range of software was used to filter the raw data and create libraries of operational taxonomic units (OTUs) for bioinformatic analysis. The software tools used for these purposes included QIIME (26%), USEARCH (14.5%), Mothur (13.6%), and DADA2 (6.8%).

In 56.1% of the reviewed records, data were compared with the SILVA (Max Planck Institute for Marine Microbiology, Bremen, Germany) bioinformatics database followed by the GreenGenes (Lawrence Berkeley National Laboratory, USA) database as used in 23.3% of studies; other, less frequently used databases, included the ChunLab 16S rRNA database (ChunLab Inc, Seoul, South Korea), the ExTaxon database (ChunLab Inc., Seoul, South Korea), GenBank, and the NR Library of NCBI (NCBI, Bethesda, MD, USA).

## DISCUSSION

The current clinical applications of the molecular methods for the analysis of genetic material include primarily the diagnosis of viral and bacterial diseases [102], when it is important that the etiological agent of infection be identified as timely as possible (e.g., in sepsis) [102]. Molecular methods facilitate a more accurate assessment of the bacterial populations present at the examined site compared to traditional cultures, although their clinical usefulness may be limited by their inability to determine metabolic activity [103].

The methods also play an important role in understanding the microbiota of the nasal cavity and paranasal sinuses [95, 100, 104]. Since traditional cultures can identify only 30% of the bacterial species inhabiting the human body [3, 4] molecular methods involving the analysis of genetic material expand the possibilities of qualitative and quantitative assessment of the microbiome understood as the pooled genetic material present at the studied site. The study of the active microbiota, defined as all living microorganisms inhabiting a specific environment, is achievable by evaluating chemical compounds found only within systems presenting with active metabolism [44]. This is possible owing to the development of relatively new methods for analyzing the metabolome, proteome, and transcriptome of the microbiome, i.e., the collection of all metabolites, proteins, and RNA molecules of microorganisms inhabiting a particular environment [105]. However, the method of gene sequencing as a tool for identification of bacterial pathogens in clinical samples has its methodological limitations [106, 107], which may contribute to falsification of the results of the analysis.

### Sampling

Both the upper and lower respiratory tracts are inhabited by low-density microbiota [108].

Therefore, when approaching an analysis of the microbiome of the paranasal sinuses, the samples should be collected by trained personnel [108] to prevent potential contamination with the microorganisms dwelling on the skin of the face and within the vestibule of the nasal cavity [109]. Rombaux et al. described the potential impact of disinfection of these regions on the composition of the microbiome of the bulla [109]. Endoscopic assistance and dilatation of the nasal vestibule using an ear speculum can reduce the risk of contamination of the collected material with microorganisms from the nasal vestibule [13, 27, 110, 111].

An important issue consists in determining the optimal tool for the collection of the material. Adequate results can be obtained from microbiological examinations of nasal blown secretions, swabs, biopsies, mucus aspirates, and sinonasal lavage [112]. Due to the high probability of contamination, microbiological testing of nasal blown secretions is recommended only in children [113]. The maxillary sinus puncture with the collection of secretions is rarely used in current research due to the fact that it yields analogous results to a swab from under the middle nasal turbinate [113]. The material collected from nasal lavage is usually too contaminated to properly reflect the composition of sinus microbiota [113, 114]. According to some authors, direct sinus lavage with the collection of a sample for examination may be more sensitive than a swab taken from under the middle nasal turbinate, especially with regard to the detection of anaerobic microorganisms [113]. The technique requires precise injection of fluid into the sinus lumen as well as a certain amount of time passing prior to collection, which makes it relatively difficult from the technical point of view [112]. The swab is the most common method of microbiological diagnosis, as evidenced by its being used in about 70% of the reviewed articles [11, 13]. This is due to the test's simplicity, low invasiveness, and relatively good tolerance by patients [113]. In addition, the lack of significant differences in the composition of the bacterial microbiota within the middle nasal meatus and paranasal sinuses [47] indicates that the former may be considered a representative sampling site for the identification of pathogens associated with sinusitis.

Tissue biopsies are an invasive alternative to swabs. Bassiouni et al. [18] showed that there was no statistically significant difference in the mean relative abundance of microorganisms detected in biopsy and swab materials, which may be indicative of the equivalence of both methods. According to observations by Wos-Oxley et al. [115], biopsy specimens are characterized by greater species richness and diversity compared to swab samples. The differences in the results in the aforementioned papers may be due to the size of the group (n = 6 *vs* n = 79) and/or the potentially deeper tissue penetration of the biopsy [116]. A limitation of the method consists in the difficulty in obtaining adequate concentrations of DNA in the specimens despite various material extraction methods [32, 80].

One of the key methodological aspects in collecting specimens for microbiological studies from the nasal cavity and paranasal sinuses is the precise determination of the site of material collection, which is important for the representativeness and diagnostic value of the results [117]. The fact that 26.8% of the analyzed publications do not provide detailed information on the location of sampling indicates potential methodological heterogeneity that may affect the interpretation of the sinus microbiome analysis results.

The most common site for the collection of specimens for microbiological examination in the analyzed material was the middle nasal meatus, as reported in about 40% of the reviewed papers. The location is easily accessible to simple instruments, and the composition of its microbiota is comparable to that of the material obtained from paranasal sinuses [31]. Ramakrishnan et al. [31] compared the relative abundance of microorganisms in swabs collected from under the middle turbinate, ethmoid cells, as well as maxillary, frontal, and sphenoid sinuses to observe no statistically significant differences between these sites. Smears collected from under the middle turbinate presented with a higher relative abundance of *Corynebacterium* as compared to the maxillary, frontal, and sphenoid sinuses; at the same time, said relative abundance was lower than that in the anterior nares [31]. *Pseudomonodales* and *Stenotrophomonas* bacteria were more abundant in the deeper sinuses than in the middle nasal meatus [31]. These significant similarities in the mucosal colonization of the different sinuses are explained by the flooding theory, in which, in the course of an infection with a runny nose, bacteria are transferred to different parts of the nasal cavity and sinuses [32, 118].

The most common sites chosen for the collection of materials in the course of surgical procedures included the ethmoid cells (20%) and the maxillary sinus (about 18%). While the former location is convenient for the collection of material due to the swab insertion angle of 30–48° relative to the nasal axis in the sagittal plane [117], it requires a small surgical intervention, namely the removal of the uncinate process, being performed beforehand. An angle of 0–30° is required for the collection of material from the maxillary sinus. The third and fourth most common sampling locations were the nasopharynx and the inferior nasal turbinate (5% each). The nasopharynx was the most frequently chosen collection site in pediatric patients with pharyngeal tonsillar hypertrophy or chronic sinusitis [119]. The microbiota of the inferior nasal trubinate was examined, among other things, in the screening for the presence of fungi within the nasal cavity [120] in the assessments of the effects of medications [121].

**Tab. II.** Methods pursued to collect the material for microbiome testing. The columns show the different material collection techniques along with their absolute and relative frequencies.

| COLLECTION TECHNIQUE | NUMBER OF OCCURRENCES IN THE ANALYZED DATA (ABSOLUTE FREQUENCY) | PERCENTAGE OCCURRENCE (%) (RELATIVE FREQUENCY) |
|---|---|---|
| Swab | 44 | 35.7 |
| Swab + biopsy | 37 | 30 |
| Mucus aspirate | 12 | 9.8 |
| Brush swab | 9 | 7.3 |
| Tissue biopsy | 9 | 7.3 |
| Mucus aspirate + swab | 4 | 3.3 |
| Nasal lavage | 3 | 2.4 |
| N/A | 5 | 4.1 |

About 56% of the material was collected using synthetic fiber swabs, as their specifications declare a lower risk of contamination compared to swabs made using natural fibers. Most commonly, the swabs were taken under endoscope guidance, which facilitated precise selection of the collection site and minimized contamination from other regions of the nasal cavity [111]. In 1/6 of rticles, the samples were frozen immediately after collection to ensure the integrity of the DNA and RNA of microorganisms [7]. In more than 80% of cases, the material was put on ice in the first moments after collection and then transported to the storage area. This treatment was also effective in preserving the structure of the genetic material. In about 20% of cases, alternative methods were used, such as the storage in RNAlater solution to stabilize the genetic material at room temperature [41] or in RNA lysis buffers, directly preparing the material for sequencing. The impact of the type of swabs used to collect material from the nasal cavity and paranasal sinuses, as well as the impact of the storage method, have not been studied in more detail to date.

## DNA extraction

The choice of the method for extracting the genetic material from samples can affect the outcome of sequencing [122]. The bacterial lysis methods used in the studies present with different efficiencies depending on the structure of the cell wall. Enzymatic methods (e.g., the use of trypsin in the extraction of *Bacteroidetes*) are more effective for relatively thin bacterial walls with liposaccharide-containing membranes [122]. They are more effective in the case of materials rich in human eukaryotic DNA, which can effectively contaminate the test sample and hinder the analysis of the bacterial microbiome [122]. Mechanical lysis methods are more effective for microorganisms with thick bacterial walls (e.g., *Firmicutes*) [122]. A variety of methods had been used in the reviewed studies, including physical, chemical, and enzymatic methods. Mechanical homogenization (bead-beating) was the most common physical method, used in about 60% of cases. Enzymatic methods, such as proteinase K lysis, were used in 30% of the studies, especially in cases where a more delicate approach to the test material was required. Phenol-chloroform extraction was used less frequently (10% of cases) due to its labor-intensive nature and the need to use toxic chemicals.

### Sequencing

In the studies reviewed, the Illumina MiSeq (70.37%), pyrosequencing (12.96%), and Ion Torrent (5.56%) platforms were used most frequently for the sequencing of *16S rRNA* hypervariable fragments. Shotgun sequencing methods were used in single cases. In most cases, the sequencing was carried out on short V3 and V4 fragments of *16S rRNA* (about 1500 bp), which ensured the speed of this method [122]. Owing to its ubiquitous use in scientific papers, the approach facilitates the comparisons of multicenter data in meta-analyses. On the other hand, it has a relatively poor taxonomic resolution, making it difficult, for example, to determine the species composition of the microbiota [122]. Due to the different frequencies of the V3 and V4 genes in the bacterial genome, estimation of true abundance can also be difficult [122].

The shotgun method is based on the sequencing of the entire genetic material. As a result, a more accurate composition of the microbiome can be obtained, including not only the bacterial species, but also those belonging to other kingdoms. The limitations of this method include its high cost and differences in sequencing results depending on the differences in laboratory protocols on top of biological variability of microorganisms [122].

### Bioinformatic analysis

Bioinformatic analysis aims to process the genetic code obtained from the sequenced material so as to assign it to individual microorganisms. The analysis consists of several steps: data filtering and cleaning, clustering, and taxonomic classification based on reference databases. Both specialized single-function programs and integrated bioinformatics platforms facilitating complex multistep analyses are used to perform these tasks. In the reviewed material, a significant part of data processing was carried out using the QIIME platform, which reflected the frequency of the use of Illumina-based sequencing technologies frequently used together with the platform. USEARCH, Mothur, and DADA2 software tools are also compatible with data generated by platforms such as Illumina. However, it should be noted that the use of individual tools is not determined by the type of input files that are compatible with their analysis algorithms rather by the specific type of the sequencing platform.

The dominant frequency of use of the SILVA reference database (56.3%) in the reviewed publications is due to its high curation quality, comprehensiveness, and regular updates. Curation quality refers to the degree to which the data in a biological database has been verified, organized, updated, and correctly labeled by experts or bioinformatics algorithms. The SILVA database, developed by the Max Planck Institute for Marine Microbiology (Bremen, Germany), contains carefully curated ribosomal sequences (*16S/18S rRNA*) characterized by high concordance with phylogenetic taxonomy, which makes it the preferred reference source for microbiome studies. Other databases used in the study are less frequently updated (GreenGenes [Lawrence Berkeley National Laboratory, USA]), or are primarily available on a commercial basis (the ChunLab 16S rRNA database [ChunLab Inc., Seoul, South Korea], the ExTaxon database [ChunLab Inc., Seoul, South Korea]).

## CONCLUSIONS

In summary, molecular methods such as DNA sequencing, metagenomics, and PCR, combined with advanced bioinformatics and statistical tools, facilitate comprehensive analysis of the sinus microbiome. Despite the challenges, such as the complexity of bacterial biofilms and the interpretation of large data sets, these techniques have significantly advanced our knowledge of the role of the microbiota in healthy organisms as well as those burdened by other diseases. For the correct use of these methods, it is necessary to establish the correct methodology for the collection, storage, and analysis of biological material. A review of the subject literature is a key part of research planning, allowing the research strategy to be properly focused and avoiding repetition of past mistakes.

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Table of content: https://otorhinolaryngologypl.com/issue/17010 Tables: 2 Figures: 1 References: 122

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Competing interests: The authors declare that they have no competing interests.

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Cite this article as: Sokolowski J.: Molecular Methods for the Detection and Analysis of Sinonasal Microbiota: A Systematic Review; Pol Otorhino Rev 2025; 14 (4): 1–10; DOI: 10.5604/01.3001.0055.4600