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Reporting and Interpretation of Respiratory Metagenomic Results in Clinical Practice: A Systematic Review and Consensus Statement from the UK NHS Respiratory Metagenomics Network.

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Reporting and Interpretation of Respiratory Metagenomic Results in Clinical Practice: A Systematic Review and Consensus Statement from the UK NHS Respiratory Metagenomics Network.

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Abstract (241/250)

Background: Metagenomic next-generation sequencing (mNGS) offers culture-independent pathogen detection and is increasingly being adopted in clinical practice. However, approaches to reporting and interpreting metagenomic results remain poorly standardised. No previous society or professional guidelines exist for reporting and interpretation of metagenomic data. We aimed to synthesise current practice and evidence, identify gaps in knowledge, and to develop national consensus statement for clinical reporting and interpretation of respiratory metagenomics.

Methods: We undertook a systematic review of studies using metagenomic sequencing for clinical reporting and interpretation of human respiratory samples following PRISMA guidelines. The findings were informed by two workshops and a three-round Delphi process involving clinicians (physicians, microbiologists, virologists and clinical scientists) within the UK National Health System (NHS) Respiratory Metagenomics Network.

Results: For the systematic review, 53 studies including 6,000 participants met inclusion criteria but showed high heterogeneity in reporting and interpretation practices with a high risk of bias. Nineteen panellists from six NHS sites participated in the workshops and Delphi process with 27 statements reaching consensus. The guidelines provide principles for setting detection thresholds, using negative controls, reporting of detections, and interpretation of findings. A decision algorithm for reporting respiratory metagenomic results is presented. Knowledge gaps and priorities for future research were identified.

Conclusions: Use of respiratory metagenomic tests in practice is heterogeneous and often at risk of bias or subjective interpretation. Our consensus statement supports transparent, standardised interpretation and reporting of respiratory metagenomic results, and highlights priorities for future research and validation studies.

Background

Respiratory infections are a leading cause of hospital and ICU admission, yet conventional microbiology often misses the causative pathogen or turnaround times are too long to guide treatment [1]. Metagenomic next-generation sequencing (mNGS) has emerged as an agnostic, culture-independent assay that can detect bacteria, viruses, fungi and parasites in a single test [2]. Over the last decade, multiple studies have demonstrated the analytical performance of respiratory metagenomics, including improved pathogen detection over culture and identification of atypical or mixed infections [3–6]. Clinical services are now incorporating respiratory metagenomics into routine decision making [6].

However, implementation has outpaced interpretive frameworks. In contrast to culture-based microbiology, where long-standing guidance exists on differentiating colonisation from infection, applying quantitative thresholds, and handling mixed growth [7,8], there is no agreed protocol on how metagenomic outputs should be interpreted and reported to clinicians. For laboratories, it creates uncertainty about which organisms to report and how to synthesise outputs in a way that is clinically useful. For clinicians, it complicates the integration of metagenomic results with existing diagnostic pathways and may undermine trust in the technology. Inconsistent reporting rules and thresholds also make it difficult to compare studies or aggregate data. There is no accepted guidance on how to move from sequence data to clinically actionable reports.

Against this backdrop, there is a clear need to synthesise current practice and evidence, and to develop consensus-based principles that can support more standardised, reproducible interpretation and reporting of respiratory metagenomic results. We combined a systematic review of respiratory metagenomic diagnostics with virtual workshops and a Delphi consensus process in the United Kingdom (UK) National Health Service (NHS) Respiratory Metagenomics Network (https://metagenomics.nhs.uk). Together, these aimed to describe current use of metagenomics in hospital settings, and agree practical principles for reporting and interpreting metagenomic results from respiratory samples in clinical practice. The resulting consensus statement, endorsed by the UK NHS Respiratory Metagenomics Network, are intended to support laboratories and clinicians in the UK and other regions in implementing respiratory metagenomics and to highlight priorities for research where evidence remains insufficient to make specific recommendations.

Methods

The UK NHS Respiratory Metagenomics Network (https://metagenomics.nhs.uk) is a national programme to implement clinical metagenomics in public hospitals. To standardise practice across the UK, formation of guidelines adopted the following process. Briefly, we conducted a systematic evidence review to gather and appraise relevant literature on respiratory metagenomic diagnostic reporting and interpretation following PRISMA [9] and assessing bias using QUADAS-2 for diagnostic studies [10]. Next a Delphi consensus process combined with virtual workshops was undertaken following the CREDES process [11], to synthesize expert opinion, identify areas of uncertainty, and reach agreement on reporting standards and interpretation. The study was registered with the Open Science Framework (https://osf.io/2f4zt) and rated using the AGREE II instrument by the Network programme director (RB, Appendix). Full methods detailing systematic review and Delphi consensus processes are available in Supplementary Methods.

To inform the guidelines, we determined the following foreground questions after initial workshop, framed using a PICO structure and focusing on patients with suspected respiratory infection undergoing metagenomic testing in acute care settings:

- Reporting thresholds: In patients undergoing respiratory metagenomic testing, what predefined detection thresholds are used to decide which pathogen detections are reported as clinically significant?
- Use of negative controls and background microbiome: How are negative controls and background contamination data used in interpretation and reporting to reduce false positive calls and increase confidence in reported pathogens?
- Organisms that may represent colonisation: For organisms recognised to behave as both colonisers and pathogens in the respiratory tract (for example oral flora, anaerobes, *Pneumocystis jirovecii*, herpesviruses and *Tropheryma whipplei*), what structured reporting rules and standardised interpretation are employed for clinical reporting to help distinguish colonisation, contamination and probable infection?
- Reporting antimicrobial resistance determinants: How are metagenomic outputs used to report antimicrobial resistance?
- Report structure and validation workflows: Do studies use standardised reporting processes (for example pathogen lists, decision algorithms and standardised interpretative comments) and validation workflows to provide consistency in reporting and interpretation across laboratories?

Given the heterogeneity in technologies and reporting practices, we focused on a qualitative synthesis to identify common challenges, areas of agreement and disagreement, and recurring themes. The primary studies generally provided low quality, low certainty evidence, so a formal GRADE assessment was not undertaken. Instead, we used a structured Delphi process to generate consensus statements that combine the available evidence with expert opinion.

Results

Literature review of clinical metagenomics in respiratory samples

From 894 publications identified by database search, 53 studies involving over 6,000 participants fulfilled inclusion criteria and were taken forward to review (

Figure 1, Supplementary Table 1, Appendix). From the studies that reported sex distribution, 62% of participants were male. 27 studies explicitly reported immunocompromise status totalling 942 participants. Across the 53 studies, Illumina was used in 25 (47%), BGI Tech in 18 (34%), Oxford Nanopore in 4 (8%), and unspecified platforms in 6 studies (11%).

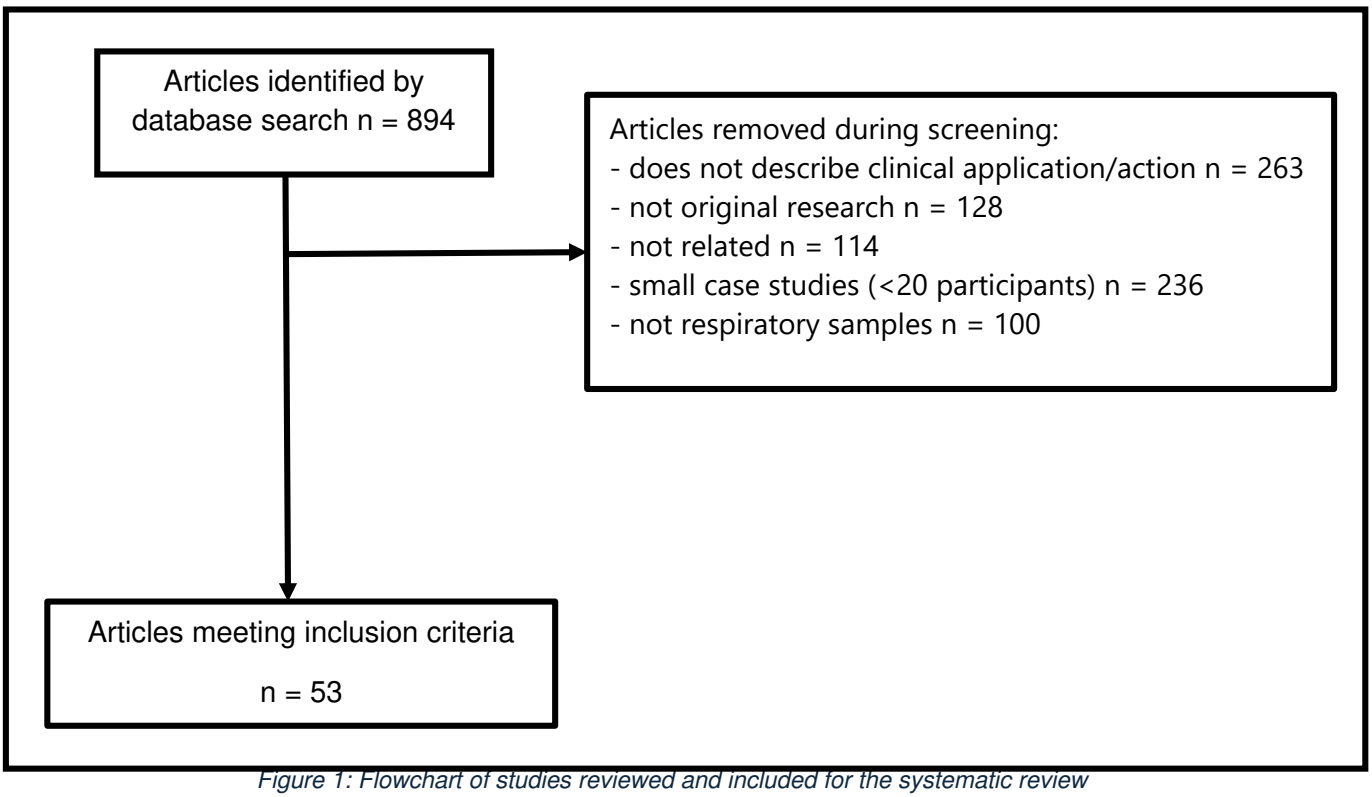


Figure 1: Flowchart of studies reviewed and included for the systematic review

Risk of bias and applicability

Fifty-three studies were assessed using the QUADAS-2 tool (Supplementary Results, Appendix). Overall, the risk of bias was substantial. The literature is heavily limited by non-

consecutive or poorly described patient selection, heterogeneous and often weak reference standards, and frequent lack of blinding, or fully pre-specified thresholds.

Delphi process

For the Delphi rounds, statements with aggregate results including percentage agreement, median Likert and IQR available in Supplementary Table 2 with participant's comments available in the Appendix. In the first Delphi round, 19 participants rated, with twenty statements (20/27, 74%) accepted (Table 1). The remaining seven statements were reviewed, revised and proceeded to round two of the Delphi, with six (6/7, 86%) being accepted. In the third round, one statement was voted on and accepted.

Detection and reporting thresholds for pathogens of different kingdoms.

Evidence from the review

Metagenomic workflows apply thresholds to metagenomic outputs to call detections of organisms. Many metagenomic workflows call detections when a taxon exceeds a set proportion of microbial reads. Some authors define a detection when a genus represents ≥30% of microbial read [12–19] whilst the thresholds used by others is lower at 1% [20]. Higher thresholds improve specificity but risk missing minority pathogens [14,18], and optimal values remain uncertain. One group derived thresholds by ROC analysis against culture [20]. Others use rank-based rules, for example reporting only the top ten organisms [21,22]. Dominance rules may also be employed, such as requiring a target to exceed the next organism by ≥5–10x coverage [13,23,24],

These strategies are straightforward, but sensitive to background signal and may miss legitimate low-level pathogens or pathogens in polymicrobial samples, for instance when commensals or contaminants dominate [13,15,18,25]. Z-score methods, which compare the abundance of each organism to background distributions attempt to normalise for this background signal [26], but these also vary between laboratories which limits comparability.

Absolute read-count thresholds [5,12–14,16,18,19,25,27–30] are intuitive but influenced by sequencing depth, contamination, and flow cell yield. Adding requirements for non-overlapping or uniquely mapped reads likely improves specificity [16,21,30–32]. These composite rules reduce spurious assignments but may under-detect low-biomass fungi or RNA viruses, which are often assigned lower thresholds [6]. Organism-specific exceptions allow detections from fewer reads for obligate pathogens such as *Mycobacterium tuberculosis* or *Legionella sp.* [5,14–16,29,30,33,34], maximising sensitivity but risking false positives from misclassification or environmental DNA.

Several studies use predefined pathogen lists or explicitly exclude commensal organisms [3,15,20,24,35,36], which improve interpretability but are anatomical site and specimen specific. This strategy also risks omission of atypical or zoonotic pathogens and

miscategorization of commensal organisms that can also act as pathogens in the respiratory tract [4,24].

Conversely, a minority use clinician adjudication, integrating imaging and conventional results rather than fixed cut-offs [37–39]. This can improve clinical interpretability but reduces reproducibility and comparability across centres. Routine adjudication of all detections also limits scalability. Where detections are uncertain, orthogonal testing can confirm them [20].

Overall, detection criteria remain inconsistent and often organism-specific.

Consensus statements

The working group considered the above and felt defining principles for setting detection thresholds was important, though noting there was insufficient evidence to recommend specific strategies. The following statements were accepted:

- Thresholds for reporting detections of micro-organisms should be based on evidence from validation studies against standard-of-care diagnostics.
- Obligate pathogens (e.g. *Mycobacterium tuberculosis*) should be reported whenever detected, regardless of abundance.
- Detections of organisms on a pre-defined respiratory pathogen list that meet reporting thresholds should be reported directly, whereas detections of other pathogens should be assessed further at clinical validation.
- Noting that thresholds may differ for obligate and opportunistic pathogens, detections of organisms below the pre-specified thresholds should not be reported.
- Reporting decisions may rely on expert clinical adjudication rather than fixed numeric thresholds, particularly where quantitative validation is unfeasible (e.g. rare organisms).

Implications & research gaps

There is a clinical need to establish validated clinical thresholds against standard of care diagnostics for different sample types and microbial kingdoms using ROC analyses. Given organisms have different genome sizes and are likely to be differentially processed by metagenomic workflows, organism specific thresholds should also be validated, which requires large and likely multi-site datasets. Organism specific limits of detection, whilst known for some organisms for current workflows, should also be established to understand the differential outputs for different organisms. The effect on antibiotic exposure on abundance of organism should also be established to prevent causative organisms being missed when sampling occurs after treatment has been initiated.

Detection and reporting thresholds for pathogens of different kingdoms.
Thresholds for reporting detections of micro-organisms should be based on evidence from validation studies against standard-of-care diagnostics.
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Reporting decisions may rely on expert clinical adjudication rather than fixed numeric thresholds, particularly where quantitative validation is unfeasible (e.g. rare organisms).
Detections of organisms on a pre-defined respiratory pathogen list that meet reporting thresholds should be reported directly, whereas detections of other pathogens should be assessed further at clinical validation.
Negative control handling
Organisms present in negative controls and patient samples at comparable levels should not be reported.
Reporting potential pathogens amongst oral flora
Reports may summarise mixed oral flora collectively rather than listing each species.
Detections of potential pathogens (e.g. *Streptoccus pneumoniae*, *Haemophilus influenzae*, *Streptococcus dysgalactiae*) above threshold but amongst oral flora should be available to the validating clinician.
The clinician validating results should consider specimen type, relative abundance, and clinical context before deciding whether to report potential pathogens amongst oral flora.
Reporting of Candida in respiratory samples.
Candida sp. detected in respiratory samples should be reported when found above threshold.
Detections of Streptococcus pyogenes
Streptococcus pyogenes should always be reported when detected in respiratory samples.
Anaerobic bacteria
Where a mixture of anaerobes with non-anaerobic commensal bacteria is present, this could be reported as “oral flora including anaerobes.”
When only anaerobes are detected, “Anaerobes detected” may be reported.
Species of anaerobes detected in pleural fluids should be named at the genus or species level.
When anaerobes are predominant amongst oral flora, they may be reported as oral flora with predominant anaerobes.
Pneumocystis jirovecii
Detections of *Pneumocystis jirovecii* above threshold should be reported in lower respiratory-tract samples.
Reporting of *Pneumocystis jirovecii* should be accompanied by an interpretative comment noting potential colonisation.
Detections of *Pneumocystis jirovecii* in upper respiratory tract samples should be assessed at clinical validation and may be reported if considered potentially clinically significant.
Human herpesviruses
Detections of herpesviruses should be assessed by the clinician validating results.

Detections of herpesviruses should be interpreted with consideration of the patient's immune status and clinical context.
Special populations (e.g. allogeneic haemopoietic stem cell transplant recipients and neonates) may require different reporting rules for herpesviruses compared to the general ICU population.
Where clinical significance is suspected, the diagnosis of herpesvirus disease should always be confirmed with additional testing (e.g. viral load, serology, cytopathology).
Where herpesviruses are reported, an interpretative comment should be added (e.g. "Detection of herpesviruses is common in critically unwell patients and likely reflects reactivation; please correlate clinically").
Herpesviruses can be suppressed and not released to clinicians outside the infection team if judged to be non-significant at clinical validation
Tropheryma whipplei
Tropheryma whipplei should be assessed at clinical validation and if judged to be clinically insignificant, it may be suppressed or considered part of oral flora.
Prediction of antimicrobial resistance
Genotypic predictions of antimicrobial resistance may be reported if they have been formally validated.

234 *Table 1: Consensus statements for the reporting and interpretation of respiratory metagenomic results.*

Negative control handling

Evidence from the review

Negative controls are essential to judge contamination to prevent these organisms from being reported. Several groups exclude sequences originating from organisms commonly encountered as laboratory contaminants. For example, some groups pre-define specific organisms as contaminants [22,35] such as *Xanthomonas campestris, Cutibacterium acnes*, *Mycoplasma wenyonii*, and *Acinetobacter johnsonii* that are consistently detected in negative controls. Suppressing such organisms is straightforward when they are not pathogens, but becomes problematic when they overlap with clinically relevant species. One group highlighted this challenge [40], explicitly removing *Escherichia coli* from all reporting because it was also present in the negative control.

Other pipelines compare samples against negative controls, reporting positives only when reads or coverage are ≥5–10x higher than in the negative control [21,25,29,30,32,41,42]; this enhances robustness but depends on negative control quality and subject to batch variation.

Some pipelines maintained internal 'background databases' to filter contamination. However the background was variously defined, for instance if a taxa is present in 10% [43], ≥25% [26] or over half [5] of samples. One group [42] collected environmental swabs to define a lab-specific background list, though this will not identify the contaminants present in reagents.

Consensus statements

The working group recognised the importance in assessing detections in negative controls to identify contamination and therefore prevent reporting spurious detections. It was felt there was insufficient evidence to suggest which strategy was optimum for handling negative control data. Notably the working group did not agree on the notion of pre-defining exclusion lists of organisms (e.g. commensals or contaminants), as in some circumstances many may act as opportunistic pathogens, especially in the immunosuppressed. The following statement was agreed:

- Organisms present in negative controls and patient samples at comparable levels should not be reported.

Reporting potential pathogens amongst oral flora

Evidence from the review

Streptococcus pneumoniae, *Haemophilus influenzae* and *Staphylococcus aureus* are often present in respiratory flora yet remain important causes of pneumonia. Guidelines for lower respiratory tract specimens address this by recommending reporting organisms by specimen type and quantity of growth, accounting for pure or predominant growth [7]. They also advise assessing epithelial cells as markers of contamination and neutrophils as markers of inflammation to aid interpretation [7,44].

By contrast, interpretation of metagenomic data is more limited. Sequencing does not provide epithelial cell or neutrophil counts, and evidence is insufficient that metagenomic profiles mirror culture-based quantification or composition. Many studies therefore require clinician review, integrating specimen type and detections with clinical context [15,33,42,45]. Some studies limited reporting to predefined organisms to avoid naming oral flora [15,20,34], but this may prevent reporting taxa that can act as both commensals and pathogens. Dominance criteria, reporting these pathogens only when highly abundant relative to other oral flora, may mitigate this [18], though whether metagenomic outputs correlate with quantitative growth is yet to be established.

Consensus statements

The following statements were agreed:

- Reports may summarise mixed oral flora collectively rather than listing each species.
- Detections of potential pathogens (e.g. *S. pneumoniae*, *H. influenzae*, *S. dysgalactiae*) above threshold but amongst oral flora should be available to the validating clinician.
- The clinician validating results should consider specimen type, relative abundance, and clinical context before deciding whether to report potential pathogens amongst oral flora.

Implications & research gaps

Given the importance of determining the relative abundance of potential pathogens, further work is required to establish whether the relative abundance described in metagenomic outputs accurately reflects microbial communities in samples. Furthermore, correlating read counts with quantitative growth in agar culture may add further resolution to decision making, based on established microbiological practice of assigning significance based on quantification. At the very least, consensus on what abundance thresholds should be used to describe dominance should be agreed.

Reporting of Candida in respiratory samples.

Evidence from the review

Candida spp. are frequently detected in respiratory samples but are usually interpreted as airway colonizers rather than causes of pneumonia [20,26,46]. *Candida spp.* is often reported but categorised as a non-significant organism in metagenomic reports, supporting antifungal de-escalation [20,36]. Of the reviewed literature some suppressed *Candida* spp. in respiratory samples [26]. Detections are similarly not taken to indicate invasive disease [15,31]. Most studies only consider *Candida* spp. alongside clinical, radiologic, and other microbiological and pathological features when considering invasive disease [17,19,23,31,46]. This is consistent with EORTC/MSGERC criteria [47]; proven invasive candidiasis hinges on detection in the bloodstream or normally sterile sites (e.g., candidemia or sterile-site histopathology).

Consensus statements

The working group held similar views, accepting the following statement:

- *Candida* sp. detected in respiratory samples should be reported when found above threshold.

Implications & research gaps

Accepting that *Candida* sp. should be reported is consistent with practice across UK microbiology labs. However, whether reporting in metagenomics leads to increased treatment with antifungals should be assessed. The use of interpretative comments could aid stewardship.

Detections of Streptococcus pyogenes

Evidence from the review

Streptococcus pyogenes (Group A *Streptococcus*) is a recognised cause of pneumonia [48] though carrier status is noted [49]. Most diagnostic laboratories report any isolation from throat swabs, though also note that detection alone does not confirm infection [49,50]. It was only reported in a few metagenomic studies in lower respiratory tract and the studies treated this organism as a pathogen when detected [20,32,38].

Consensus statements

The following statement was agreed:

- *Streptococcus pyogenes* should always be reported when detected in respiratory samples.

Anaerobic bacteria

Evidence from the review

Anaerobes are normal inhabitants of the upper airway and oropharynx but can cause disease in aspiration pneumonia, lung abscesses, and empyema. Certain species, such as *Fusobacterium necrophorum*, are linked to specific clinical syndromes. Both BTS and ATS guidelines include empirical regimens for severe community acquired pneumonia for which the spectrum does not include anaerobic cover [51,52], unless there is suspicion of a specific condition where anaerobes are implicated. Thus, findings of anaerobes may support clinical decision-making, though their significance depends on both the clinical scenario (e.g., suspicion of aspiration or abscess) and specimen quality.

Notably, studies report finding anaerobes more frequently in metagenomic testing compared to conventional methods [35,43]. In the metagenomic studies some considered anaerobes

as commensals in the respiratory tract [15,35,41]. Others report anaerobes but note the difficulty in distinguishing them as colonizers or pathogens [12]. One study which reported anaerobes felt that they were clinically relevant in a number of cases [20]. Overall, the balance of these studies is to consider anaerobes as commensals unless there is strong supporting clinical evidence to treat them as true lower respiratory tract pathogens.

In contrast, the pleural space is normally sterile; any anaerobic growth should be considered significant and may indicate empyema [53]. Species-level identification can be useful for guiding therapy due to differences in antimicrobial susceptibility [54].

Consensus statements

The following statements regarding anaerobes were agreed:

- Where a mixture of anaerobes with non-anaerobic commensal bacteria is present, this could be reported as “oral flora including anaerobes.”
- When only anaerobes are detected, “Anaerobes detected” may be reported.
- When anaerobes are predominant amongst oral flora, they may be reported as oral flora with predominant anaerobes.
- Species of anaerobes detected in pleural fluids should be named at the genus or species level.

Implications & research gaps

The role of anaerobes in pneumonia remains controversial, and it is unclear whether targeted antimicrobial therapy with anaerobic coverage provides additional benefit. Future studies could assess this, for instance comparing use of cephalosporins without anaerobic spectra with beta-lactam inhibitor combinations. Alternatively consensus opinions should be sought on how detections should affect treatment in different clinical contexts and different sample types. Given the increasing, poorly understood, and varied resistance patterns in anaerobes [54], improved genotypic resistance predictions could also impact treatment.

Pneumocystis jirovecii

Evidence from the review

Pneumocystis jirovecii is a non-culturable pathogen detected by PCR, immunofluorescence, or special staining. Colonization is common, particularly in children, and carriage without clinical or radiographic evidence of pneumonia is well documented [55].

The UK SMI recommends testing only in immunocompromised patients, with BAL and induced sputum the preferred specimens due to higher sensitivity [7]. From the included studies, three explicitly investigated *Pneumocystis,* though many more reported detections of this organism [5,18,26,32–34,43,46,56–60]. One explicitly discussed the common finding of colonization in children [23]. Some note the importance of clinical correlation to adjudicate significance [4]. In one study antimicrobials were changed in half of *Pneumocystis* detections [61], whereas another altered antimicrobial treatment after almost every detection in a

paediatric cohort [25]. One study implicated other species of this genus with significant infection, namely *Pneumocystis yezoensis* [62].

While semi-quantitative methods may suggest organism burden and help differentiate colonization from infection [63], most routine diagnostic methods do not currently provide reliable quantification.

Consensus statements

The following statements were agreed:

- Detections of *Pneumocystis jirovecii* above threshold should be reported in lower respiratory-tract samples.
- Reporting of *Pneumocystis jirovecii* should be accompanied by an interpretative comment noting potential colonisation.
- Detections of *P. jirovecii* in upper respiratory tract samples should be assessed at clinical validation and may be reported if considered potentially clinically significant.

Implications & research gaps

Future avenues for research include defining precisely the performance of different sample types for detection of *Pneumocystis jirovecii* with metagenomics. Decision support tools may also be useful for determining whether a detection is likely to be clinically significant, given the heterogeneity of populations and the increasing use of immunomodulation. Correlation between metagenomics and current semi-quantitative methods may help inform decisions on significance.

Human herpesviruses

Evidence from the review

Human herpesviruses (HHVs) establish latency and may reactivate, causing intermittent detection in respiratory samples. Differentiating asymptomatic shedding from disease is often difficult [22,64]. Interpretation of HHV detections require close clinical context, with greatest weight in immunocompromised or neonatal patients [13,16,17,25,38,46,61,64] and may be interpreted alongside blood viral load, cytology, immunohistochemistry and imaging [20,28,38,62,64].

In high risk services such as transplant, CMV is the main clinically actionable herpesvirus and demands close clinical and radiological correlation [25,26,35,43]. Other HHVs also cause respiratory disease, for example HSV-1 pneumonitis in transplant recipients, which may lead to treatment when detected by respiratory metagenomics [39,43,61,65]. Most often though, HSV-1 was judged to represent reactivation [28,32,61]. HSV-2, reported rarely from the respiratory tract, may indicate disseminated disease in neonates or immunosuppressed host [20]. Varicella pneumonia occurs in immunocompetent adults and during pregnancy, with respiratory detection outside primary infection infrequently reported [38].

The relevance of other herpesviruses, namely EBV, HHV-6 and HHV-7, is unclear. They are reported in respiratory metagenomic studies, often in haematology patients [32,38,46,64]. HHV-6 is commonly detected in oropharyngeal secretions due to periodic reactivation [38,64]; pneumonia is rare and limited to case reports in severely immunocompromised individuals. Less is known about KSHV in respiratory samples; the virus is typically associated with haematological malignancy in advanced HIV or other immunosuppressed states.

Consensus statements

In most cases, detection of HHVs in respiratory specimens should be interpreted with caution and in close clinical context, with greatest significance in immunocompromised or neonatal patients. The following statements were agreed:

- Detections of herpesviruses should be assessed by the clinician validating results.
- Detections of herpesviruses should be interpreted with consideration of the patient's immune status and clinical context.
- Special populations (e.g. allogeneic HSCT recipients and neonates) may require different reporting rules for herpesviruses compared to the general ICU population.
- Where clinical significance is suspected, the diagnosis of herpesvirus disease should always be confirmed with additional testing (e.g. viral load, serology, cytopathology).
- Where herpesviruses are reported, an interpretative comment should be added (e.g. "Detection of herpesviruses is common in critically unwell patients and likely reflects reactivation; please correlate clinically").
- Herpesviruses can be suppressed and not released to clinicians outside the infection team if judged to be non-significant at clinical validation.

Implications & research gaps

Future avenues of research should include correlating metagenomic outputs such as read counts and relative abundance with quantitative viral loads in both the respiratory compartment and the blood. Distinguishing pathology from bystander reactivation also remains challenging, and the role of cytopathology in determining direct pathological effects could be considered. With more frequent detection the question of whether treatment of certain human herpesviruses like HSV-1 and CMV is clinically indicated in ICU populations is likely to re-emerge. Whether detection of herpesviruses in metagenomic outputs leads to increased use of antivirals should also be assessed. The association of detection of herpesviruses in metagenomic tests with prognosis, even when judged to be reactivation, could also be studied.

Tropheryma whipplei

Evidence from the review

Tropheryma whipplei is the causative agent of Whipple's disease, a rare systemic disorder affecting the intestine, joints, and central nervous system. While classically associated with the gastrointestinal tract, the organism has also been detected in blood and other body sites

in a small percentage of the population [66]. Epidemiological studies have associated this agent with respiratory tract infection [67] or bacteremia with fever and cough [68]. In respiratory metagenomic studies, there are sporadic reports describing *T. whipplei* in BAL or other respiratory samples. In two studies it was reported as the sole organism detected in 5 patients, however only 2 patients had clear evidence of respiratory infection [20,27]. One other study reports detection of *T. whipplei* without comment on significance [32].

Consensus statements

Current understanding therefore suggests detections represent carriage or colonization, but the role of *Tropheryma whipplei* in causing respiratory disease is still uncertain. The following statement was agreed:

- *Tropheryma whipplei* should be assessed at clinical validation and if judged to be clinically insignificant, it may be suppressed or considered part of oral flora.

Implications & research gaps

Future avenues for research include studies to establish the role of *Tropheryma whipplei* in lower respiratory tract infection. For instance, case–control cohort studies that compare *Tropheryma whipplei* detection in pneumonia cases versus non-infectious controls are needed to clarify causality. Pairing such studies with analysis of co-localized inflammation or immune activation, as judged by traditional histopathological methods or newer transcriptomic measures, would advance understanding of its potential to cause disease.

Prediction of antimicrobial resistance

Evidence from the review

Studies that utilise metagenomic data for antimicrobial resistance prediction focus on proven high confidence genotype–phenotype correlations. Our practice is to predict MRSA with *mecA*-mediated methicillin resistance in *S. aureus*, resistance to penicillin-inhibitor combinations in Enterobacterales with ESBLs (e.g., CTX-M, SHV), and vancomycin resistance in *Enterococcus* sp. with *vanA* [20]. In contrast, several other groups did not report explicit genotype–phenotype resistance predicitons [13,15,18,19,24,31,38,42,56,69], instead deferring wider susceptibility assessment to organism identification plus conventional antimicrobial testing [15,31]. One study analysed antimicrobial resistance genes retrospectively against conventional antimicrobial susceptibility testing but without reporting concordance [33]. Essentially, findings of AMR elements are treated as actionable when the gene–phenotype link is strong [20] and explicitly de-emphasized for drug-bug combinations which are multifactorial or inconsistently expressed (e.g., *Pseudomonas*, *Acinetobacter*) [15,43].

Consensus statements

After considering the limited data the following statement was accepted:

- Genotypic predictions of antimicrobial resistance may be reported if they have been formally validated.

Implications & research gaps

Priorities for future research were set as validating further genotypic antimicrobial resistance predictions for extended drug-bug combinations. This should include not just simple concordance between genetic element detection and phenotypic resistance but other methods for inference of antimicrobial resistance inference, such as genomic neighbour typing [70] and machine learning approaches [71]. Similarly, standards for the genome completeness required for each drug-bug prediction are also needed; establishing robust confidence scores for such predictions is also necessary to aid interpretation and prevent major and very major errors. Strategies to confidently assign detected resistance elements to specific bacterial detections would also be welcome, for instance to predict *mecA* conferring resistance to methicillin in *S. aureus r*ather than in co-localised coagulase-negative *Staphylococcus.*

Distinguishing colonization from infection

Evidence from the review

A common theme identified the need for information to aid interpretation and distinguish colonization, contamination and *bona fide* infection [5,18,72]. Many studies attempt to classify organisms into these categories, mostly using pre-defined lists which specify certain organisms as colonizers [15,20,24,33,41,43,62,64]. Some define commensal lists empirically based on what is usually found in samples, so called ‘background’ databases [28]. Most rely on clinical adjudication rather than data from the metagenomic output [12,13,16,35,37,38,42,69]. This is a perennial problem in microbiology, and currently some laboratories utilise microscopy to identify polymorphonuclear leucocytes as a measure of infection and epithelial cells to indicate contamination [7].

Implications & research gaps

Whether microscopy also aids metagenomic interpretation should be assessed. Incorporating other methods of judging whether an immune response against pathogens is present, for instance immune profiling or transcriptomics.

Decision algorithm for reporting of metagenomic results

Evidence from the review

For many studies the first test for reporting is whether an organism meets the predefined detection thresholds [5,12–14,16,18,19,25,27–30]. As discussed in previous sections, some studies use predefined lists of pathogens [20], commensals or contaminants [4,12,36] to aid reporting decisions. However, not all studies report using internal or external controls [17], or explain how these are handled.

Consensus statements

The working group formalised a standard approach for considering quality controls and reprting samples aiming to harmonize processes across sites (Figure 2), based on the findings of this review and the consensus statements agreed.

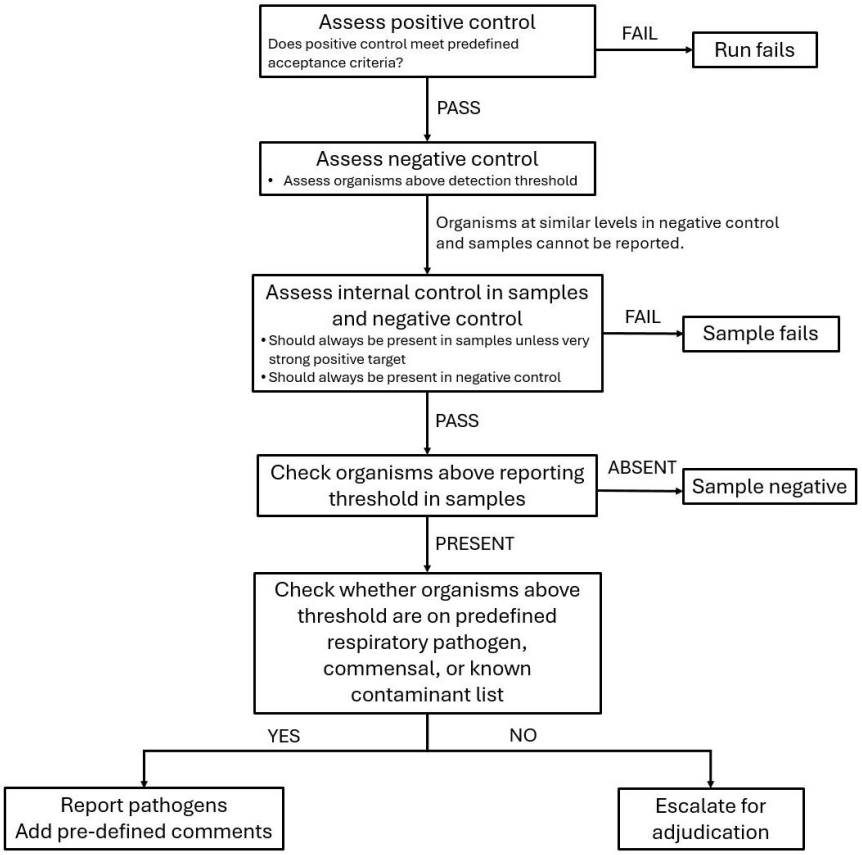


Figure 2: Decision algorithm for quality control and reporting of respiratory metagenomic results

Discussion

We combined a systematic review of respiratory metagenomic diagnostics with a Delphi consensus process to develop practical consensus statement for interpreting and reporting metagenomic results. We found wide variation in thresholds, treatment of oral flora and contaminants, and reporting rules, with metagenomics used mainly for pathogen detection rather than characterisation or genotypic resistance prediction. The Delphi panel built on these findings to agree reporting principles, endorsed by the UK NHS Respiratory Metagenomics Network, and highlighting the need for structured, reproducible interpretation.

A central message is that thresholds cannot be treated as simple technical cut offs. Multiple approaches are in use, with different trade-offs between sensitivity and specificity and variable performance across microbial kingdoms. The panel did not endorse a single numerical framework but agreed that thresholds should, wherever possible, be validated against standard of care diagnostics, that obligate pathogens should be reported irrespective of abundance, that likely contaminants must be accounted for, and that detections below prespecified thresholds should usually be suppressed. These principles allow harmonisation while permitting local optimisation of practices.

Both the literature and consensus emphasised the continuing need for expert clinical adjudication. Many studies integrated metagenomic outputs with radiology, conventional microbiology and clinical assessment rather than relying solely on rigid rules for metagenomic reporting. The panel agreed that reporting may rely on adjudication where robust quantitative thresholds are not available, particularly for rare pathogens or emerging syndromes. Our guidance therefore seeks to balance standardisation and flexibility by encouraging validated threshold frameworks while recognising situations where nuanced interpretation is essential.

Reconciling metagenomic outputs with culture-based frameworks for flora and colonisation remains challenging, especially for organisms that can act as both commensals and pathogens, such as oral flora, anaerobes and herpesviruses. The specific organism recommendations aim to integrate metagenomic findings into existing interpretive paradigms and favour clinically contextualised reporting over exhaustive disclosure of all taxa detected. *Pneumocystis jirovecii* exemplifies this approach, with above threshold detections in lower respiratory tract samples reported with an interpretative comment and a more cautious strategy for upper respiratory tract samples.

Prediction of antimicrobial resistance from metagenomic data remains an area of development rather than routine practice. Most studies either restricted themselves to a small number of high confidence genotypic predictions or did not attempt formal prediction. The panel agreed that genotypic resistance calls should only be reported where formally validated, and our guidance supports targeted, conservative use of metagenomic resistance predictions to complement, rather than replace, phenotypic testing.

Key research priorities include prospective multicentre studies to define organism and specimen specific thresholds and to evaluate how these influence clinical behaviour,

antimicrobial use, and patient outcomes. Work is needed to relate metagenomic abundance measures to quantitative culture and sample composition. Systematic validation of resistance prediction with clear standards for genome completeness and confidence metrics will be crucial if metagenomics is to inform initial antibiotic selection more directly.

In summary, respiratory metagenomics continues to mature and the main challenge has shifted from detection to interpretation, reporting and clinical action. This Delphi process provides consensus-based guidelines to support more consistent, clinically relevant reporting, while ongoing research refines the evidence base for future iterations.

Abbreviations

AGREE II	Appraisal of Guidelines for Research and Evaluation II
AMR	Antimicrobial resistance
ATS	American Thoracic Society
BAL	Bronchoalveolar lavage
BGI	Beijing Genomics Institute
BTS	British Thoracic Society
CMV	Cytomegalovirus
CREDES	Conducting and REporting DElphi Studies
CTX M	CTX M family extended spectrum beta lactamase
DNA	Deoxyribonucleic acid
EBV	Epstein Barr virus
EORTC/MSGERC	European Organisation for Research and Treatment of Cancer / Mycoses Study Group Education and Research Consortium
ESBLs	Extended spectrum beta lactamases
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HHV	Human herpesvirus
HHVs	Human herpesviruses
HIV	Human immunodeficiency virus
HSCT	Haemopoietic stem cell transplant
HSV	Herpes simplex virus
ICU	Intensive care unit
IQR	Interquartile range
KSHV	Kaposi sarcoma associated herpesvirus (human herpesvirus 8)

mNGS	Metagenomic next generation sequencing
MRSA	Methicillin resistant *Staphylococcus aureus*
NHS	National Health Service
PCR	Polymerase chain reaction
PICO	Population, Intervention, Comparator, Outcome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta Analyses
QUADAS 2	Quality Assessment of Diagnostic Accuracy Studies 2
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
SHV	Sulfhydryl variable beta lactamase family (SHV type ESBLs)
SMI	Standards for Microbiology Investigations
UK	United Kingdom

Declarations

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Competing interest: Jonathan Edgeworth is an employee of Oxford Nanopore Technologies. AAM and RB have a patent pending for the mechanical human DNA depletion method (PCT/GB2023/051417). Some of the authors (LB/RB/AAM/GN/JE) are authors of studies captured in this systematic review.

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Authors' contributions: LBS conceived and designed the study, acquired, analysed and interpreted the data, and drafted the manuscript. AAM contributed to study design and to data acquisition, analysis and interpretation. RB, JB, JRB, JDE, VG, TG, PG, SG, RH, JH, CH, MI, ALS, SM, RM, CM, GN, NJN, PP, SR, KS, MV, and TGSW contributed to data acquisition, interpretation, and

critically revised the manuscript. All authors gave final approval of the version to be published and agree to be accountable for all aspects of the work. JE and RB were responsible for funding.

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