Polymerase-chain reaction/electrospray ionization-mass spectrometry for the detection of bacteria and fungi in bronchoalveolar lavage fluids: a prospective observational study

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Abstract

PLEX-ID uses polymerase chain reaction-electrospray ionization/mass spectrometry for rapid identification of infectious agents in clinical samples. We evaluated its concordance with our centre's standard methods (SM) for bacterial and fungal detection in bronchoalveolar lavage (BAL) fluid in a prospective observational cohort study. The primary outcome was concordance (%) between SM and PLEX-ID. Secondary outcomes included concordance when excluding commensal oral flora, detection of resistance genes, and PLEX-ID's potential impact on clinical management, as determined by two independent reviewers. Included were 101 specimens from 94 patients. BALs were performed primarily for suspected pneumonia (76/101, 75%) and lung transplant work-ups (12/101, 12%). Most specimens yielded at least one organism by either method (92/101, 91%). Among all microorganisms detected (n = 218), 83% and 17% were bacterial and fungal, respectively. Overall concordance between SM and PLEX-ID was 45% (45/101). Concordance increased to 66% (67/101) when discordance for commensal flora was excluded. PLEX-ID failed to detect 21% of all 183 SM-identified organisms, while SM did not identify 28% of the 191 PLEX-ID-identified organisms ($p < 0.001$). There was low concordance for *mecA* detection. Two infectious-disease specialists' analyses concluded that in most of the 31 discordant, non-commensal cases, PLEX-ID results would have had little or no impact on patient management; in eight cases, however, PLEX-ID would have led to 'wrong decision-making'. The tested version of PLEX-ID concurred weakly with standard methods in the detection of bacteria and fungi in BAL specimens, and is not likely to be useful as a standalone tool for microbiological diagnosis in suspected respiratory infections.

Keywords: Bacteria, bronchoalveolar lavage, concordance, discordance, fungi, molecular diagnostic technique, PLEX-ID, polymerase chain reaction-electrospray ionization/mass spectrometry

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Introduction

Definitive identification of pathogens causing lower respiratory tract infections (LRTIs) requires time and specialized laboratory personnel. In the days preceding microbiological diagnosis, patients often receive broad-spectrum antimicrobials that may nonetheless be ineffective. Molecular diagnostic tools such as polymerase chain reaction (PCR) have increased sensitivity and reduced turnaround time, but even multiplex PCR remains only partially broad-range, as it requires anticipation of specific pathogens for their detection [1].

Currently research-use-only (RUO), the Abbott PLEX-ID system is a platform for pathogen detection combining broad-range PCR with electrospray ionization-mass spectrometry (PCR/ESI-MS). Minute quantities of deoxyribonucleic acid (DNA) are extracted from clinical specimens and amplified via PCR [2]. Amplicons undergo ESI-MS, allowing

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for the determination of their base composition. Computerized triangulation employs an internal database containing listings of base compositions with linking orders for known microbes to determine pathogens' genotypic identity.

PLEX-ID remains experimental and requires validation in the clinic. A recent retrospective study comparing PLEX-ID with standard blood-culturing techniques in blood-borne bacterial and yeast infections yielded concordances of 98.7% and 96.6% at genus and species levels, respectively [3]. For pure yeast detection, Simner *et al.* [4] reported a concordance of 91.8% between traditional culture and PLEX-ID's broad fungal assay in formalin-fixed, paraffin-embedded tissue samples, some of which were more than a decade old. The PLEX-ID/flu assay [5] was tested against nasopharyngeal specimens positive by PCR for the influenza virus and concurred at 91.3% and 95.3% for influenza A and B, respectively [6].

Prospective studies across a wider variety of clinical specimens are lacking, however. In this proof-of-concept study, our objectives were to quantify PLEX-ID's concordance with our centre's standard methods (SM) for bacterial and fungal detection in the bronchoalveolar lavage (BAL) fluid of patients undergoing bronchoscopy, and qualitatively evaluate the clinical consequences of PLEX-ID results in discordant cases.

Materials and Methods

Study design, patients and samples

This prospective, single-centre observational cohort study included all consecutive BAL specimens from any in- or outpatients undergoing clinically indicated bronchoscopy and with at least 5 mL of fluid remaining after extraction for the SM, collected between 1 January and 1 September 2013 at the Geneva University Hospitals, an 1800-bed tertiary-care medical centre. No more than two samples from the same patient were included. Immediately after sterile extraction of BAL fluid for SM processing, samples were stored at −80°C for later batch-testing via PLEX-ID.

Ethics

The study protocol and related materials were approved by the University of Geneva's ethics committee (reference n° 12-265); the study was carried out in accordance with the Declaration of Helsinki, 6th revision. A waiver of informed consent was granted given the study's observational nature.

Outcomes

The primary outcome was concordance (%) between SM and PLEX-ID for bacteria and fungi at genus and species levels. Secondary outcomes included concordance (i) beyond non-commensal oral flora, as SM do not typically identify all oral flora at the species and/or genus level (see below), (ii) for genus identification, and (iii) for detection of resistance genes *mecA*, *vanA*, *vanB* and *KPC* (although the latter three genes have rarely been detected at our institution). Finally, (iv) PLEX-ID's potential impact on therapeutic decision-making for discordant non-commensal specimens was qualitatively assessed via a clinical analysis undertaken independently by two infectious-disease specialists.

Definitions

Immunosuppression. In accordance with the Centers for Disease Control and Prevention's definition [7], patients were considered immunosuppressed if they had one or more of the following: neutropenia (absolute neutrophil count <500/mm^3), leukaemia, lymphoma, human immunodeficiency virus (HIV) with CD4 count <200/μL or early post-transplant state (<6 months), or were receiving cytotoxic chemotherapy or high-dose steroids.

Lower respiratory tract infection. Lower respiratory tract infection was defined, per European guidelines, as pneumonia and/or an acute illness present for 21 days or less, usually with cough as the main symptom, with at least one other lower respiratory-tract symptom (sputum production, dyspnoea, wheeze or chest discomfort/pain) and no alternative explanation (e.g. sinusitis or asthma) [8].

Standard methods

Our centre's standard diagnostic methods for BAL analysis include direct microscopic specimen examination with Gram, acridine orange and calcofluor white staining, and bacterial and fungal cultures, as well as *Mycoplasma* and *Chlamydia*-specific PCR. Bacterial and fungal cultures are performed by streaking a calibrated loop on various generic and selective media [9]; buffered charcoal yeast agar is routinely employed for the detection of *Legionella* spp. Colonies are quantitatively reported (e.g. >10^3 cfu/mL) and identified using a combination of manual (e.g. optochin, pneumo-agglutination) and molecular assays (matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF), Bruker MALDI Bio-Typer 2.0®, Billerica, MA, USA). Fungi are identified by morphology when grown on specific media as well as by MALDI-TOF (after extraction, using the commercially available Bruker database), and by 18S and/or internal transcribed spacer (ITS) gene sequencing when discordant.

Upon request, specific PCRs are performed for the detection of *Pneumocystis jirovecii*, other fungi, mycobacteria (using GeneXpert®; Cepheid, Sunnyvale, CA, USA), and a

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