Rapid, Non-invasive Detection of *Legionella* and *Nocardia* and Resolution of Species Diversity in Clinical Infections in Immunocompromised Hosts Using the Karius Test, a Plasma-based Microbial Cell-free DNA Sequencing Test for Pathogen Identification

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Background

Legionella and *Nocardia* are important causes of infection in immunocompromised hosts. *Legionella* species can cause lower respiratory tract infection or a systemic febrile illness ("Pontiac fever"). Infections occur sporadically or in outbreaks associated with environmental water sources. *Legionella* is difficult to culture and urine antigen testing (UAT) only detects *Legionella pneumophila* serogroup 1 which is thought to cause most clinical infections. Many additional *Legionella* species cause disease and are undetected by UAT[1]. A broad range of *Nocardia* species can cause pulmonary, brain, skin or disseminated infections in immunocompromised (IC) and immunocompetent hosts. Furthermore molecular methods have led to reclassification of *Nocardia* and identification of new species. *Nocardia asteroides* complex – the cause of the majority of invasive disease – has been reclassified into six phenotypic groups based on antibiotic sensitivity profile. Definitive diagnosis can be challenging, requiring direct testing of respiratory, brain or skin samples. Modified acid-fast staining can also be performed but has limited yield[2]. The gold standard for both *Legionella* and *Nocardia* detection involves culture with buffered charcoal yeast extract that can be cumbersome and is not available in all microbiology laboratories. Plasma-based next-generation sequencing (NGS) of circulating microbial cell-free DNA (mcfDNA) has shown utility in diagnosing a variety of infections[3,4,5,6] and offers promise for detection of infections caused by *Legionella* and *Nocardia*.

Methods

Karius® Test (Redwood City, CA) results were reviewed for detections of *Legionella* and *Nocardia*. The Karius Test (KT) was developed and validated in Karius' CLIA certified/CAP accredited lab and detects mcfDNA. McfDNA is extracted, NGS is performed, human sequences are removed and remaining sequences are aligned to a curated pathogen database of >1400 organisms. Organisms present above a statistical threshold are reported and quantified. For > 85% of test results the time to result reporting is 24 hours from sample receipt. Clinical information was obtained from test requisition submission or from treating physicians at the time of result reporting after clinical consultation with Karius medical directors.

References

1. Mercante et al. *Clin Microbiol Rev*. 2015;28(1):95-133.
2. Brown-Elliott et al. *Clin Microbiol Rev*. 2006;19(2):259.
3. Blauwkamp et al. *Nat Microbiol.* 2019 Apr;4(4):663-674.
4. Farnaes et al. *Diagn Microbiol Infect Dis.* Jun;94(2):188-191.
5. Rossoff et al. *Open Forum Infect Dis.* 2019 Aug 1;6(8).
6. Hong et al. *Diagn Microbiol Infect Dis.* 2018 Nov 1;92(3):210-213.

Figure 1. *Legionella* detections (n=48) using The Karius Test

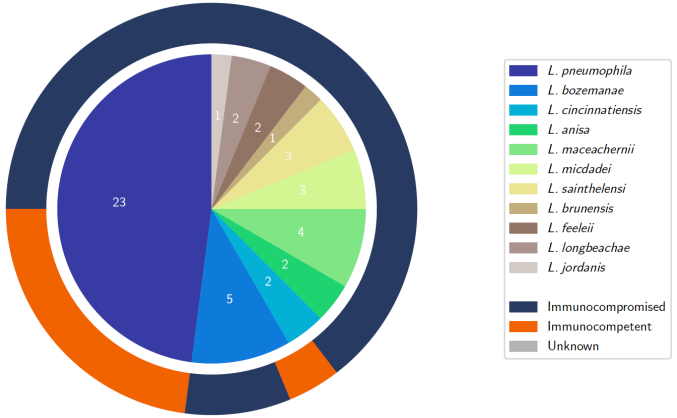
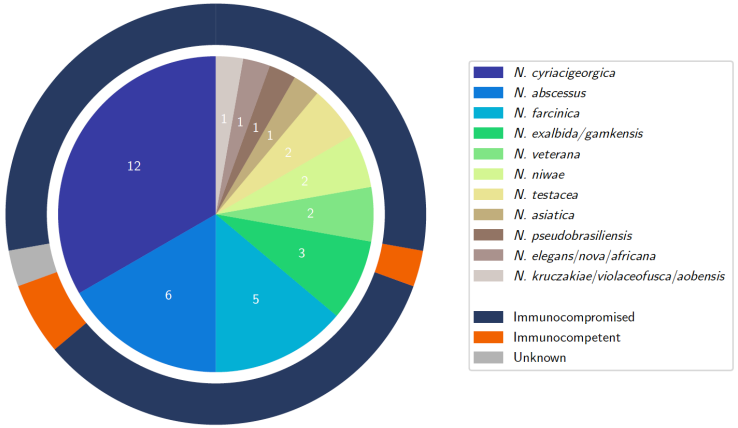


Figure 2. *Nocardia* detections (n=36) using The Karius Test



Results

The KT detected *Legionella* in 48 samples (46 patients) including 39 adults (85%) and seven children. Thirty-four patients (74%) were immunocompromised; 39 detections (81%) were associated with a pulmonary focus and a presumptive lower respiratory tract infection. Twenty-three samples were positive for *L. pneumophila* (48%), five samples were positive for *L. bozemanae* (10%), four samples were positive for *L. maceachernii* (8%), three samples each were positive for *L. micdadei* (6%) and *L. sainthelensi* (6%), two samples each were positive for *L. anisa* (4%), *L. cincinnatiensis* (4%), *L. feeleii* (4%) and *L. longbeachae* (4%), and one sample each was positive for *L. brunensis* (2%), and *L. jordanis* (2%) (Figure 1). This is the first reported detection of *L. brunensis* as a cause of human infection. The majority of infections (80%) in immunocompetent individuals were caused by *L. pneumophila* whereas *Legionella* identified in IC individuals were more commonly non-*pneumophila* species (65%). The KT detected *Nocardia* in 36 samples (32 patients) including 25 adults (78%) and seven children with an overall male predominance (63%). Twenty five patients (78%) were IC with a majority being bone marrow transplant recipients (28%). Twenty-three detections (64%) involved a pulmonary focus, four detections (11%) involved a brain focus and two (6%) involved disseminated disease. Twelve samples were positive for *N. cyriacigeorgica* (33%), six samples were positive for *N. abscessus* (17%), five samples were positive for *N. farcinica* (14%), three samples were positive for *N. exalbida/gamkensis* (closely related species) (8%), two samples each were positive for *N. niwae* (6%), *N. testacea* (6%), and *N. veterana* (6%), and one sample each were positive for *N. pseudobrasiliensis* (3%), *N. asiatica* (3%), *N. elegans/nova/africana* (closely related species) (3%) and *N. kruczakiae/violaceofusca/aobensis* (closely related species) (3%) (Figure 2).

Conclusion

The Karius Test, a plasma-based mcfDNA NGS offers a rapid, non-invasive means of detecting a broad diversity of known and potentially novel *Legionella and Nocardia* species in clinical infections. Most *Legionella* detected were non-*pneumophila* species undetectable by the UAT and were detected in the immunocompromised host. Accurate species-level identification of *Legionella* has important public health implications given the pathogen's association with environmental outbreaks. The plasma-based KT non-invasively detected Nocardiosis originating from focal infections from various anatomical sites including the brain where biopsy is challenging. The KT uncovered new insights on genus complexity potentially not resolved by current taxonomic classification. The non-invasive, unbiased approach by investigating plasma for mcfDNA can help diagnose important, under-diagnosed, difficult to culture pathogens causing focal infections in IC hosts with a very wide differential diagnosis.