Presentation Type:
Poster Presentation

Blind Spots in Methods Based on Cultivation and Metagenomic Sequencing for Surface Microbiomes in a Medical Intensive Care Unit

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Background: Cultivation of targeted pathogens has been long recognized as a gold standard for healthcare surveillance. However, there is an emergent need to characterize all viable microorganisms in healthcare facilities to understand the role that both clinical and nonclinical microorganisms play in healthcare-associated infections. Metagenomic sequencing allows detection of entire microbial communities, in contrast to targeted identification by cultivation. Widespread application of metagenomic sequencing has been impeded in part because the sensitivity and specificity are unknown, which inhibits our ability to interpret results for risk assessment. To assess the impact of sample preparation methods on sensitivity and specificity, we compared several pretreatment steps followed by metagenomic sequencing, and we performed culture-based analyses. **Methods:** We collected 120 surface swabs from the medical intensive care unit at Rush University Medical Center, which we aggregated to create a representative microbiome sample. We then subjected aliquots to different processing methods (DNA extraction methods, internal standard addition, propidium monoazide (PMA) treatment, and whole-cell serial filtration). We evaluated the effects of these methods based on DNA yields and metagenomic sequencing outcomes. We also compared the metagenomic results to the microbial identifications obtained by cultivation using environmental microbiology methods and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). **Results:** Our results demonstrate that bead-beating and heat lysis followed by liquid-liquid extraction is the optimal method for the identification of low-biomass surface-associated microbes, as opposed to widely used column-based and magnetic bead-based methods. For low-biomass surface-associated samples, ~590,000 reads per sample are sufficient for ≈90% coverage in metagenomic sequencing (Fig. 1). The ZymoBIOMICS microbial community standard is not appropriate for methods assessing membrane integrity. For the identification of putatively viable microorganisms, PMA treatment is promising, although elimination of signals from nonviable organisms will reduce the overall detectable signal. Combining PMA-treated metagenomic sequencing with cultivation yields the most comprehensive results, particularly for low-abundance taxa, despite high sequencing coverage (Fig. 2). To distribute more detection resources to bacteria, our target domain, we tried whole-cell filtration prior to extraction, attempting to isolate bacterial cells from eukaryotic cells and other particles. For low-biomass surface-associated samples, the sample loss and the difficulties in performing filtration outweigh the slight increase of bacterial signal. **Conclusions:** Despite optimization, we observed certain blind spots in both cultivation and metagenomic sequencing. This information is essential for informed risk assessment. Further research is needed to identify additional limitations to ensure that results from metagenomic sequencing can be interpreted in the context of healthcare-acquired infection prevention.

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Disclosures: None
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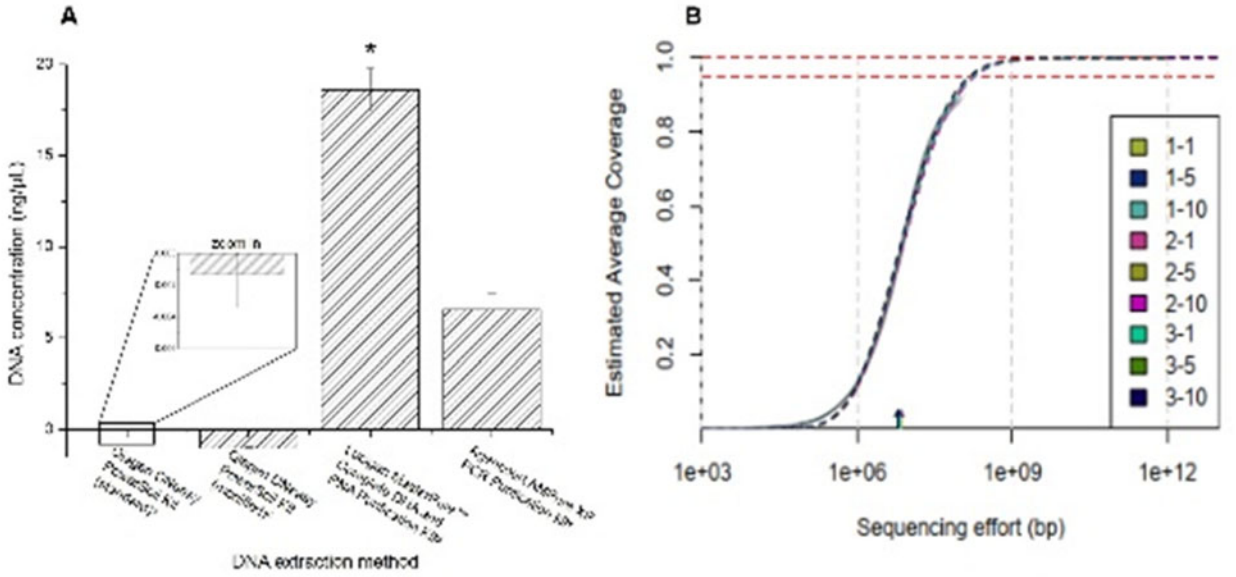

Figure 1. (A) DNA yields (quantified by NanoDrop) of low-biomass surface-associated samples extracted by different methods. Extraction kits marked with α are column-based, with β are magnetic bead-based, and with γ use liquid-liquid extraction. (B) Estimation of metagenomic sequencing coverage for triplicate samples diluted to 1X, 0.5X, or 0.1X generated using Nonpareil. All samples were extracted using Lucigen MasterPure ™ Complete DNA and RNA Purification Kit (asterisked in Fig. 1A).

Fig. 1.

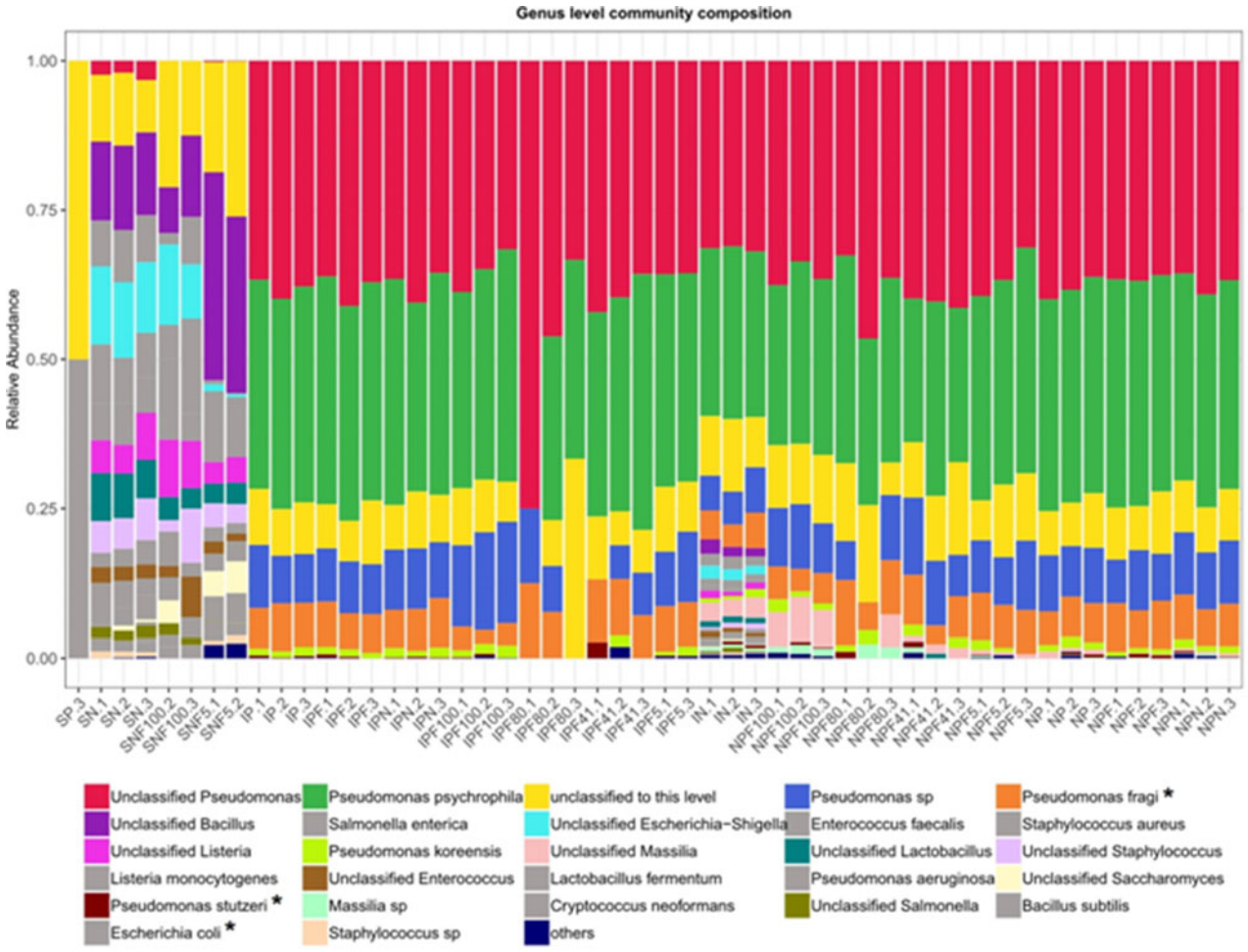

Figure 2. Taxonomic identifications using Metaxa2 resulting from metagenomic sequencing of samples subjected to different processing methods. 1) List of abbreviations. IN: internal standard added, not PMA treated. NP: no internal standard added, PMA treated. IPF: internal standard added, PMA treated, filtered. IPN: internal standard added, PMA treated, unfiltered. NPF: no internal standard added, PMA treated, filtered. Samples generated from the filter retentate are marked with "F" followed by the pore size of the filter. Samples starting with "S" represent the ZymoBIOMICS Microbial Community Standard only. 2) All taxa claimed as components by the ZymoBIOMICS Microbial Community Standard are in gray. 3) Taxa labeled with an asterisk in the legend were detected by cultivation coupled with MALDI-TOF MS. *Pantoea agglomerans, Pseudescherichia vulneris, Pseudomonas fluorescens Pseudomonas putida, Psychrobacter faecalis, Erwinia rhapontici, Bacillus simplex, Cultibacterium Avidum* were detected by cultivation but not seen by metagenomic sequencing.

Fig. 2.

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Poster Presentation

Blood Culturing Practices at an Academic Medical Center

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Background: Blood cultures are part of the evaluation of hospital patients with fever. Patients with central lines in place, frequently have blood samples for culture drawn through lines. We sought to assess blood culturing practices at our institution. **Methods:** Retrospective review of BCs performed in hospitalized patients over a 12-month period (August 2018–July 2019) at an academic, tertiary-care center with 1,297 licensed beds and >62,000 admissions a year. A specialized phlebotomy team is involved in all peripherally drawn blood samples; however, the patient's nurse obtains a blood sample through a central line. **Results:** Overall, 35,121 blood cultures were performed for an incidence rate of 106 BC per 1,000 patient days or 566 blood cultures per 1,000 admissions. Most blood samples (67%) were collected via peripheral venipuncture. We detected significant variation in culturing rates and the proportion of blood samples obtained through central lines among collecting units (Table 1). Overall, the blood culture contamination rate was 1.6%. Blood samples obtained through a central line had a higher contamination rate (2.2%) compared to samples obtained through peripheral venipuncture (1.3%; $P < .0001$). Blood culture rates were highest in intensive care units (ICUs) compared with other types of patient care units (Table 1). The blood

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