152. rapid Ultra-high Enrichment of Bacterial Pathogens at Low Concentration from Blood for Species ID and AMR Prediction Using Nanopore Sequencing

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Session: O-29. Innovations in Diagnostics

Background: Each year in the United States there are over 1.7 million cases of sepsis that account for a third of hospital deaths. A key to reducing morbidity and mortality rates is early, appropriate antibiotic therapy. Most new diagnostic approaches still suffer from insufficient sensitivity to low bacterial loads in blood and limited sets of detection targets for bacterial species identification (ID) and antimicrobial resistance (AMR) determination. As such, blood culture remains the gold standard for diagnosing bacteremia despite limitations such as > 2-day turnaround time (TAT), incompatibility with fastidious organisms, and frequent inability to recover causative pathogens.

Methods: 31 clinically relevant bacterial pathogens, made up of 17 gram-positive and 14 gram-negative bacterial species, were spiked into 2 to 4 healthy donor blood samples at 1 to 5 CFU/mL. The samples were run through our proprietary Blood2Bac™ pipeline, sequenced on a nanopore platform, and data were passed through Keynome®, our proprietary machine learning algorithm to determine species ID and AMR.

Results: By assessing the efficiency of pathogen DNA enrichment and genome coverage post sequencing, we report high performance of 3 CFU/mL for 3 bacterial species and ≤ 2 CFU/mL for the 28 remaining species, which includes *S. aureus*, *E. coli*, and *Streptococcus* spp., three of the leading causes of sepsis.

For all 31 bacterial species tested, Keynome called species ID with 100% accuracy. In addition, Keynome also predicted the AMR profile of pathogens with 100% accuracy for 19 drug/species AMR combinations, including ciprofloxacin for *E. coli*, clindamycin for *S. aureus*, and aztreonam for *K. pneumoniae*.

Conclusion: Blood2Bac is able to enrich a wide range of bacterial pathogens directly from blood and enable bacterial whole genome sequencing with an estimated TAT of 12 hours. When coupled with Keynome, our process provides accurate species ID and AMR calls for key BSI pathogens even at single-digit CFU/mL concentrations. Our species-agnostic and culture-free process enables detection of a diverse range of bacterial species with high sensitivity, providing a robust and comprehensive diagnostic.

Disclosures: Chiahao Tsui, n/a, Day Zero Diagnostics (Employee, Shareholder) Lisa S. Cunden, PhD, Day Zero Diagnostics (Shareholder) Nicole Billings, PhD, Day Zero Diagnostics (Employee) Imaly A. Nanayakkara, PhD, Day Zero Diagnostics (Employee, Shareholder) Ian Herriott, BS, Day Zero Diagnostics (Employee, Shareholder) Rachel R. Martin, n/a, Day Zero DIagnostics (Employee) Michelle Chen, MS, Day Zero Diagnostics (Employee, Shareholder) Febriana Pangestu, n/a, Day Zero Diagnostics (Employee, Shareholder) Paul Knysh, PhD, Day Zero Diagnostics (Employee) Cabell Maddux, n/a, Day Zero Diagnostics (Employee, Shareholder) Zachary Munro, n/a, Day Zero Diagnostics Inc. (Employee, Shareholder) Miriam Huntley, PhD, Day Zero Diagnostics (Employee, Shareholder)

153. pilot Study of a Novel Whole-genome Sequencing Based Rapid Bacterial Identification Assay in Patients with Bacteremia

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Session: O-29. Innovations in Diagnostics

Background: Blood stream infections (BSI) are among the leading cause of morbidity and mortality, yet gold standard culture-based diagnostics have limited ability to guide therapeutic intervention due to multi-day turnaround time and low sensitivity. Day Zero Diagnostics has developed Blood2Bac™, a culture-free, species agnostic process to enrich bacteria direct from whole blood. Coupled with whole genome sequencing (WGS) and Day Zero Diagnostics' Keynome® algorithmic tools for species ID and antimicrobial resistance (AMR), we conducted the first proof-of-concept feasibility study in an inpatient clinical setting.

Methods: Study participants were enrolled and specimens collected from Boston Medical Center. Eligibility criteria included hospitalized adults with suspected and/or documented BSI, irrespective of empiric antibiotic therapy duration. Whole blood samples were processed with Blood2Bac, sequenced on a nanopore platform, and bacterial ID determined with Keynome ID. Keynome ID results were compared with blood culture results to measure concordance.

Results: Specimens from 21 participants were processed with Blood2Bac and nanopore sequencing. For 20/21 samples, Keynome ID calls were concordant with clinical blood culture, where 6 concordant positive and 14 were concordant negative. In 3 concordant samples, Keynome ID called positive while concurrent blood cultures were negative. However, all IDs corresponded with positive blood culture results from the day prior, suggesting potentially higher sensitivity for the Blood2Bac compared to blood culture. Two concordant positive IDs, resulted in >95% of the genome recovered and Keynome concomitantly resulted in AMR predictions with 100% accuracy compared to pathogen phenotype. In 1 discordant specimen, the Keynome ID result was negative while blood cultures 8 hours before were positive. In this case, the patient was on empiric therapy for 8 days prior to samples collection and cultures were negative 19-hours post specimen collection.

Conclusion: These results highlight the sensitivity of a real-time blood WGS approach to identify BSI and its utility as a diagnostic to minimize unnecessary antibiotic exposure contributing to the antibiotic resistance crisis.

Disclosures: **Archana Asundi, MD**, **Gilead** (Scientific Research Study Investigator)**Merck** (Scientific Research Study Investigator)**ViiV** (Scientific Research Study Investigator) **Nicole Billings, PhD**, **Day Zero Diagnostics** (Employee) **Zoe H. Rogers, MPH**, **Day Zero Diagnostics** (Employee, Shareholder) **Lisa S. Cunden, PhD**, **Day Zero Diagnostics** (Shareholder) **Imaly A. Nanayakkara, PhD**, **Day Zero Diagnostics** (Employee, Shareholder) **Chiahao Tsui, n/a**, **Day Zero Diagnostics** (Employee, Shareholder) **Paul Knysh, PhD**, **Day Zero Diagnostics** (Employee) **Cabell Maddux, n/a**, **Day Zero Diagnostics** (Employee, Shareholder) **Zachary Munro, n/a**, **Day Zero Diagnostics Inc.** (Employee, Shareholder) **Ian Herriott, BS**, **Day Zero Diagnostics** (Employee, Shareholder) **Miriam Huntley, PhD**, **Day Zero Diagnostics** (Employee, Shareholder) **Nina H. Lin, MD**, **Gilead Sciences** (Scientific Research Study Investigator)**ViiV** (Scientific Research Study Investigator)

154. comparative Genomics Reveals Extra-hospital Transmission Networks of Carbapenem-resistant *acinetobacter Baumannii* sustained over Multiple Years in a US Midwest City

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Session: O-30. MDRO Epidemiology and Transmission

Background: The transmission dynamics of *Acinetobacter baumannii* (*Ab*) outside the setting of hospital outbreaks is underinvestigated. The BJC Healthcare System in St. Louis, MO has not experienced an *Ab* hospital outbreak since 2012. Despite this, nearly 60% of all BJC *Ab* isolates are carbapenem-resistant *Ab* (CrAb).

Methods: We acquired whole genome sequences (WGSs) of 110 *Ab* isolates identified in five BJC hospitals from July 2017 to May 2019. We performed multilocus sequence typing, core genome alignment and pairwise average nucleotide identity analysis to compare WGSs from BJC isolates and GenBank-available WGSs of *Ab* isolates from other US hospitals. Further epidemiologic characterization was performed using BJC electronic medical records and detailed chart review.

Results: Though the majority of CrAb isolates in other US studies belonged to globally-prevalent sequence type 2 (ST2 [Pasteur scheme]), 62% and 26% of BJC CrAb index isolates belonged to the unrelated ST499 and ST406, respectively. BJC ST499 and ST406 isolates were phylogenetically distinct compared to corresponding isolates from other US hospitals. Under the assumption that *Ab* transmission occurs primarily through nosocomial spread, we expected BJC isolates from the same hospital and timespan to share the highest degree of homogeneity. However, geotemporal proximity between ST499 or ST406 BJC isolates was a poor predictor of their genetic relatedness, according to multiple comparative methods. Review of patient metadata did not identify epidemiological links between BJC isolates within phylogenetic subgroups.

Conclusion: We combined comparative genomics and detailed clinical chart review to characterize the transmission dynamics of two emerging US CrAb sequence types, ST499 and ST406. Though these highly homogeneous *Ab* isolates were identified over two years in multiple BJC hospitals, we found no evidence of robust intra-hospital transmission networks. Instead, it appears that these CrAb isolates independently emerged from yet-to-be-identified regional, extra-hospital *Ab* populations. To neutralize the threat of drug-resistant infections in the US, it is essential to identify, characterize and disrupt emergent CrAb transmission networks that exist outside of hospital environments.

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155. Public Health Action-based System for Tracking and Responding to U.S. candida Drug Resistance: AR Lab Network, 2016–2019

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Session: O-30. MDRO Epidemiology and Transmission

Background: Many U.S. clinical laboratories lack capacity to definitively identify fungi or perform antifungal susceptibility testing (AFST). To expand testing access, CDC's Antibiotic Resistance Laboratory Network (AR Lab Network) provides *Candida* species identification and AFST to U.S. facilities for clinical and public health purposes. We describe the first three years of *Candida* AR Lab Network resistance data.

Methods: Isolates from any body site with species identification and AFST performed July 2016–June 2019 are included. Submissions were based on clinical

and public health need. Patients may have multiple isolates. The 7 AR Lab Network regional laboratories used matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) or DNA sequencing for species identification. AFST was performed using broth microdilution for azoles and echinocandins (anidulafungin and micafungin) and Etest for amphotericin B. This analysis focuses on non-*albicans Candida* species with Clinical and Laboratory Standards Institute M60 minimum inhibitory concentration breakpoints and *C. auris*, which has CDC-proposed tentative breakpoints.

Results: Participation increased from healthcare facilities from 2 states submitting in 2016 to 35 states in 2019. Species identification was performed on 5,234 non-*albicans* isolates. AFST was performed on 4,222 (81%) isolates, including 2,395 *C. glabrata*, 815 *C. auris*, 267 *C. parapsilosis*, 125 *C. tropicalis*, 35 *C. guilliermondii*, and 32 *C. krusei*. Of isolates with AFST and body site indicated, 22% (900/4,102) were from blood. We found 85% of *C. auris*, 8% of *C. glabrata*, and 5% of *C. parapsilosis* isolates were resistant to azoles; 33% of *C. auris* isolates were resistant to amphotericin B; and 2% of *C. glabrata*, 1% of *C. auris*, and 1% of *C. parapsilosis* isolates were resistant to echinocandins. Although intrinsically resistant to fluconazole, *C. krusei* isolates were not resistant to voriconazole. Multidrug resistance was present in 32% of *C. auris* and 1% of *C. glabrata* isolates.

Conclusion: AR Lab Network has expanded access to rapid *Candida* testing, including AFST, and provides real-time surveillance. Results can be used to detect emerging species and resistance and guide public health action and healthcare practices.

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156. How Does Exposure to C. Diffogenic Antibiotics Impact Multidrug-resistant Organism Colonization and Environment Contamination in Nursing Homes?

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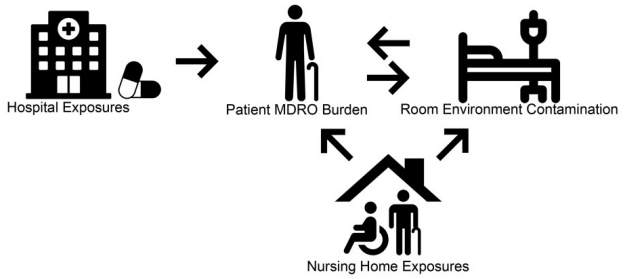
Session: O-30. MDRO Epidemiology and Transmission

Background: Antimicrobial stewardship program (ASP) outcomes are often measured in the acute care setting, less is known about the effect of acute care antibiotic exposures on multidrug-resistant organism (MDROs) colonization of nursing home (NH) patients. We assessed exposure to antibiotics commonly associated with *Clostridioides difficile* (C. diffogenic agents) on post-acute care patient colonization and room environment contamination (Figure 1).

Figure 1. Conceptual Diagram of Hospital Antibiotic Exposure's Influence on Patient Colonization and Room Environment Contamination with Multidrug-Resistant Organisms



Methods: MDRO surveillance of post-acute care patients in 6 NHs between 2013–16. We screened patient hands, nares, oropharynx, groin, perianal area, and high-touch room environment surfaces for methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and resistant Gram-negative bacilli (rGNB). C. diffogenic agents were defined as fluoroquinolones, 3rd/4th generation cephalosporins, penicillin combinations, lincosamides, and carbapenems. Multivariable logistic regression was used to assess whether hospital antibiotic exposure is an independent risk factor for MDRO colonization and room environment contamination on study enrollment.

Results: We enrolled 618 patients: average age was 74.4 years; 57.4% female; 62.3% white; 9.9% had indwelling devices (Table 1). Three hundred-fifty patients (56.6%) were MDRO colonized on enrollment: 98 (15.9%), MRSA; 208 (33.7%); VRE; 196 (31.7%), rGNB. Sixty-eight percent of patient rooms were MDRO contaminated: 166 (26.9%), MRSA; 293, (47.4%). VRE; 182 (29.5%), rGNB.

A majority (59.4%) of patients were exposed to an antibiotic before admission. Of which, 239 (65.1%) were exposed to a C. diffogenic antibiotic. In multivariable analysis, C. diffogenic antibiotic exposure was an independent risk factor for MDRO colonization (OR, 1.94; 95% CI, 1.35–2.79), MDRO room environment contamination (OR, 1.94; 95% CI, 1.43–2.63), VRE colonization (OR, 4.23; 95% CI, 2.59–6.90), and VRE room environment contamination (OR, 2.58; 95% CI, 2.00–3.33).

Table 1. Clinical Characteristics and MDRO Burden on Study Enrollment, Stratified by Hospital Antibiotic Exposure Status

Characteristic	All Patients (N=618)	No Antibiotic Exposure History (N=251)[1]	Low-Risk Antibiotic Exposure History (N=128)[1]	High-Risk Antibiotic Exposure History (N=239)[1]
Patient Characteristics				
Age, y, mean (SD)	74.4 (12.1)	74.5 (12.8)	73.9 (11.2)	74.5 (11.9)
Male Sex, No. (%)	263 (42.6)	104 (41.4)	61 (47.7)	98 (41.0)
Non-Hispanic White, No. (%)	385 (62.3)	135 (53.8)	91 (71.1)	159 (66.5)
Charlson Comorbidity Score, mean (SD)	2.6 (2.1)	2.6 (2.1)	2.3 (2.0)	2.7 (2.1)
Physical Self-Maintenance Score, mean (SD)	14.3 (4.5)	14.1 (4.2)	13.6 (4.4)	14.9 (4.8)
Indwelling Device Use, No. (%)[2]	61 (9.9)	15 (6.0)	13 (10.2)	33 (13.8)
Hospital Stay > 2 weeks, No. (%)	60 (9.7)	12 (4.8)	12 (9.4)	36 (15.1)
NH Days to Enrollment, mean (SD)	5.6 (3.0)	5.7 (3.1)	5.0 (2.9)	5.7 (2.9)
Proximal Outcome: Patient Colonization				
Any MDRO Colonization, No. (%)	350 (56.6)	119 (47.4)	71 (55.5)	160 (67.0)
MRSA, No. (%)	98 (15.9)	42 (16.7)	15 (11.7)	41 (17.2)
VRE, No. (%)	208 (33.7)	46 (18.3)	44 (34.4)	118 (49.4)
rGNB, No. (%)	196 (31.7)	71 (28.3)	40 (31.3)	85 (35.6)
Distal Outcome: Room Environment Contamination				
Any MDRO Contamination, No. (%)	418 (67.6)	150 (59.8)	87 (68.0)	181 (75.7)
MRSA, No. (%)	166 (26.9)	63 (25.1)	32 (25.0)	71 (29.7)
VRE, No. (%)	293 (47.4)	87 (34.7)	66 (51.6)	140 (58.6)
rGNB, No. (%)	182 (29.5)	77 (30.7)	32 (25.0)	73 (30.5)

[1] High-risk antibiotic exposure was defined as exposure to one or more of the following antibiotics that predispose patients to a high-risk of *Clostridioides difficile* infection prior to admission to the nursing facility: fluoroquinolones, 3rd/4th generation cephalosporins, penicillin combinations, lincosamides, and carbapenems. Patients without a high-risk antibiotic exposure, but with exposure to other antibiotics were classified as having a low-risk antibiotic exposure history.

[2] Indwelling device use was defined as the presence of a feeding tube or indwelling urinary catheter on study enrollment.

Notes: MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; NH, nursing home; rGNB, resistant Gram-negative bacilli; SD, standard deviation; VRE, vancomycin-resistant enterococci.

Multivariable Analysis of Hospital Antibiotic Exposure Status as Risk Factor for Proximal and Distal MDRO Outcomes

Characteristic	Any MDRO aOR (95% CI)[1]	MRSA aOR (95% CI)[1]	VRE aOR (95% CI)[1]	rGNB aOR (95% CI)[1]
Proximal Outcome: Patient Colonization				
No Antibiotic Exposure	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Low-Risk Exposure History[2]	1.34 (0.91-1.96)	**0.66 (0.44-0.98)**	**2.54 (1.42-4.56)**	1.10 (0.77-1.55)
High Risk Exposure History[2]	**1.94 (1.35-2.79)**	0.94 (0.51-1.73)	**4.23 (2.59-6.90)**	1.22 (0.80-1.85)
Distal Outcome: Room Environment Contamination				
No Antibiotic Exposure	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
Low-Risk Exposure History[2]	1.45 (0.79-2.64)	0.96 (0.55-1.67)	**2.24 (1.21-4.14)**	0.77 (0.43-1.38)
High Risk Exposure History[2]	**1.94 (1.43-2.63)**	1.19 (0.89-1.60)	**2.58 (2.00-3.33)**	0.91 (0.53-1.56)

[1] Multivariable logistic regression model was adjusted for age, sex, race, Charlson Comorbidity Index score, Physical Self-Maintenance score, indwelling device (urinary catheter or feeding tube) present on enrollment, hospital stay greater than 14 days, and nursing home days to enrollment. All regression analyses were cluster adjusted.

[2] High-risk antibiotic exposure was defined as exposure to one or more of the following antibiotics that predispose patients to a high-risk of *Clostridioides difficile* infection prior to admission to the nursing facility: fluoroquinolones, 3rd/4th generation cephalosporins, penicillin combinations, lincosamides, and carbapenems. Patients without a high-risk antibiotic exposure, but with exposure to other antibiotics were classified as having a low-risk antibiotic exposure history.

Notes: aOR, adjusted odds ratio; Bold, p < 0.05; CI, confidence interval; MDRO, multidrug-resistant organism; MRSA, methicillin-resistant *Staphylococcus aureus*; rGNB, resistant Gram-negative bacilli; VRE, vancomycin-resistant enterococci.

Conclusion: Hospital exposure to antibiotics is associated with an increased risk of VRE colonization and room environment contamination on NH study enrollment. These observations highlight the potential influence of hospital-based ASPs on MDRO prevalence and transmission in NHs.

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157. patient to Environment Transmission of Multidrug-resistant Bacteria Within Intensive Care Units

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Session: O-30. MDRO Epidemiology and Transmission

Background: Identifying risk factors for environmental contamination with multidrug-resistant organisms (MDROs) is essential to prioritize methods for prevention of hospital transmission.

Methods: Patients admitted to an ICU with an MDRO detected on clinical culture in the prior 30 days were enrolled. Patients (4 body sites) and high-touch objects (HTO) (3 composite sites) in ICU rooms were sampled. Environmental transmission was defined by shared MDRO species cultured on patient and HTO cultures obtained on multiple time points during the patient's stay. Risk factors for environmental transmission were identified with logistic regression.

Results: Forty-five patients were included (median 2 days of longitudinal sampling [IQR 1–4 days]). Enrollment anatomic cultures included extended-spectrum beta-lactamase-producing Enterobacterales (ESBLE) (n=12, 27%), carbapenem-resistant organisms (CRO) (n=4, 9%), methicillin-resistant *S.aureus* (MRSA) (n=11, 24%), vancomycin-resistant Enterococci (VRE) (n=4, 9%), and *C.difficile* (CDIFF) (n=14, 31%). Patient colonization during serial sampling was common with CRO (n=21, 47%), ESBLE (n=16, 36%), and VRE (n=16, 36%) and less so with MRSA (n=7, 16%) and CDIFF (n=5, 11%). Detection of MDROs on environmental surfaces was also common with identification of CRO in 47% of patient rooms (n=21) and ESBLE in 29% (n=13); MRSA (n=2, 4%), VRE (n=9, 20%), and CDIFF (n=3, 7%) were rarer. Patient to environment transmission was observed in 40% of rooms (n=18). Thirteen (29%) rooms had foreign MDRO contamination (i.e., one not detected on a body culture), most (n=10) with CRO. Environmental MDROs were most common in bathroom/