657. Evaluation of Nanopore-Based 16S Ribosomal RNA (rRNA) Gene Sequencing for the Development of a Rapid Infection Intervention Clinical Service

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Background. Rapid and accurate identification of bacteria is the basis of appropriate antibiotic treatment and effective clinical decision-making. Next-generation sequencing (NGS) platforms such as Oxford Nanopore Technologies (ONT) holds the promise of a diagnostic revolution by overcoming the limitations of culture-based identification with rapid molecular detection of bacteria. We have developed a pilot to evaluate an ONT 16S rRNA gene assay with the ability to provide real-time analysis and identification of bacterial species. Our aim was to investigate whether long-read sequencing and high-speed analysis can be combined to create a clinically useful, rapid diagnostic tool.

Methods. A collection of bacterial isolates representing pathogenic species received by the clinical laboratory over 1 year was assembled. Sample preparation was as described in the ONT 16S protocol and included bead beating sample disruption, MagNA Pure automated nucleic acid extraction (Roche), and PCR amplification (Thermo). Sequencing was performed on the MinION and GridION X5 platforms. Output was analyzed with ONT's automated EPI2ME 16S pipeline which assigns reads to taxa using BLAST results and the NCBI 16S Bacterial database.

Results. A total of 155 clinical samples with 139 species were sequenced. 119 species were identified at the species level. For 20 samples, a species in the same genus claimed the majority of reads, with the true species being matched to 3%-41% of reads. The average proportion of reads assigned to the correct species was 62.2%, specifically 67% for non-Enterobacteriaceae and 33% for Enterobacteriaceae. 4 clinical samples (3 Bronchoalveolar lavages (BALs), positive for (1) *K. pneumoniae*, (2) *S. pneumoniae*, and (3) *S. pneumoniae*, *S. enterica*, and *S. typhimurium*, and 1 bone positive for *P. aeruginosa*) were also analyzed with sequencing results matching culture.

Conclusion. Early results show that 16S rRNA sequencing coupled with real-time analysis was able to accelerate pathogen detection and was able to discriminate the majority of species from a relevant clinical collection. Pipeline refinement is required and subsequent confirmatory consensus-based identification may be a helpful adjunct. Nanopore sequencing shows promise as a rapid bacterial pathogen detection platform for clinical service.

Disclosures. **All authors:** No reported disclosures.

658. Cost-effectiveness of Molecular Diagnostic Assays for the Therapy of Severe Sepsis and Septic Shock in the Emergency Department

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Background. Sepsis presents a major burden to the emergency department (ED). Because empiric inappropriate antimicrobial therapy (IAAT) is associated with increased mortality, rapid molecular assays may decrease IAAT and improve outcomes.We evaluated the cost-effectiveness of molecular testing as an adjunct to blood cultures in patients with severe sepsis or septic shock evaluated in the ED.

Methods. We developed a decision analysis model with the primary outcome the incremental cost-effectiveness ratio expressed in terms of deaths averted. Costs were dependent on the assay price and the patients' length of stay (LOS). Three base-case scenarios regarding the difference in LOS between patients receiving appropriate (AAT) and IAAT were described.Sensitivity analyses regarding the assay cost and sensitivity, and its ability to guide changes from IAAT to AAT were performed.

Results. Under baseline assumptions, molecular testing was cost-saving when the LOS differed by 4 days between patients receiving IAAT and AAT (ICER −$7,302/death averted). Our results remained robust in sensitivity analyses for assay sensitivity ≥52%, panel efficiency ≥39%, and assay cost ≤$270. In the extreme case that the LOS of patients receiving AAT and IAAT was the same, the ICER remained ≤$20,000/death averted for every studied sensitivity (i.e., 0.5–0.95), panel efficiency ≥34%, and assay cost ≤$313. For 2 days difference in LOS, the bundle approach was dominant when the assay cost was ≤$135 and the panel efficiency was ≥77%.

Conclusion. The incorporation of molecular tests in the management of sepsis in the ED has the potential to improve outcomes and be cost-effective for a wide range of clinical scenarios.

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659. Evaluation of a Rapid Diagnostic Assay for Early Detection of Bacteriuria

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Background. Patients with suspected urinary tract infection (UTI) are often prescribed an empiric antibiotic treatment due to delays in obtaining results of urine cultures. The BacterioScan System measures the turbidity of incubating urine specimens to provide a qualitative determination of bacteriuria at a density of >5 × 10^4 colony-forming units (CFU)/mL within approximately 3 hours. We examined the utility of the BacterioScan assay in predicting bacteriuria and assessed the potential impact of this test to reduce the number of urine cultures processed.

Methods. Urine samples received for culture in the microbiology laboratory of the Cleveland VA Medical Center were collected daily between September 2018 and December 2018. For each specimen, we performed a bacterioscan diagnostic test and compared it with the result of the traditional culture and urinalysis if available. Urinary cultures were categorized into 4 groups as defined in Figure 1. We compared the sensitivity and specificity of the bacterioscan vs. urinalysis (leukocyte esterase and/or pyuria) results.

Results. 120 urine samples were tested. As shown in Table 1, the BacterioScan had better sensitivity and specificity than the urinalysis for detection of positive urine cultures. The use of the BacterioScan to rule out UTI could have accurately spared 69 of 120 (57.5%) samples from traditional culture and prevented 26 of 120 (21.6%) from possible misinterpretation as infection due to reporting of growth. BacterioScan resulted in 4 of 31 (12.9%) false negatives, but all occurred when positive cultures were due to viridans streptococci or uropathogens in numbers below 100,000 CFU.ml.

Conclusion. The BacterioScan system is a rapid diagnostic test that provides early information on urine culture results that could help to avoid overuse of empirical antimicrobials in patients with suspected UTI and decrease the workload of the Microbiology Laboratory.

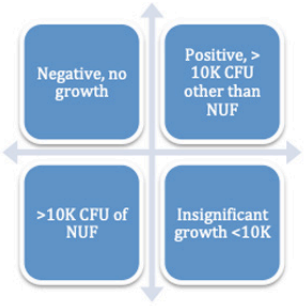

Figure1. Categories of Culture Results. Colony forming units (CFU) per ml. Normal Urogenital Flora (NUF)

Table 1.		BS – (%)	BS +(%)	UA – (%)	UA+ (%)	Comments
NEGATIVE CULTURE	No growth n=50	43 (86)	7 (14)	26 (52)	19 (38)	5 UA not completed
	Insignificant growth n=32	26 (81.2)	6 (18.7)	16 (50)	10 (31.2)	6 UA no WBC data
POSITIVE CULTURE	Colonization/ Overgrowth n=7	0 (0)	7 (100)	1 (14.3)	6 (85.7)	--
	Positive, growth n=31	4 (12.9)	27(87.1)	2 (6.5)	23 (74.2)	3 UA no|WBC data 3 UA not completed
PERFORMANCE	Sensitivity	89.5%		90.6%		
	Specificity	84.1%		59.1%		

BS: Bacterioscan; UA: Urinalysis; WBC: White Blood Cell Count

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660. Extraction-Free 16S Ribosomal RNA (rRNA) Gene Amplification and Sequencing from Resected Cardiac Implantable Electronic Device (CIED) Sonicate Fluid

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Background. We recently demonstrated that 16S rRNA PCR/sequencing performed on biofilms dislodged from extracted CIEDs into a salt solution—referred to as "sonicate fluid' (SF)—may be used to detect pathogens in culture-negative CIED infection (*Clinical Infectious Diseases*, ciz266, doi:10.1093/cid/ciz266). The method we described included a DNA extraction/purification step, which can be time consuming and costly. Here, we evaluated an extraction-free approach to 16S rRNA gene PCR/sequencing.

Methods. 28 SF samples derived from explanted clinically-infected CIEDs were tested. Cases were categorized as "culture-positive" (C-P) if ≥20 cfu/10 mL were recovered, and as "culture-negative" (C-N), if <20 cfu/10 mL were detected in SF culture. The extraction-free method consisted of a single step of lysis at 1,000 rpm, 95°C for 5 minutes using an Eppendorf ThermoMixer®. DNA extraction (the comparator method) was performed using the ZymoBIOMICS™ Kit with modifications. Samples were processed using both methods, followed by amplification of the 16S rRNA gene and bi-directional Sanger sequencing. Crossing points (CPs) generated by the two approaches were compared. Organisms detected by the two PCR methods were compared with those detected with culture.