238. Direct identification of Bacterial Species with MinION Nanopore Sequencer In Clinical Specimens Suspected of Polybacterial Infection

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Background. Conventional culture tests usually identify only a few bacterial species, which can grow well in the culture system, in the cases of polybacterial infection. 16S rRNA gene nanopore sequencing enables semi-quantitative identification of bacterial genetic materials. We aimed to evaluate usefulness of 16s rRNA gene nanopore sequencing in the cases suspected of polybacterial infection.

Methods. The research was conducted in a single university hospital for one year. Conventional bacterial culture identification and nanopore sequencing of 16s rRNA gene were carried out simultaneously for cases where polybacterial infection is strongly suspected. Blood agar plate was used for conventional culture, and Microscan (Beckman Coulter, United States) and Vitek 2 (Biomerieux, FR) automated systems were used for identification. For nanopore sequencing, 16S rRNA gene PCR was performed from the clinical specimens, and sequencing libraries were generated from the PCR products using the rapid barcoding sequencing kit (Oxford nanopore technologies, UK). MinION sequencing was performed for 1–3 hours and the generated reads were analyzed using the EPI2ME 16S BLAST workflow.

Results. Specimens were obtained from 15 patients; 6 liver abscess, 2 psoas abscess, 2 thigh abcess, 1 paraspinal abscess, 1 mycotic aneurysm, 1 necrotizing fasciitis, 1 fingertip gangrene and 1 abscess in coccyx area. 16s rRNA gene nanopore sequencing showed monobacterial organism in 8 (53.3%) specimens and polybacterial organisms in 7 (46.6%) specimens. In three (37.5%) cases of 8 cases with monobacterial infections identified by 16s rRNA gene sequencing, no organism was grown in conventional culture, possibly due to previous antibiotic administration. Notably, among 8 cases with polybacterial infection by 16s rRNA gene nanopore sequencing test, traditional culture test showed polybacterial infection in only two (25%) cases and single bacterial organism was identified in the other 6 (75%) cases.

Conclusion. Nanopore sequencing of 16s rRNA gene using the MinION sequencer may be useful for identification of causing microorganism and differentiation between monobacterial and polybacterial infection when polybacterial infection is suspected.

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239. Epidemiologic Analysis of a Worldwide Collection of Escherichia coli ST131 Using the 1928D Core Genome (cg) Multilocus Sequence Type (MLST) Reveals Country Specific and Globally Disseminated Clades

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Background. Increasing antimicrobial resistance (R) among *Escherichia coli* (EC) isolates can be associated with the expansion of the pandemic sequence type (ST) 131 that harbors virulence factors and causes more severe infections when compared with other antimicrobial-R EC. We evaluated the core genome MLST (cgMLST) profiles and R genes using the bioinformatics tool 1928D to evaluate the epidemiology of a global ST131 EC collection and unrelated STs.

Methods. A total of 259 EC clinical isolates belonging to ST131 (n = 206), ST131-single loci variant (SLV; n = 25), and 28 non-ST131 isolates collected from 27 countries during 2016–2018 were selected. Whole-genome sequencing FASTQ files were uploaded to the 1928D pipeline to generate MLST, cgMLST and R gene prediction. cgMLST assignment was based on comparing >2,500 genes.

Results. Among 231 ST131 and SLV EC isolates, 7 clades were identified (3 major [178 isolates]; Table) applying cgMLST allele distance (ad) of ≤50 as a cutoff. A total of 21 isolates were not assigned to clades (>50 ad from ST131 and SLV). Based on >95% concordance, 11 alleles differentiated clades II and III from clade I, while 6 alleles separated clades I and III from clade II. Isolates in clades I to IV were ciprofloxacin R (MIC, ≥4 mg/L); clades I and III predominantly carried $bla_{\text{CTX-M-15}}$ (39/43 and 61/66), $bla_{\text{OXA-1}}$ (35/43 and 43/66), and *aac(6')-Ib-cr* (39/43, 45/66) while clade II carried $bla_{\text{CTX-M-14}}$-like and rarely *aac(6')Ib-cr* (3/69). The most ad between the 7 ST131 clades was 216, while unrelated STs showed variable ad among isolates within that ST. Isolates bellowing to ST1193 were closely related genetically (ad of 30), but other STs had more variability among isolates (ST167, ad 552; ST38, ad 150; and ST69, ad 179).

Conclusion. 1928D is a robust platform for epidemiological analysis of isolates, providing additional granularity when compared with MLST. Clades II and III were closely related, but carried different $bla_{\text{CTX-M}}$ genes, while clades I and III were not as closely related, but both carried $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, and *aac(6')Ib-cr*. These findings suggest that these clades might have acquired R genes at different points in their genetic evolution. A threshold of ≤50 (cgMLST distance) was useful for classifying isolates into clades.

Clade	Allele distance[a]	Geographic distribution	No. of isolates	Predominant resistance mechanism (no. positive)	
				ESBL	Quinolone R
I	43	9 countries including USA	43	$bla_{\text{CTX-M-15}}$ (39), $bla_{\text{OXA-1}}$ (35)	*aac(6')Ib-cr* (39)
II	47	11 countries including USA	69	$bla_{\text{CTX-M-14}}$-like[b] (43)	*aac(6')Ib-cr* (3), *qnrB19* (1)
III	49 (53)	13 countries including USA	66	$bla_{\text{CTX-M-15}}$ (61), $bla_{\text{OXA-1}}$ (43)	*aac(6')Ib-cr* (45)
IV	40	Australia and USA	5	$bla_{\text{CTX-M-15}}$ (3), $bla_{\text{CTX-M-14}}$-like[b] (1)	*qnrS1* (1)
V	45 (55)	8 countries including USA	16	$bla_{\text{CTX-M-14}}$-like (6)	-
VI	49	Costa Rica and USA	6	$bla_{\text{CTX-M-211}}$ and $bla_{\text{CTX-M-3}}$ (1)	-
VII	28	Costa Rica and USA	6	$bla_{\text{CTX-M-15}}$ (4)	-

[a]Number in parentheses is allele distance (cgMLST) for outliers of the clade.
[b]Includes $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-24}}$, $bla_{\text{CTX-M-27}}$, and $bla_{\text{CTX-M-134}}$.
ESBL, extended-spectrum β-lactamase; R, resistant

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240. The Clinical Utility of Molecular Testing in the Diagnosis and Management of Infectious Diseases: Plasma-based Next-Generation Sequencing (PNGS)

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Background. Molecular diagnostic tests can provide microbiologic results rapidly and with greater sensitivity than traditional methods. However, these tests come with considerable costs, so thoughtful diagnostic stewardship is essential to ensure that resources and outcomes are optimized. We sought to evaluate the impact of PNGS testing on patient management.

Methods. From February 2017 to January 2019, physicians in our group ordered 164 PNGS tests (Karius, Redwood City CA) on 125 patients. A retrospective chart review was performed to determine the clinical indication and utility of the test.

Results. The assay failure rate was 4.9% (8/164). Positive (pos) results were noted in 34% (53/156), of which 23 (43.4%) represented false pos results; 28 were true pos (52.8%) but 2 were unnecessary (also had pos blood cultures). The most common reason for testing was to assess for Mycobacterium chimera (Mc) infection, representing 94 of 156 (60.3%) tests. Of the 21 patients with known Mc, only 10/21 had pos initial tests (47.6%); if patients with Mc localized to the sternum were excluded (8 patients), 76.9% with deep organ involvement had pos initial tests. Five patients with deep Mc infection had persistently pos results while on therapy; 4 of these had not had surgery; 1 was 6 months s/p valve replacement for Mc. The next most common indication was to r/o endocarditis in 18/156 (11.5%) and had an impact in 8/18 (44.4%), including 4 patients whose PNGS result identified a likely pathogen in culture negative endocarditis (CNE). Of the 62 tests done for non-Mc patients, 33.9% (21/62) were useful for management decisions. Among patients who eventually had a diagnosis made but had negative PNGS results included patients with Whipple's (1), CNS infection (2), and fungal infections (5).

Conclusion. Overall, only 17.9% (28/156) of tests yielded true pos results. The most common reason was to evaluate for Mc infection. PNGS did not detect Mc in patients with proven local disease and was pos in >75% with deep/disseminated disease. However, a negative result did not exclude significant Mc infection. Repeat testing can be considered if clinical suspicion is high but should not be done before standard blood cultures are negative. While more than 60% of the non-Mc tests were not clinically useful, there was modest added utility where infection is high on the differential especially patients with CNE.

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241. Molecular patterns of Streptococcus agalactiae (GBS) Strains Associated with Different Clinical Syndromes: Early-Onset Disease in Neonates, Intrauterine Infection, and Vaginal Colonization, an Orthodox Jewish Community (OJC) Residing in Bney Brak

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Background. Rectovaginal colonization during pregnancy with Group B *Streptococcus* (GBS) is a risk factor for early neonatal sepsis, and may also cause chorioamnionitis and fetal death. In Israel, the reported colonization rate in pregnant women is low, and therefore routine screening of pregnant women for GBS colonization is not recommended. We noticed higher rates of early-onset disease (EOD) due to GBS in newborns of women hospitalized in Maayaney Hayeshua Medical Center, which serves an Orthodox Jewish Community (OJC) in Israel. Therefore, our aim was to