Microbiology Resource Announcements

| Pathogenesis and Host Response | Announcement

Biofilm-derived bile duct microbiota in liver transplantation: high-quality genomes of *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Enterococcus faecium*

Anna Baborski,[1] Oliver Rohland,[2] Tia Wuenschmann,[1] Michael Bauer,[3,4] Rosalind J. Allen,[1,3] Anne Busch[1,3]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT We present draft genomes of *Klebsiella pneumoniae (K. pneumoniae*), *Enterococcus faecalis* (*E. faecalis*), and *Enterococcus faecium* (*E. faecium*) isolated from a bilioenteric catheter after liver transplantation. Genome sizes were 5.58 Mb, 2.95 Mb, and 2.78 Mb, with G+C contents of 57.25%, 37.6%, and 37.99%, respectively, highlighting biofilm-associated bile duct colonizers.

KEYWORDS biofilm, liver transplant patients, *Klebsiella*, *Enterococcus*, catheter

Liver transplant patients receive antibiotic treatment and immunosuppressive medication to prevent organ rejection (1, 2). However, this can lead to an increased risk of infection. *Enterobacteriaceae* and *Enterococcaceae* are the most prevalent families within biliary diseases (3, 4). We describe the genetic characteristics of *Klebsiella pneumoniae* (MS-Stent-001-342), *Enterococcus faecalis* (MS-Stent-001-343), and *Enterococcus faecium* (MS-Stent-001-344), three bacterial isolates from bilioenteric catheters from a liver transplant recipient. These strains were successfully used to establish an *in vitro* model for studying infection and bacterial interactions. The catheter, collected post-surgery from a liver transplant patient as part of the MS-Stent study at Jena University Hospital, was removed, rinsed, and processed with ultrasonication to detach the biofilm. Subsequently, the biofilm was stored in PBS or in MAST CRYOBANK at −70°C. For isolation, MS-Stent-001-342 was cultured on MacConkey Agar (Carl Roh, Karlsruhe, Germany). Meanwhile, MS-Stent-001-343 and MS-Stent-001-344 were grown on Enterococcus Selective Agar (NutriSelect, Darmstadt, Germany). All strains were incubated for 24 h at 37°C under aerobic conditions.

Each strain was cultured in BHI medium for 24 h at 37°C. To perform gDNA isolation, the protocol of the PureLink Genomic DNA Kit (Thermo Fisher Scientific, 2024) was followed. Here, 20 µL of ≥45,000 U lysozyme (50 mg/mL) was added to each sample instead of the lysis buffer to increase cell lysis. Using the multiplex PCR approach, neither of the isolated *Enterococcaceae* strains contains VanA or VanB resistance genes (5). This approach targets *vanA*, *vanB*, *vanC1*, *vanC2/C3*, *E. faecalis*, *E. faecium,* and *rrs* (16S rRNA) with primers as described before (5).

Following the gDNA isolation, strain specification was done using 16S gene sequencing by EUROFINS (Ebersberg, Germany) as previously described (6), identifying MS-Stent-001-342 as *K. pneumoniae*, MS-Stent-001-343 as *E. faecalis* and MS-Stent-001-344 as *E. faecium* using NCBI BLASTn (7).

Whole genome short read sequencing of all three strains was performed on the iSeq100 platform with a 300-cycle iSeq reagent kit generating paired-end (2 × 150 bp) raw reads. Here, libraries were prepared using standard protocols for the Illumina DNA Prep Kit and the Nextera CD indexes. A long-read sequencing library was prepared with a one-dimensional ligation sequencing kit SQK-LSK109 (Oxford Nanopore Technologies

Editor Daria Van Tyne, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Address correspondence to Anne Busch, anne.busch@uni-jena.de.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 8 August 2025
Accepted 14 November 2025
Published 22 January 2026

TABLE 1 Genomic characteristics of bilioenteric catheter-derived isolates on hybrid genomes

Characteristic	Value or result for bacterial strain:		
	MS-Stent-001-342	MS-Stent-001-343	MS-Stent-001-344
Species-level identification from genome assemblies	*K. pneumoniae*	*E. faecalis*	*E. faecium*
MLST	1,630	21	Unknown
Illumina raw reads	2,229,530	2,163,742	2,402,168
ONT raw reads, average length of ONT reads (bp)	149,581, 6,004.0 ± 8,240.2	266,093, 5,184.6 ± 6,491.6	376,061, 3,877.6 ± 5,522.4
Average read coverage of hybrid assemblies	58.0 ± 12.0	105.2 ± 24.2	208.6 ± 114.5
DNA size (bp)	5,581,245	2,946,259	2,782,127
G+C content (%)	57.25	37.6	37.99
Contig count	3	5	17
Largest contig	5,321,311	2,762,364	795,579
Contig N50 (bp)	5,321,311	2,762,364	447,908
Contig L50 (bp)	1	1	3
Total protein-encoding genes predicted	5,118	2,757	2,700
rRNA	25	12	3
tRNA	86	61	58
tmRNA	1	1	1
Contamination	Not contaminated	Not contaminated	No contamination detected

[ONT]) and NEBNext Companion Module for ONT Ligation Sequencing kit (New England BioLabs, USA) following each manufacturer's protocol. Subsequently, the library was loaded into a FLO-MIN106 flow cell (R9.4.1, ONT) on a MinION sequencer (ONT). Base-calling of the fast5 reads was done using MinKNOW (v.19.10.1 [ONT]) with Guppy v.3.3.3 in high-accuracy base-calling mode.

FastQC was used for read quality assessment (v.0.74) (8). *De novo* assembly of short reads was performed with SPAdes (v.3.15.5) (9), while long-read sequence *de novo* assembly was done using Flye (v.2.9.5) (10). Hybrid assemblies were then polished using pilon (v.1.20.1) (11). Subsequently, species identification on genome assemblies was done using NCBI BLASTn (7). The genome assemblies were checked using QUAST (v.5.3.0) (12), and annotations were performed with Prokka (v.1.14.6) (13). The multi-locus sequence types (MLSTs) of all three species were determined using MLST online service from Center for Genomic Epidemiology from the Technical University of Denmark (14). Contamination was checked with EZbio ContEST16S (15). Unless otherwise noted, default parameters were used for all software (Table 1).

ACKNOWLEDGMENTS

R.J.A. and A. Busch were supported by the Deutsche Forschungsgemeinschaft (German Research Foundation) under Germany's Excellence Strategy (EXC 2051—Project-ID 390713860).

During the preparation of this work, the authors used https://chat.openai.com/ for proofreading. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

We thank Lisa Karnbach for excellent technical assistance.

O.R. was responsible for sample collection and study support.

AUTHOR AFFILIATIONS

[1]Theoretical Microbial Ecology, Friedrich Schiller University Jena, Jena, Germany

[2]Department of General, Visceral and Vascular Surgery, Jena University Hospital, Jena, Germany
[3]Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena, Jena, Germany
[4]Department of Anaesthesiology and Intensive Care Medicine, University Hospital Jena, Jena, Germany

AUTHOR ORCIDs

Anna Baborski http://orcid.org/0000-0003-2993-3181
Anne Busch http://orcid.org/0000-0001-9560-0057

FUNDING

Funder	Grant(s)	Author(s)
Deutsche Forschungsgemeinschaft	390713860.	Anna Baborski
		Michael Bauer
		Tia Wuenschmann
		Rosalind J. Allen
		Anne Busch

AUTHOR CONTRIBUTIONS

Anna Baborski, Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing – review and editing | Oliver Rohland, Methodology, Writing – review and editing | Tia Wuenschmann, Software | Michael Bauer, Conceptualization, Funding acquisition, Writing – review and editing | Rosalind J. Allen, Conceptualization, Funding acquisition, Writing – review and editing | Anne Busch, Conceptualization, Investigation, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

Sequencing data for this study have been deposited in the NCBI. The BioProject PRJNA1280976 contains three BioSamples: SAMN49540934 (accession JBPKJC000000000, SRA runs SRR34394232 and SRR34394233), corresponding to *Klebsiella pneumoniae* MS-Stent-001-342; SAMN49540972 (accession JBPKJD000000000, SRA runs SRR34395282 and SRR34395283), corresponding to *Enterococcus faecalis* MS-Stent-001-343; and SAMN49541016 (accession JBPKJE000000000, SRA runs SRR34395232 and SRR34395233), corresponding to *Enterococcus faecium* MS-Stent-001-344.

ETHICS APPROVAL

Patient sampling was approved by consent of the patients and the competent ethics committee of the Jena University Hospital under the registration 2022-2824_1 Material.

REFERENCES

1. Shbaklo N, Tandoi F, Lupia T, Corcione S, Romagnoli R, De Rosa FG. 2022. Bacterial and viral infections in liver transplantation: new insights from clinical and surgical perspectives. Biomedicines 10:1561. https://doi.org/10.3390/biomedicines10071561
2. Hernandez MDP, Martin P, Simkins J. 2015. Infectious complications after liver transplantation. Gastroenterol Hepatol (N Y) 11:741–753.
3. Dondorf F, Graf M, Deeb AA, Rohland O, Felgendreff P, Ardelt M, Settmacher U, Rauchfuss F. 2023. Pathogen detection in patients with perihilar cholangiocarcinoma: implications for targeted perioperative antibiotic therapy. Hepatobiliary Pancreat Dis Int 22:512–518. https://doi.org/10.1016/j.hbpd.2022.01.005
4. Eberhardt N, Santamarina BG, Enghardt M-L, Rohland O, Hussain I, Tannert A, Thieme L, Rubio I, Arndt H-D, Bauer M, Busch A, Jürgen Rödel, Bettina Löffler. 2024. The effects of photoactivated ciprofloxacin and bile acids on biofilms on bile duct catheters. Int J Antimicrob Agents 63:107086. https://doi.org/10.1016/j.ijantimicag.2024.107086
5. Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H. 2000. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. J Clin Microbiol 38:3092–3095. https://doi.org/10.1128/JCM.38.8.3092-3095.2000
6. Dumann G, Rohland O, Abdel-Glil MY, Allen RJ, Bauer M, Busch A. 2024. Draft genomes of the bile duct microbiome strains *Klebsiella pneumoniae* and *Enterococcus lactis* isolated from bilioenteric drainages with biofilm-forming abilities. Microbiol Resour Announc 13:e0020224. https://doi.org/10.1128/mra.00202-24

7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
8. Babraham Bioinformatics. 2011. FastQC: a quality control tool for high throughput sequence data
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021
10. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads using repeat graphs. Nat Biotechnol 37:540–546. https://doi.org/10.1038/s41587-019-0072-8
11. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963
12. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086
13. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153
14. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11
15. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H, Chun J. 2017. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. Int J Syst Evol Microbiol 67:2053–2057. https://doi.org/10.1099/ijsem.0.001872