

Supplemental Methods

Endotoxin Quantification The UCS1 *E. coli* phage cocktail was serially diluted 3 logs (10^{-3}) in endotoxin-free water so that the endotoxin concentration fell within the range of the Endosafe Cartridge (PTS201, 0.1-1 EU/mL), then submitted to the Cell and Gene Therapy Center at Baylor College of Medicine for analysis. A Limulus Amoebocyte Assay (LAL) was performed on the diluted cocktail using a PTS201 Endosafe cartridge. The analysis certificate is in **Figure S4**.

Sterility Testing 100 μ L of UCS1 phage cocktail from each aliquot was sent to NOVA biologicals (Conroe, TX) for USP 71 sterility testing, with 50 μ L each inoculated into Fluid Thioglycollate medium and Tryptic Soy Broth. 100 μ L of the cocktail was also sent to NOVA for Acid Fast Bacteria (mycobacteria) testing. The analysis certificates are in **Figure S4**.

Genomic DNA preparation and sequencing

Phage genomic DNA was extracted and sequenced as described in Green et al 2017 [17]. Paired-end libraries were constructed from purified DNA. Amplicons were visualized via gel electrophoresis and purified to remove primer-dimers and non-specific amplicons (ChargeSwitch PCR Clean-up Kit, Invitrogen). Quantification utilized the Qubit® Fluorometer resulting in pooled samples at DNA mass of 100 ng per sample. The amplicon pool was sequenced on the Illumina MiSeq platform using reagent kit v2 (2 \times 250 bp) paired-end protocol yielding paired-end reads that overlap almost completely.

Bacterial genomic DNA was purified using Omega E.Z.N.A. Bacterial DNA Kit (Cat#D3350) according to manufacturer's protocol, and sequencing performed by Novogene Corporation Inc.

Supplementary Figures

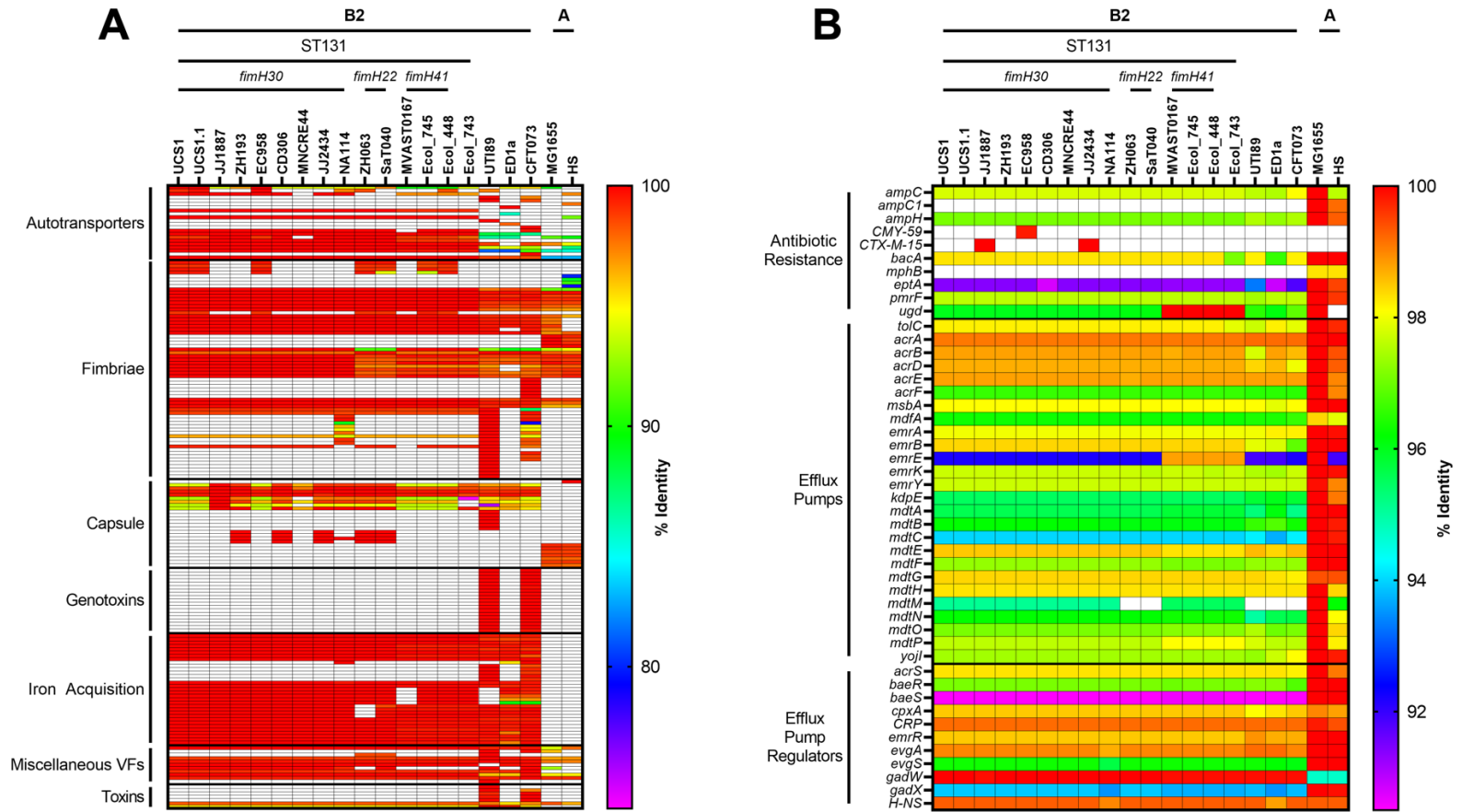


Figure S1. Comparative pathogenomics and resistomics of UCS1 and UCS1.1 with other *E. coli* species.

Several ST131 strains, including archetypal members of well-known ExPEC-associated ST95 (UTI89) and ST73 (CFT073) sequence types, and 3 non-pathogenic strains (ED1a, HS, and K-12 MG1655) were used as queries against either a custom *E. coli* virulence factor (VF) database (A) [37] or against the Comprehensive Antibiotic Resistance Database (CARD) (B). Heatmaps show the percent identity relative to the reference gene in the database. To generate the heatmaps with Graphpad Prism 9.2.0, megaBLAST aligned VFs or antibiotic resistance genes against each strain. The percent identity of High-scoring Segment Pairs (HSPs) that covered >90% of the reference gene was extracted and mapped.

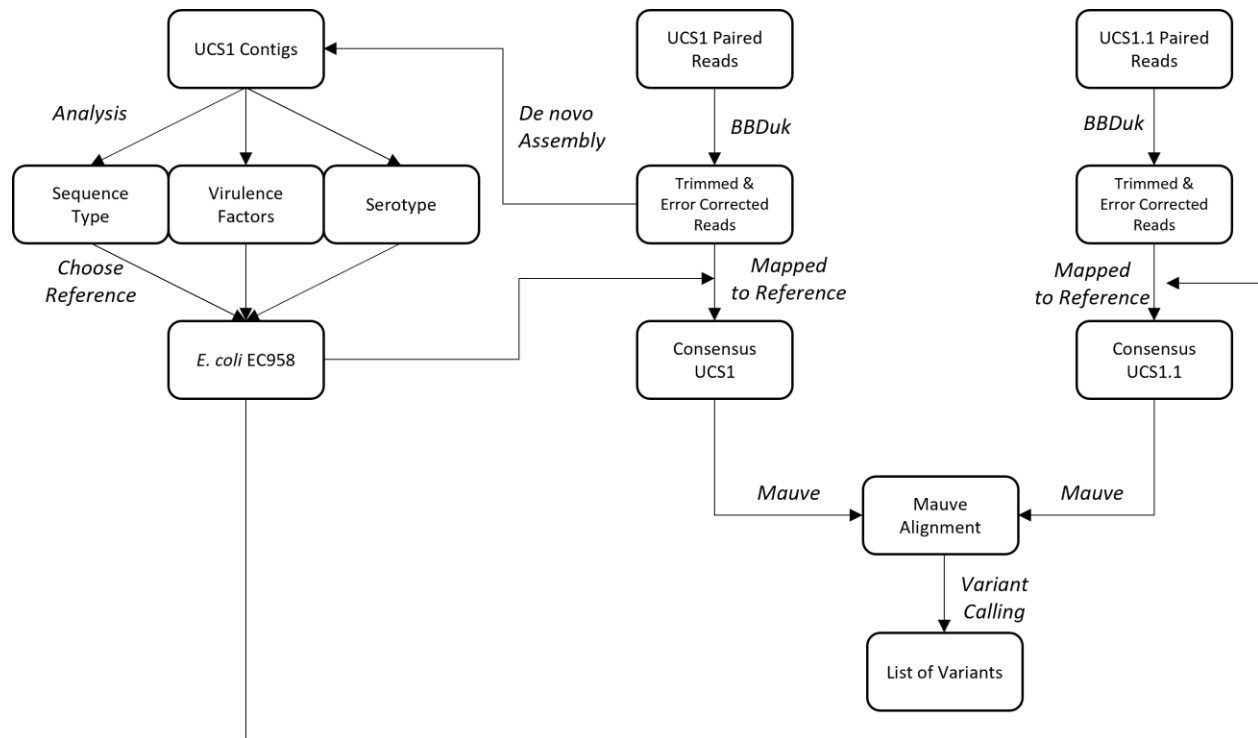


Figure S2. Bioinformatic pipeline for genomic comparison between UCS1 and UCS1.1

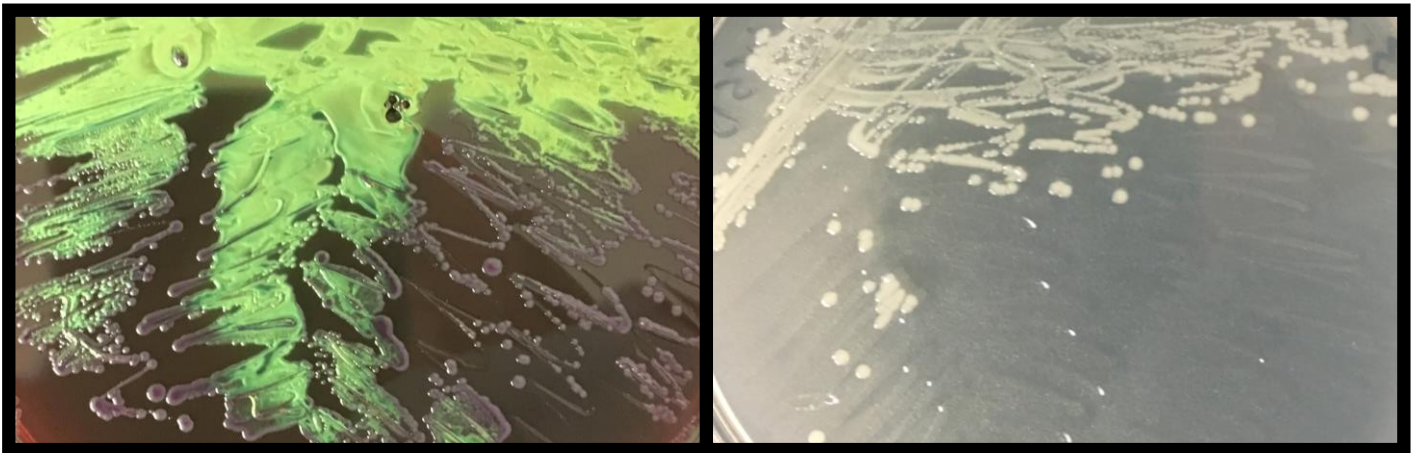


Figure S3. Colony Morphology of UCS1 *E. coli*. Clonal UCS1 colonies were streaked onto EMB agar (left) and LB agar (right) and incubated overnight at 37C.

A
B
C

WORKSHEET: BW03.32.27A ENDOTOXIN TESTING USING THE ENDOSAFe PTS DEVICE

EndoSafe Unit: ☐ PTS Reader SERIAL # 0203 ☐ BNC000# E000000000
☐ PTS Reader SERIAL # 2287 ☐ BNC000# E000000000
☐ PTS Reader SERIAL # 2717 ☐ BNC000# E000000001
☐ PTS Reader SERIAL # 2888 ☐ LOANER (RECORD SERIAL # ON PRODUCTION REPORT)

Sample Description
Test #: ESF9v8
AOAC #: 10.0.3.3.3 IN N/A CP N/A Loop UCS1.5-4.2.3.0.1
Third Party Donor ID: 2.1.1.8

Specimen Handling: Check all that apply:
☐ 1-40 Dilution in LAL Water ☐ Heat Inactivated Sample ☐ 1-40 Dilution in MFC ☐ add Dilution in LAL Water

Final Results: 0.455 EU/ml
(Print out results on the back of this form. Results were necessary)

Final Spike Recovery: 13.7 %
(Minimum Spike Recovery: 100%)

Acceptance Criteria:
Sample Run Time CV <25%
Spike Run Time CV <25%
Spike Recovery: 90% - 200%

Label If Available:
Phage Culture Room
Mikessio Lab
4/14/19

☐ Confirmatory Testing on Back Page
☐ Assay Failure: Repeats on Back Page

Analyst: Scott Sample **Date:** 10/24/19 **Reviewed by:** [Signature]
This form is double-sided

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Sample collection Date	Sample Received Date	Completed Date	Requisition
12-18-2019	12-19-2019	01-03-2020	M40228

Customer: Baylor College of Medicine
Street: One Baylor Plaza
City, State, Zip: Houston, TX 77030

Laboratory Report
USP<71>Sterility Testing, Direct Inoculation

Procedure: One Hundred (100 µL) microdilutors was aseptically removed from each vial. From each vial, fifty (50 µL) microdilutors was aseptically transferred to an appropriate volume of culture media, and incubated for no less than 14 days at the indicated temperature. At intervals during the incubation period and at its conclusion, the media was examined for macroscopic evidence of microbial growth. Appropriate negative controls and growth promotion of the media were included.

Modification: Per client request, sample volume diluted to 50 µL per media type.

Sample	Lot/Batch Number	Sample Extract Volume	Sample Results	Status
SLC Cocktail	8/59/19	100 µL	No Evidence of Microbial Growth	Pass
UCS Cocktail	10/23/19	100 µL	No Evidence of Microbial Growth	Pass

Interpretation:
The sample meets the requirements of the test for USP <71> Sterility testing if there is no evidence of microbial growth following 14 days incubation in Thioglycollate broth at 32.5°C ±1.2.5, and Tryptic Soy broth at 32.5°C ±1.2.5.

References:
USP <71> Sterility Test, 2017, United States Pharmacopeia - National Formulary (USP 40-NF15)

QA Review: [Signature] **Approved by:** [Signature] **Date:** 01/03/2020
Paul J. Tenen, Ph.D.
Laboratory Director
Specialist in Microbiology (SMA/ASCP Board of Certification)

The results shown on this report refer only to the sample(s) tested unless otherwise stated. No further evaluation of these results is made by Nova Biologicals, Inc. This report cannot be reproduced except in full, without prior written consent of Nova Biologicals, Inc.

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Sample received		Completed		Requisition
Date	Time	Date	Time	
12-19-2019	0900	02-03-2020	1400	M40229

Customer: Baylor College of Medicine
Street: 21300 BI 45 North
City, State, Zip: Spring, TX 77373
Phone: (281)353-3300

Laboratory Results

Sample: SLC Cocktail
Sample Lot/ Batch #: 08/30/19
Sample Matrix: Aqueous

Analyte	Test	AFB Smear	AFB Culture	Qualifiers
Acid Fast Bacteria	ASM Method for AFB	None Seen	Not Detected	None

Sample: UCS Cocktail
Sample Lot/ Batch #: 10/23/19
Sample Matrix: Aqueous

Analyte	Test	AFB Smear	AFB Culture	Qualifiers
Acid Fast Bacteria	ASM Method for AFB	None Seen	Not Detected	None

Interpretation:
Lowenstein Jensen Media is recommended for use in the cultivation and isolation of Mycobacterium species. Samples were incubated at 35° to 37° in CO₂ atmosphere for 8 weeks. Complete reporting of deviations from method requirements, regardless of the suspected impact on the data is required. Quality control failures not reported within a report summary are noted here.

The following quality control failures were noted: None
The following qualifiers were identified: None

Reference: American Society for Microbiology, Manual of Microbiology, 9th, 2007.

QA Review: [Signature] **Approved by:** [Signature] **Date:** 02/24/2020
Paul J. Tenen, Ph.D.
Laboratory Director
Specialist in Microbiology (SMA/ASCP Board of Certification)

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Figure S4. Characterization of UCS1 cocktail by certified laboratories. A. UCS1 phage cocktail was subjected to USP 85 endotoxin testing by limulus amoebocyte assay (Endosafe cartridges), **B.** USP 71 sterility testing by direct inoculation in rich media (Tryptic Soy Broth, Thioglycollate Broth), and **C.** Acid Fast Bacteria testing (Lowenstein Jensen Media)