

Clinical and Genomic Characterization of Persistent Enterococcal Bacteremia in the VENOUS Cohort

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BACKGROUND

- Enterococci are a leading cause of hospital-acquired bloodstream infections and a pathogen of increasing importance.
- The genomes of enterococci are highly dynamic, facilitating adaptation under environmental, antibiotic, and immune selective pressures.
- Adaptive evolution of these pathogens results in infections that persist even after the initiation of antimicrobial therapy.
- Previous studies have shown gain of, or changes in, genes associated with specific functional groups, such as in genes involved in carbohydrate or alternative nutrient utilization in enterococci *in vivo*.
- There has been little research done on the genomic and clinical dynamics of enterococcal bloodstream infections on a large or comprehensive scale.

RESULTS

Table 1. Clinical characteristics of persistent and non-persistent enterococcal infections.

Institution	Persistent (n=42)	Non-persistent (n=80)	Univariable p-value	Adjusted OR-GLMM
Cologne [n (%)]	8 (19.0)	12* (15.0)		
Henry Ford	7 (16.7)	14 (17.5)		
MDACC	15 (35.7)	30 (37.5)		
Memorial Hermann	5 (11.9)	10 (12.5)	0.99	Random effect to account for any site-specific clustering
Houston Methodist	4 (9.5)	8 (10.0)		
Fred Hutch	1 (2.4)	2 (2.5)		
Miami JHS	1 (2.4)	2 (2.5)		
Mississippi	1 (2.4)	2 (2.5)		
Species				
<i>E. faecalis</i> [n (%)]	14 (33.3)	28 (35.0)	1	
<i>E. faecium</i>	28 (66.7)	52* (65.0)		
Vancomycin susceptibility of index culture				
VRE	19 (45.2)	39 (48.8)	0.86	
VSE	23 (54.8)	41 (51.2)		
Patient gender (male) [n (%)]	30 (71.4)	37 (46.3)	0.01	3.04 (1.14-8.07)
Neutropenia (WBC <1.5 cells/uL) [n (%)]	15 (37.5)*	26 (35.6)**	1	
Charlson comorbidity index ≥ 3 [n (median; range)]	41 (8; 2-13)	78 (7; 0-14)	1	
Presence of an invasive device [n (%)]	33 (78.6)	68 (85.0)	0.52	
History of hospitalization in previous 365 days [n (%)]	32 (76.2)	57 (71.3)	1	
Index positive culture taken >2 days after admission [n (%)]	29 (69.0)	49 (61.3)	0.51	
Median (range) length of hospitalization after index positive culture, days	50.5 (10-354)	20 (3-101)	<0.001	1.04 (1.02-1.07)
Development of endocarditis during hospitalization [n (%)]	6 (14.3)	3 (3.8)	0.06	8.12 (1.65-39.9)
In-hospital mortality [n (%)]	17 (40.5)	19 (23.8)	0.09	1.23 (0.42-3.62)

*2 missing, **7 missing

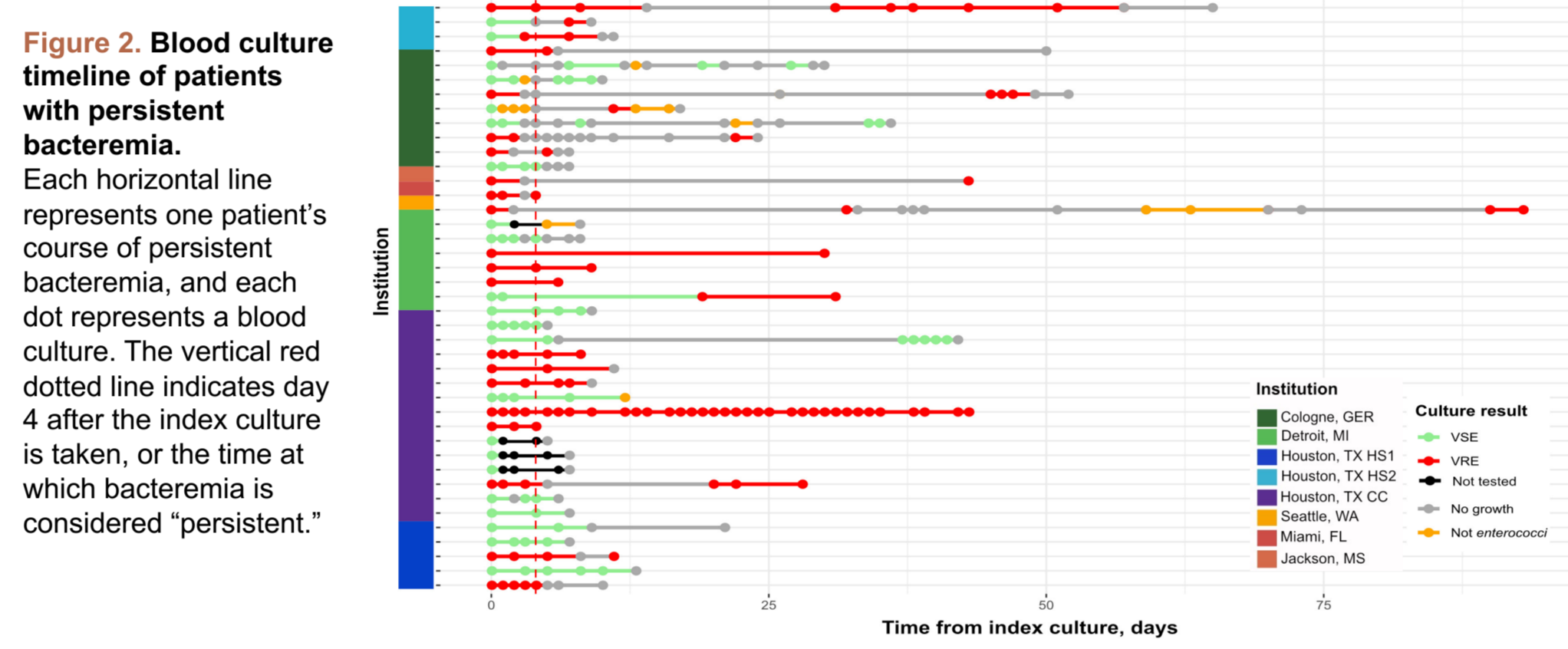


Figure 2. Blood culture timeline of patients with persistent bacteremia. Each horizontal line represents one patient's course of persistent bacteremia, and each dot represents a blood culture. The vertical red dotted line indicates day 4 after the index culture is taken, or the time at which bacteremia is considered "persistent."

Table 2. Descriptive genomic characteristics of persistent and non-persistent index blood cultures from patients with enterococcal bacteremia.

van gene	Persistent (n=28)		Non-persistent (n=52*)		Difference	P-value
	vanA	vanB	vanA	vanB		
vanA	17 (60.7%)	30 (57.7%)	3 (5.8%)	5 (9.6%)	3.0%	0.81
vanB	2 (7.1%)	5 (9.6%)	17 (32.7%)	1 (1.9%)	1.1%	1
None	9 (32.1%)	17 (32.7%)	1 (1.9%)	1 (1.9%)	1.9%	1
Average genome size (range), bp	3,171,909 (3,015,067-3,359,805)	3,081,634 (2,546,048-3,300,684)	3,171,909 (3,015,067-3,359,805)	3,081,634 (2,546,048-3,300,684)	90,275	0.31
Proportion of genome comprised of accessory genes	37.8%	35.5%	37.8%	35.5%	2.3%	0.22
Average number of plasmids (median [range])	4.9 (4.5; 2-9)	4.0 (4; 0-8)	4.9 (4.5; 2-9)	4.0 (4; 0-8)	0.9 (0.5)	0.12

Table 2B. *E. faecalis*

van gene	Persistent (n=14)		Non-persistent (n=28)		Difference	P-value
	vanA	vanB	vanA	vanB		
vanA	1 (7.1%)	5 (17.9%)	1 (3.6%)	0 (0%)	10.8%	0.65
vanB	1 (7.1%)	0 (0%)	12 (42.9%)	23 (82.1%)	7.1%	0.33
None	12 (85.7%)	23 (82.1%)	1 (3.6%)	5 (17.9%)	3.6%	1
Average genome size (range), bp	3,067,277 (2,873,527-3,339,286)	3,101,762 (2,733,050-3,452,763)	3,067,277 (2,873,527-3,339,286)	3,101,762 (2,733,050-3,452,763)	34,485	0.722
Proportion of genome comprised of accessory genes	26.9%	28.0%	26.9%	28.0%	1.1%	0.51
Average number of plasmids (median [range])	1.5 (1; 0-3)	1.7 (2; 0-5)	1.5 (1; 0-3)	1.7 (2; 0-5)	0.2 (1)	0.72

Table 3. Descriptive genomic characteristics of blood culture isolates from patients with persistent enterococcal bacteremia.

van gene	Index culture (n=28)		Persistent culture (day 4+) (n=28)		Change	P-value
	vanA	vanB	vanA	vanB		
vanA	17 (60.7%)	20 (71.4%)	2 (7.1%)	3 (10.7%)	+3	1
vanB	2 (7.1%)	3 (10.7%)	9 (32.1%)	5 (17.9%)	+1	1
None	9 (32.1%)	5 (17.9%)	1 (3.6%)	11 (39.3%)	-4	0.76
Average genome size (range), bp	3,171,909 (3,015,067-3,359,805)	3,179,570 (3,034,579-3,456,686)	3,171,909 (3,015,067-3,359,805)	3,179,570 (3,034,579-3,456,686)	+7,661	0.89
Proportion of genome that is comprised of accessory genes	33.4%	33.6%	33.4%	33.6%	+0.2%	0.88
Average number of plasmids (median [range])	4.9 (4.5; 2-9)	4 (4.7; 2-9)	4.9 (4.5; 2-9)	4 (4.7; 2-9)	-0.9 (0.5)	0.59

Table 3B. *E. faecalis*

van gene	Index culture (n=14)		Persistent culture (day 4+) (n=14)		Change	P-value
	vanA	vanB	vanA	vanB		
vanA	1 (7.1%)	2 (14.3%)	1 (7.1%)	1 (7.1%)	+1	1
vanB	1 (7.1%)	1 (7.1%)	12 (85.7%)	11 (78.6%)	+0	1
None	12 (85.7%)	11 (78.6%)	1 (3.6%)	2 (14.3%)	-1	1
Average genome size (range), bp	3,067,277 (2,873,527-3,339,286)	3,068,360 (2,873,526-3,339,275)	3,067,277 (2,873,527-3,339,286)	3,068,360 (2,873,526-3,339,275)	+1,083	0.98
Proportion of genome that is comprised of accessory genes	26.8%	26.9%	26.8%	26.9%	+0.1%	0.93
Average number of plasmids (median [range])	1.5 (1; 0-3)	1.64 (1.5; 0-4)	1.5 (1; 0-3)	1.64 (1.5; 0-4)	+0.14 (0.5)	0.81

METHODS

- Enterococcal bloodstream isolates collected through the Vancomycin Resistant Enterococcal Bacteremia Outcomes Study (VENOUS).
- Persistent bacteremia defined as "two blood cultures positive for enterococci of the same species and MLST sequence type (ST) ≥ four days apart in the same hospitalization period."
- Isolates were subjected to both short- and long-read whole-genome sequencing on Illumina (HiSeq 2000, NextSeq 2000, MiSeq) and Oxford Nanopore Technologies (GridION, R9.4.1 flow cells) platforms.
- Hybrid genomic assembly was performed using a bespoke pipeline, which utilizes flye v. 2.9.1 for long-read assembly, followed by iterative short- and long-read polishing steps.
- Mutations in each persistent isolate relative to the index isolate were identified using snippy v4.6.0. Clusters of Orthologous Groups (COGs) were inferred from mutation-containing genes and annotated using eggNOG-mapper v2.1.9.
- Maximum likelihood phylogenetic trees were generated using RAxML v.8.2.12 with a core genome alignment from Roary v.3.13.0.

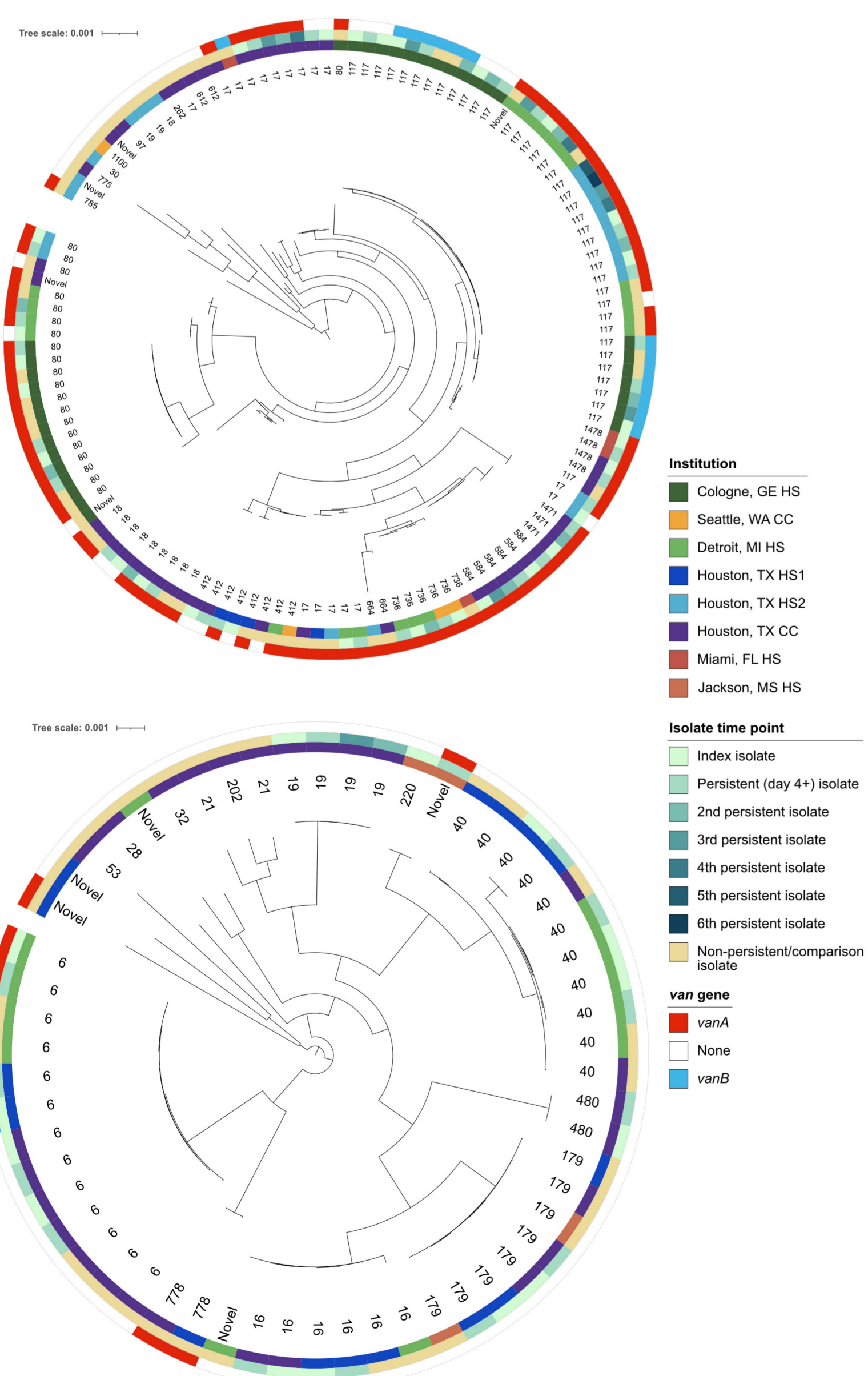
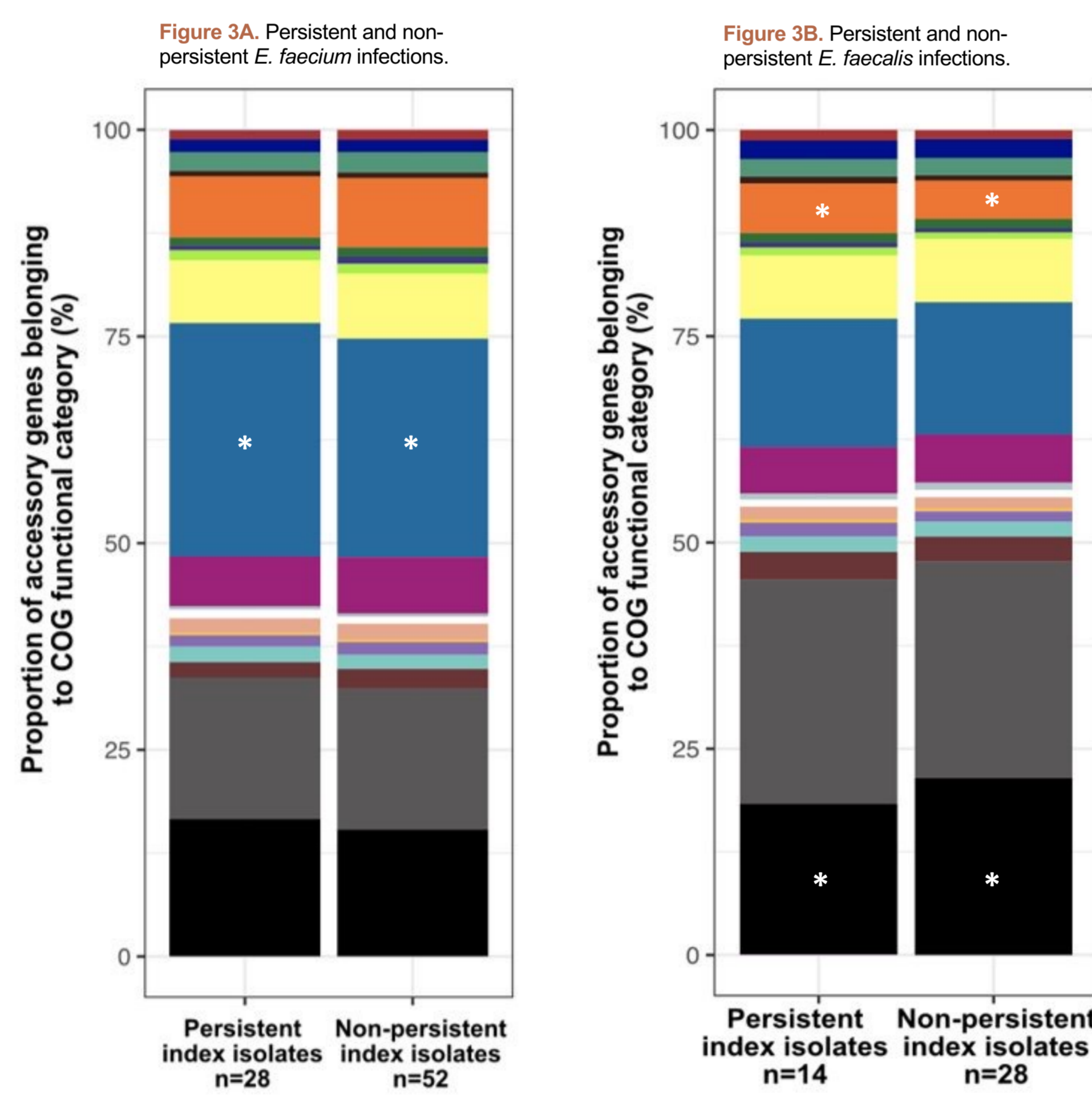


Figure 1. Midpoint-rooted, maximum likelihood phylogenetic trees of persistent and non-persistent enterococcal isolates from the VENOUS cohort.

Figure 1A represents *E. faecium* VENOUS isolates; Figure 1B represents *E. faecalis* VENOUS isolates. Isolates belonging to persistent bacteremia infections are denoted with a color strip on the outermost layer of each of the trees.

CC- cancer center; HS- hospital system

Figure 3. Functional characterization comparison of accessory gene presence/absence in persistent and non-persistent enterococcal bacteremia isolates.

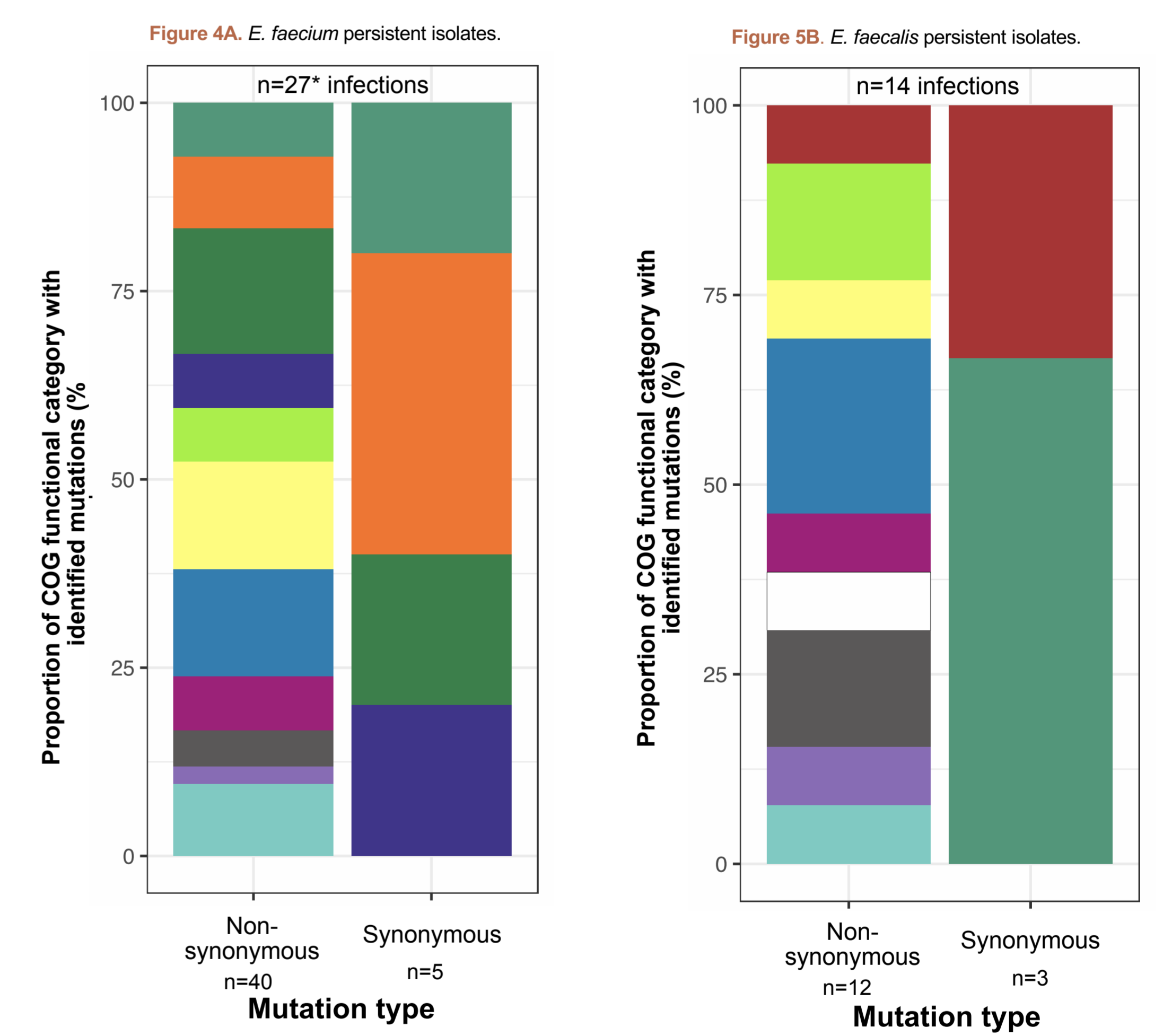


*significant difference (p < 0.05) between persistent and non-persistent groups.

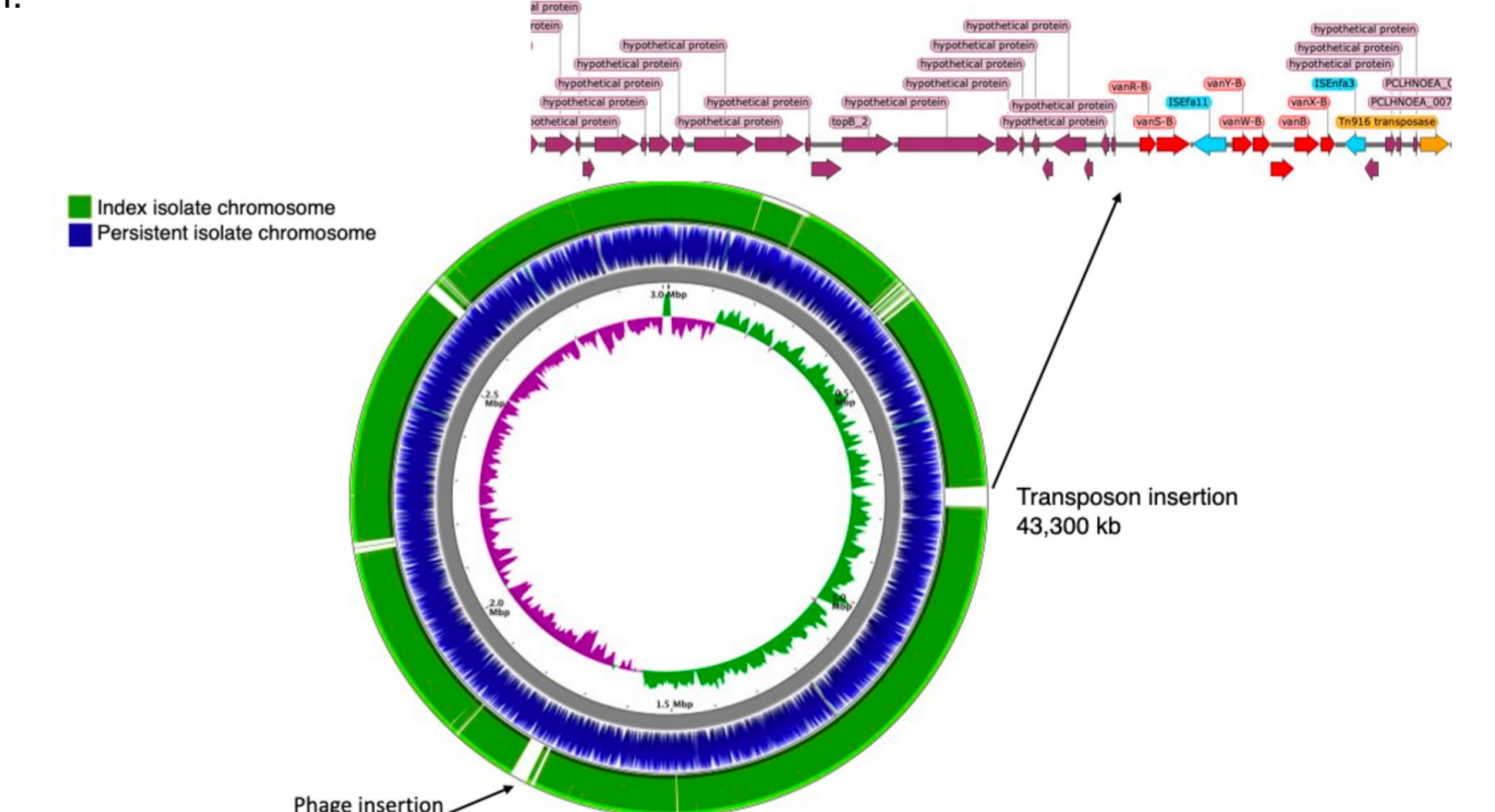
COG Functional Groups

- C- Energy production & conversion
- D- Cell cycle control/cell division
- E- Amino acid transport & metabolism
- F- Nucleotide transport & metabolism
- G- Carbohydrate transport & metabolism
- H- Coenzyme transport & metabolism
- I- Lipid transport & metabolism
- J- Translation/ribosomal structure & biogenesis
- K- Transcription
- L- Replication, recombination & repair
- M- Cell wall/membrane/envelope biogenesis
- N- Cell motility
- O- Post-translational modification
- P- Inorganic ion transport & metabolism
- Q- Secondary metabolites biosynthesis/transport
- T- Signal transduction mechanisms
- U- Intracellular trafficking/secretion
- V- Defense mechanisms
- W- Extracellular structures
- S- Function unknown
- Undefined

Figure 4. Functional characterization comparison of genes with mutations acquired during persistent infection.



* One infection was not included in the *E. faecium* mutation analysis, as the index/persistent isolate pair exhibited well above the average expected number of mutations (1,301) relative to the average of the remaining cohort (8.81). Over 80% of observed mutations were found in the chromosome, and large insertions of mobile elements were observed (Figure 5), including the insertion of a 43.3kb transposon carrying the *vanB* operon.



CONCLUSIONS

- Over 75% of patients with persistent bacteremia for which data was available had a recent history of hospitalization. Patients with persistent bacteremia more often developed endocarditis during hospitalization relative to patients with non-persistent bacteremia.
- Median length of hospitalization after first positive culture was higher for those with persistent *E. faecium* bacteremia relative to *E. faecalis* (45 vs. 16.5 days).
- Proportion of genes belonging to COG L was significantly higher in persistent *E. faecium* index isolates relative to non-persistent isolates (28.2% vs. 26.4%). In *E. faecalis*, the proportion of genes belonging to COG G was significantly higher in persistent vs. non-persistent isolates (6.03% vs. 4.67%).
- Mutational changes were markedly different across species, suggesting species-specific pathways of adaptation during the course of recalcitrant bloodstream infections.
 - E. faecium*: more COGs represented in identified mutations. Larger proportion of missense mutations in genes related to transcription (COG K) and carbohydrate transport/utilization (COG G).
 - E. faecalis*: over 2x more missense mutations identified than synonymous; largest proportion of missense mutations seen in genes associated with replication, recombination, and repair (COG L). Notably, mutations belonging to COG G (carbohydrate utilization) are absent.

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