Association of PBP4 Variants and β-Lactam Susceptibility in *Enterococcus faecalis*

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Tran TT[1,2], Simar SR[3], Egge SL[1,2,4], Atterstrom RL[1,2], Dinh AQ[1,2], Contreras G[4], Hanson BM[3], Zervos M[5], Abbo LM[6,7], Shimose L[8], Shelburne SA[9], Arias CA[1,2], Miller WR[1,2]

[1]Center for Infectious Diseases Research, Houston Methodist Research Institute, Houston, TX [2]Division of Infectious Diseases, Houston Methodist Hospital, Houston, TX [3]University of Texas School of Public Health, Houston, TX [4]Division of Infectious Diseases, University of Texas McGovern Medical School, Houston, TX [5]Division of Infectious Diseases, Henry Ford Hospital, Detroit, MI [6]Division of Infectious Diseases, Department of Medicine, University of Miami Miller School of Medicine, Miami, FL [7]Jackson Health System, Miami Transplant Institute, Miami, FL [8]Division of Infectious Diseases, Department of Medicine, University of Mississippi Medical Center, Jackson, MI [9]University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Background: Penicillin-binding protein 4 (PBP4) is a low affinity PBP that has been associated with decreased susceptibility to penicillins in *Enterococcus faecalis* (Efs). In vitro data have shown that changes in the promoter region leading to increased *pbp4* gene expression and amino acid changes resulting in active site remodeling contribute to this phenotype. There is limited data on the prevalence of these strains in the United States. We investigated β-lactam susceptibility trends in association with variations in PBP4 (allotypes) and the upstream promoter region.

Methods: Efs bloodstream isolates (n=184) were selected from the multicenter VENOUS cohort from 2016 to 2021. Whole genome sequencing (WGS) was performed on all isolates, and changes in the *pbp4* gene and promoter region 200 bp upstream of the start codon were identified using Efs JH2-2 as reference. Broth microdilution (BMD) testing for ampicillin (AMP), penicillin (PCN), piperacillin (PIP), and imipenem (IMI) was performed for 81 isolates. Analysis of MICs vs. WGS results was performed.

Results: A total of 31 PBP4 allotypes and 10 promoter variations were identified. ST6 isolates most frequently carried the promoter mutation ΔA117 (P6), previously shown to increase expression of the *pbp4* gene, with allotype 1 PBP4 (**Fig 1**). ST179 isolates most frequently carried the JH2-2 wild type promoter (P1) with the allotype 30 PBP4. All isolates were susceptible to AMP (MIC_{50} ≤1 µg/mL, MIC_{90} 2 µg/mL) and PCN (MIC_{50} ≤2 µg/mL, MIC_{90} 4 µg/mL; **Table 1**). PIP was the least potent β-lactam, with an MIC_{50} 4 µg/mL and an MIC_{90} of 8 µg/mL. Isolates with the P6 promoter had significantly higher piperacillin MICs (p<0.0001) as compared to P1.

Conclusions: Changes in the *pbp4* gene promoter correlated with an increase in PIP MICs. Caution should be used when choosing β-lactams other than AMP for definitive treatment deep-seated Efs infections.

Background

- Enterococci are among the most common causes of hospital-associated infections, with the most significant proportion of cases due to *Enterococcus faecalis*.[1]
- Enterococci are successful nosocomial pathogens since they are intrinsically resistant to many antimicrobial agents (i.e. cephalosporins) and can also acquire nonsusceptibility to other drugs, such as quinolones, glycopeptides, and aminoglycosides.[1,2]
- Ampicillin (AMP) resistance has been rarely reported in *E. faecalis*. Thus, severe *E. facalis* infectious are often treated with the combination of AMP and ceftriaxone.[3]
- However, the emergence of AMP-susceptible penicillin-resistant (ASPR) *E. faecalis* clinical isolates which exhibit increasing levels of resistance to penicillin threatens the use of β-lactams as a treatment option.[4-9]
- 2 main mechanisms attributed to reduced susceptibility to β-lactams:
  a. production of β-lactamases[10]
  b. Overproduction of PBP4, a low-affinity class B penicillin-binding protein[11,12]
- The ASPR phenotype, while uncommon, has been reported in many geographical regions of the world. However, its epidemiological impact in the US is unknown.

Objective/Aims

To investigate the β-lactam susceptibility trends in association with variations in PBP4 and upstream promoter region in a collection of clinical isolates of *Enterococcus faecalis* recovered from patients with bacteremia.

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Methods

Bacterial isolates: 184 *E. faecalis* isolates from the VENOUS cohort. The Vancomycin-Resistant Enterococcal BSI Outcomes Study (VENOUS) is a prospective cohort of study of adult patients with blood cultures positive for enterococci. Isolates were collected from 2016 – 2020.

Whole-genome sequencing: Extraction of genomic DNA, library preparation, and genome sequencing (Illumina platform) were performed. Paired-end sequencing data and genome assemblies are under National Center for Biotechnology Information Bioproject PRJNA665052. Midpoint-rooted maximum-likelihood phylogenetic tree based on core genomes was created using RAxML version 8.2.12 with 100 bootstrap iterations. Changes in *pbp4* gene and promoter region 200 bp upstream of the start codon were identified using *E. faecalis* JH2-2 as reference.

Susceptibility testing: minimum inhibitory concentrations (MICs) of ampicillin (AMP), penicillin, piperacillin, and imipenem were determined using broth microdilution as described by the Clinical and Laboratory Standards Institute in a subset of representative strains (n = 80).

Results

Table 1. Geographical distribution of *E. faecalis* strains recovered from VENOUS cohort

SITE	City, State	No. Strains
A	Miami, FL	3
B	Jackson, MS	13
C	Detroit, MI	39
D	Houston, TX	58
E	Houston, TX	71

Table 2. PBP4 variation (allotypes) of *E. faecalis*

Allotype	Amino Acid Changes	No. Strains
1	A369V	74
2	V19I	1
3	V70I	1
4	A26T, A501T	1
5	T50I	2
6	T50I, T418A, L475M, A488T, D666P	1
7	T53K	1
8	T53K, E289K	2
9	T53E, L570I	4
10	S59T, E62K	1
11	S59T, E62K, E289K	3
12	S59T, E62K, T119N, E289K	1
13	S59T, E62K, E289K, A437T	4
14	delW38	1
15	T52N, A73S, A150S	7
16	T146I	1
17	G200A	1
18	D164G, P520S	1
19	K152N, I166V, Q228R, E286D, E289K	2
20	E289K	7
21	V223I	15
22	V223I, L570I	1
23	V223I, S204F	1
24	S204F	2
25	A488T	1
26	V582I	2
27	V582I, A677S	2
28	D573E	1
29	A501T	6
30	P520S	36
31	T665I	1

Results

Table 3. Promoter variation of *E. faecalis*

Category	Nucleotide Changes	No. Strains
P1	Reference, JH2-2	72
P2	A30C	35
P3	A30C, A143G	1
P4	A30C, A177G	1
P5	A30C, C44T, A92T	1
P6	delA117	42
P7	A30C, delA117	5
P8	InsA14	23
P9	InsA14, delA117	1
P10	InsT161	1
P11*	A30C, del197-208	1
P12*	A30C, del114-208	1

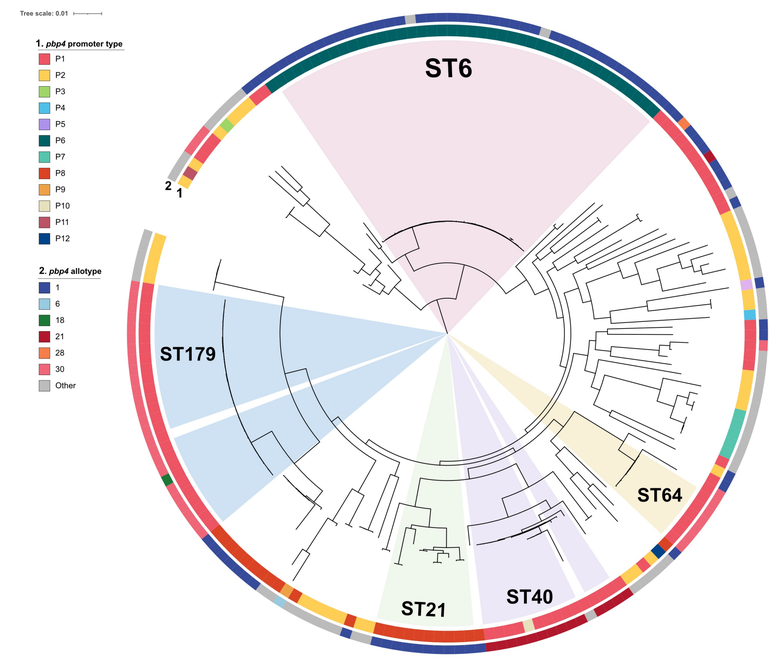

Table 4. β-lactam Susceptibility of *E. faecalis* by PBP4 Allotype and Promoter Variation (n = 80)

Promoter Type	PBP4 Allotype	No. isolates with MIC (µg/ml) of antimicrobial																			
		Ampicillin					Penicillin					Piperacillin					Imipenem				
		≤1	2	4	8	≥ 16	≤1	2	4	8	≥ 16	≤1	2	4	8	≥ 16	≤1	2	4	8	≥ 16
P1	All	27	7				1	23	10				4	20	9	1	11	21	2		
	1	5	1				1	4	1				2	2	2		3	3			
	18		1					1							1			1			
	21	8						7	1				1	7			3	5			
	28	1						1						1			1				
	30	11	4					8	7					9	4	1	3	10	2		
P2	All	16	1				2	12	2				5	10	1		5	12			
	20	3					1	2					1	2			1	2			
	15	2	1					3						3			1	2			
	27	2						2					2					2			
P3	All		1						1						1			1			
P6	All	14							14						12	2	1	9	4		
	1	14							14						12	2	1	9	4		
P8	All	13	2					13	2			1		10	4		9	5	1		
	1	11	2					11	2					10	3		8	4	1		
	5	2						2				1			1		1	1			

Conclusions

Changes in *pbp4* gene promoter correlated with an increased in piperacillin MICs. Caution should be used when choosing β-lactams other than ampicillin for definitive treatment of deep-seated *E. faecalis.*

References

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