

Supplementary information for

Genetic diversity among *S. aureus* isolates showing oxacillin and/or cefoxitin resistance not linked to the presence of *mec* genes

M. Angeles Argudín^{a#}, S. Roisin^a, L. Nienhaus^a, M. Dodémont^a,

R. de Mendonça^a, C. Nonhoff^a, A. Deplano^a, O. Denis^{a,b}

^a National Reference Centre – *Staphylococcus aureus*, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium

^b Ecole de Santé Publique, Université Libre de Bruxelles, Avenue Franklin Roosevelt 50, 1050 Brussels, Belgium

#Address correspondence to M. A. Argudín, maria.argudin@erasme.ulb.ac.be

Table of contents

Supplementary: Materials and Methods	2
Supplementary: Nucleotide accession numbers	3
References	5

Supplementary: Materials and Methods

The *pbp1*, *pbp2*, *pbp3*, *pbp4*, *gdpP* and *yjbH* genes and the *pbp4* promoter were amplified and sequenced using primers previously described (**Table S1**). Amplicons were obtained by using the PCR Master Mix Promega Benelux BV, and later purified with the illustra™ ExoStar™ 1-Step kit (GE Healthcare). Custom primers were designed to sequence each entire gene. Sequencing was performed by using the Dye Terminator DNA sequencing kit V1.1 (Applied Biosystems) followed by a purification using the Performa DTR Ultra 96-well Plate kit (Edge Bio). Sequences were assembled and analysed using the BioNumerics 7.6 software (Applied Maths). The sequences obtained were compared to available sequences of reference strains from the NCBI database (1). The strains ATCC 25923, ATCC 9144, NCTC 8325 and MSSA 476 were used as reference for *pbp1*, *pbp2*, *pbp3*, *pbp4* (including its promoter), *gdpP* and *yjbH* genes.

Table S1. Oligonucleotides used for sequencing in this study.

Gene detected	Primers	Sequence (5'-3')	Reference
<i>pbp1</i>	pbp1_Fw	GATACGCGAGGAAAGATTGC	(2)
	pbp1_Rv	TTTACGGCATAAGAGGCCAG	(2)
<i>pbp2</i> ^a	pbp2_Fw	TCGAAGTATTTTGGGAAGAG	(2)
	pbp2_Rv	GTGAATGACTGATTTTACG	(2)
	pbp2_Fw2	AAAGTGAGGACCGCGTATGAC	(3)
<i>pbp3</i>	pbp2_Rv2	AGCACCGTAAGTACCAGTACC	(3)
	pbp3_Fw	GTATGATTACTTGTTTCGGTCTC	(2)
<i>pbp4</i> ^b	pbp3_Rv	CAACCATGCGCTACACAATC	(2)
	AbcA-UpF	TGATTTGCTGCTTGACAGG	(4)
<i>gdpP</i>	P1	CAGATTGTGTAAGTGTGATATCTTTTG	(5)
	pbp4_Fw	GAGTAAGTTTGCTCTTCG	(2)
	pbp4_Rv	GTACAGAAGGCATTTTCGACG	(2)
	gdpP_Fw1	CCTACGACGCATATTGTAGGAC	(3)
<i>yjbIH</i>	gdpP_Rev	GCTTCGGCAATTTGTTTTGT	(6)
	yjbIH_SA2	CTGCACTACGCATAAGAGTTAAAG	(7)
	yjbIH_SA3	CCGGATCCACACTTCTATATGAATTATTATG	(7)

^a Two different pair of primers were used for amplified the entire *pbp2* gene region.

^b Two different pair of primers were used for amplified the entire *pbp4* gene and its promoter region.

Supplementary: Nucleotide accession numbers

Table S2. Nucleotide accession numbers of the sequences generated in this study and deposited in GenBank.

Strain	Gene	Nucleotide accession number	Strain	Gene	Nucleotide accession number
BORSA-001	<i>pbp1</i>	MF070915.1	BORSA-008	<i>pbp1</i>	MF070922.1
	<i>pbp2</i>	MF070947.1		<i>pbp2</i>	MF070954.1
	<i>pbp3</i>	MF070979.1		<i>pbp3</i>	MF070986.1
	<i>pbp4</i>	MF071011.1		<i>pbp4</i>	MF071018.1
	<i>gdpP</i>	MF071075.1		<i>gdpP</i>	MF071082.1
	<i>yjbIH</i>	MF071043.1		<i>yjbIH</i>	MF071050.1
BORSA-002	<i>pbp1</i>	MF070916.1	BORSA-009	<i>pbp1</i>	MF070923.1
	<i>pbp2</i>	MF070948.1		<i>pbp2</i>	MF070955.1
	<i>pbp3</i>	MF070980.1		<i>pbp3</i>	MF070987.1
	<i>pbp4</i>	MF071012.1		<i>pbp4</i>	MF071019.1
	<i>gdpP</i>	MF071076.1		<i>gdpP</i>	MF071083.1
	<i>yjbIH</i>	MF071044.1		<i>yjbIH</i>	MF071051.1
BORSA-003	<i>pbp1</i>	MF070917.1	BORSA-010	<i>pbp1</i>	MF070924.1
	<i>pbp2</i>	MF070949.1		<i>pbp2</i>	MF070956.1
	<i>pbp3</i>	MF070981.1		<i>pbp3</i>	MF070988.1
	<i>pbp4</i>	MF071013.1		<i>pbp4</i>	MF071020.1
	<i>gdpP</i>	MF071077.1		<i>gdpP</i>	MF071084.1
	<i>yjbIH</i>	MF071045.1		<i>yjbIH</i>	MF071052.1
BORSA-004	<i>pbp1</i>	MF070918.1	BORSA-011	<i>pbp1</i>	MF070925.1
	<i>pbp2</i>	MF070950.1		<i>pbp2</i>	MF070957.1
	<i>pbp3</i>	MF070982.1		<i>pbp3</i>	MF070989.1
	<i>pbp4</i>	MF071014.1		<i>pbp4</i>	MF071021.1
	<i>gdpP</i>	MF071078.1		<i>gdpP</i>	MF071085.1
	<i>yjbIH</i>	MF071046.1		<i>yjbIH</i>	MF071053.1
BORSA-005	<i>pbp1</i>	MF070919.1	BORSA-012	<i>pbp1</i>	MF070926.1
	<i>pbp2</i>	MF070951.1		<i>pbp2</i>	MF070958.1
	<i>pbp3</i>	MF070983.1		<i>pbp3</i>	MF070990.1
	<i>pbp4</i>	MF071015.1		<i>pbp4</i>	MF071022.1
	<i>gdpP</i>	MF071079.1		<i>gdpP</i>	MF071086.1
	<i>yjbIH</i>	MF071047.1		<i>yjbIH</i>	MF071054.1
BORSA-006	<i>pbp1</i>	MF070920.1	BORSA-013	<i>pbp1</i>	MF070927.1
	<i>pbp2</i>	MF070952.1		<i>pbp2</i>	MF070959.1
	<i>pbp3</i>	MF070984.1		<i>pbp3</i>	MF070991.1
	<i>pbp4</i>	MF071016.1		<i>pbp4</i>	MF071023.1
	<i>gdpP</i>	MF071080.1		<i>gdpP</i>	MF071087.1
	<i>yjbIH</i>	MF071048.1		<i>yjbIH</i>	MF071055.1
BORSA-007	<i>pbp1</i>	MF070921.1	BORSA-014	<i>pbp1</i>	MF070928.1
	<i>pbp2</i>	MF070953.1		<i>pbp2</i>	MF070960.1
	<i>pbp3</i>	MF070985.1		<i>pbp3</i>	MF070992.1
	<i>pbp4</i>	MF071017.1		<i>pbp4</i>	MF071024.1
	<i>gdpP</i>	MF071081.1		<i>gdpP</i>	MF071088.1
	<i>yjbIH</i>	MF071049.1		<i>yjbIH</i>	MF071056.1

Strain	Gene	Nucleotide accession number	Strain	Gene	Nucleotide accession number
BORSA-015	<i>pbp1</i>	MF070929.1	BORSA-023	<i>pbp1</i>	MF070937.1
	<i>pbp2</i>	MF070961.1		<i>pbp2</i>	MF070969.1
	<i>pbp3</i>	MF070993.1		<i>pbp3</i>	MF071001.1
	<i>pbp4</i>	MF071025.1		<i>pbp4</i>	MF071033.1
	<i>gdpP</i>	MF071089.1		<i>gdpP</i>	MF071097.1
	<i>yjbIH</i>	MF071057.1		<i>yjbIH</i>	MF071065.1
BORSA-016	<i>pbp1</i>	MF070930.1	BORSA-024	<i>pbp1</i>	MF070938.1
	<i>pbp2</i>	MF070962.1		<i>pbp2</i>	MF070970.1
	<i>pbp3</i>	MF070994.1		<i>pbp3</i>	MF071002.1
	<i>pbp4</i>	MF071026.1		<i>pbp4</i>	MF071034.1
	<i>gdpP</i>	MF071090.1		<i>gdpP</i>	MF071098.1
	<i>yjbIH</i>	MF071058.1		<i>yjbIH</i>	MF071066.1
BORSA-017	<i>pbp1</i>	MF070931.1	BORSA-025	<i>pbp1</i>	MF070939.1
	<i>pbp2</i>	MF070963.1		<i>pbp2</i>	MF070971.1
	<i>pbp3</i>	MF070995.1		<i>pbp3</i>	MF071003.1
	<i>pbp4</i>	MF071027.1		<i>pbp4</i>	MF071035.1
	<i>gdpP</i>	MF071091.1		<i>gdpP</i>	MF071099.1
	<i>yjbIH</i>	MF071059.1		<i>yjbIH</i>	MF071067.1
BORSA-018	<i>pbp1</i>	MF070932.1	BORSA-026	<i>pbp1</i>	MF070940.1
	<i>pbp2</i>	MF070964.1		<i>pbp2</i>	MF070972.1
	<i>pbp3</i>	MF070996.1		<i>pbp3</i>	MF071004.1
	<i>pbp4</i>	MF071028.1		<i>pbp4</i>	MF071036.1
	<i>gdpP</i>	MF071092.1		<i>gdpP</i>	MF071100.1
	<i>yjbIH</i>	MF071060.1		<i>yjbIH</i>	MF071068.1
BORSA-019	<i>pbp1</i>	MF070933.1	BORSA-027	<i>pbp1</i>	MF070941.1
	<i>pbp2</i>	MF070965.1		<i>pbp2</i>	MF070973.1
	<i>pbp3</i>	MF070997.1		<i>pbp3</i>	MF071005.1
	<i>pbp4</i>	MF071029.1		<i>pbp4</i>	MF071037.1
	<i>gdpP</i>	MF071093.1		<i>gdpP</i>	MF071101.1
	<i>yjbIH</i>	MF071061.1		<i>yjbIH</i>	MF071069.1
BORSA-020	<i>pbp1</i>	MF070934.1	BORSA-028	<i>pbp1</i>	MF070942.1
	<i>pbp2</i>	MF070966.1		<i>pbp2</i>	MF070974.1
	<i>pbp3</i>	MF070998.1		<i>pbp3</i>	MF071006.1
	<i>pbp4</i>	MF071030.1		<i>pbp4</i>	MF071038.1
	<i>gdpP</i>	MF071094.1		<i>gdpP</i>	MF071102.1
	<i>yjbIH</i>	MF071062.1		<i>yjbIH</i>	MF071070.1
BORSA-021	<i>pbp1</i>	MF070935.1	BORSA-029	<i>pbp1</i>	MF070943.1
	<i>pbp2</i>	MF070967.1		<i>pbp2</i>	MF070975.1
	<i>pbp3</i>	MF070999.1		<i>pbp3</i>	MF071007.1
	<i>pbp4</i>	MF071031.1		<i>pbp4</i>	MF071039.1
	<i>gdpP</i>	MF071095.1		<i>gdpP</i>	MF071103.1
	<i>yjbIH</i>	MF071063.1		<i>yjbIH</i>	MF071071.1
BORSA-022	<i>pbp1</i>	MF070936.1	BORSA-030	<i>pbp1</i>	MF070944.1
	<i>pbp2</i>	MF070968.1		<i>pbp2</i>	MF070976.1
	<i>pbp3</i>	MF071000.1		<i>pbp3</i>	MF071008.1
	<i>pbp4</i>	MF071032.1		<i>pbp4</i>	MF071040.1
	<i>gdpP</i>	MF071096.1		<i>gdpP</i>	MF071104.1
	<i>yjbIH</i>	MF071064.1		<i>yjbIH</i>	MF071072.1

Strain	Gene	Nucleotide accession number	Strain	Gene	Nucleotide accession number
BORSA-031	<i>pbp1</i>	MF070945.1	BORSA-032	<i>pbp1</i>	MF070946.1
	<i>pbp2</i>	MF070977.1		<i>pbp2</i>	MF070978.1
	<i>pbp3</i>	MF071009.1		<i>pbp3</i>	MF071010.1
	<i>pbp4</i>	MF071041.1		<i>pbp4</i>	MF071042.1
	<i>gdpP</i>	MF071105.1		<i>gdpP</i>	MF071106.1
	<i>yjbIH</i>	MF071073.1		<i>yjbIH</i>	MF071074.1

References

1. Anonymous. National Center for Biotechnology Information. <http://www.ncbi.nlm.nih.gov/>. Accessed February 2018.
2. Fuller E, Elmer C, Nattress F, Ellis R, Horne G, Cook P, Fawcett T. 2005. Beta-lactam resistance in *Staphylococcus aureus* cells that do not require a cell wall for integrity. *Antimicrob Agents Chemother* 49:5075-80.
3. Argudín MA, Dodemont M, Taguemount M, Roisin S, de Mendonca R, Deplano A, Nonhoff C, Denis O. 2017. In vitro activity of ceftaroline against clinical *Staphylococcus aureus* isolates collected during a national survey conducted in Belgian hospitals. *J Antimicrob Chemother* 72:56-59.
4. Villet RA, Truong-Bolduc QC, Wang Y, Estabrooks Z, Medeiros H, Hooper DC. 2014. Regulation of expression of *abcA* and its response to environmental conditions. *J Bacteriol* 196:1532-9.
5. Hamilton SM, Alexander JAN, Choo EJ, Basuino L, da Costa TM, Severin A, Chung M, Aedo S, Strynadka NCJ, Tomasz A, Chatterjee SS, Chambers HF. 2017. High-Level Resistance of *Staphylococcus aureus* to beta-Lactam Antibiotics Mediated by Penicillin-Binding Protein 4 (PBP4). *Antimicrob Agents Chemother* 61.
6. Pozzi C, Waters EM, Rudkin JK, Schaeffer CR, Lohan AJ, Tong P, Loftus BJ, Pier GB, Fey PD, Massey RC, O'Gara JP. 2012. Methicillin resistance alters the biofilm phenotype and attenuates virulence in *Staphylococcus aureus* device-associated infections. *PLoS Pathog* 8:e1002626.
7. Engman J, Rogstam A, Frees D, Ingmer H, von Wachenfeldt C. 2012. The YjbH adaptor protein enhances proteolysis of the transcriptional regulator Spx in *Staphylococcus aureus*. *J Bacteriol* 194:1186-94.