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Letters to the Editor

Diversity of Penicillin Binding Proteins among Clinical *Streptococcus pneumoniae* Strains from Portugal[▽]

The main mechanism of resistance of pneumococci to penicillin is the alteration of the affinities of penicillin binding proteins (PBPs) to β-lactams by heterologous recombination events (4). For the present study, we have investigated the diversity of the *penA* (accession numbers AM779386 to AM779409), *pbpX* (AM779338 to AM779361), and *pbp1A* (AM779362 to AM779385) genes of 21 clinical Portuguese *Streptococcus pneumoniae* strains (randomly selected from the strain collection of the Antibiotic Resistance Unit at the National Institute of Health in Lisbon) in relation to their penicillin susceptibilities; four ATCC strains were also added to the sample. The analyzed sequences were compared to the R6 amino acid sequence. MICs to penicillin G were determined and interpreted as previously described (3).

The amino acid substitutions located in the vicinity of conserved PBP motifs are shown in Table 1. We found 21, 23, and 20 different alleles in PBP2B, PBP2X, and PBP1A, respectively; only 4, 5, and 8 alleles were already present in the DDBJ/EMBL/GenBank databases, respectively. In comparison to the amino acid sequence of strain R6, the average number of amino acid substitutions in strains susceptible (n = 8), intermediate (n = 10), and resistant (n = 7) to penicillin were 3, 32, and 24 for PBP2B; 15, 52, and 23 for PBP2X; and 5, 46, and 28 for PBP1A, respectively.

Among nonsusceptible strains, PBP2B presented short mosaics in Ala-Phe-Ser-Arg-Pro-Met (5/17), Ala-Phe-Ser-Val-Pro-Met (1/17), and Pro-Ala-Phe-Ser-Val-Pro-Thr (1/17) from residues 431 to 437; these mosaics were variants of those found by Dowson and others (4, 9). The Ala624Gly

TABLE 1. Deduced amino acid substitutions in PBP2B, PBP2X, and PBP1A of 25 pneumococcal strains[a]

Strain	Resistance phenotype	Serotype	MIC[b]	Amino acid substitutions in PBP2B at position:												No. of amino acid substitutions in PBP2B:		
				443	451	481	485	488	494	614	624	629	630	633	635	DM	TP	ORF
R6	S		0.0125	Q	T	E	S	G	T	L	A	A	D	Q	T			
ATCC BAA-334	S	4	0.0125	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0
URA4929	S	9V	0.0125	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0
URA4376	T, E, C	6B	0.0125	.	.	.	.	.	.	.	.	.	.	.	.	1	0	1
URA3537	S	3	0.0125	.	.	.	.	.	.	.	.	.	.	.	.	1	1	2
URA2543	S	NT	0.025	.	.	.	.	.	.	.	.	.	.	.	.	2	0	2
URA4933	E	14	0.0125	.	.	.	.	.	.	.	.	.	.	.	.	3	0	3
URA3558	P, T, E, C	23	0.2	.	.	.	.	.	.	.	.	.	.	.	.	3	1	4
URA4087	S	19F	0.025	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0
URA4566	P, T, E, C	6B	0.1	.	A	G	.	.	S	.	.	.	G	E	N	0	13	18
URA2542	S	22	0.05	.	A	G	.	.	S	S	.	E	G	E	N	1	12	17
URA3635	P	19A	0.1	.	A	G	.	.	S	.	.	.	.	.	.	6	6	13
URA4731	P	14	0.1	E	A	G	.	A	A	A	.	E	G	E	N	1	32	37
URA5391	P	23F	0.1	E	A	G	.	A	A	S	.	E	G	E	N	1	34	40
URA5779	P, T, E, C	15A	0.1	.	A	G	.	.	S	A	.	E	G	E	N	1	10	16
ATCC 700670	P, X, R, T, F	6B	2	E	A	G	.	.	S	A	.	.	G	E	N	2	11	14
ATCC700673	P, T, E, C	19A	2	.	A	G	.	.	S	.	.	.	.	.	.	2	15	18
URA5805	P, X, R, T, E, C	19A	1.6	E	A	G	A	.	A	T	G	.	G	E	N	9	31	50
ATCC 51916	P, X, R, E	23F	0.1	.	.	.	.	.	.	.	G	.	G	E	N	2	3	14
URA3543	P, X, R	14	2	E	A	G	A	.	A	T	G	.	G	E	N	5	21	37
URA6056	P, X, R	ND	3.2	E	A	G	.	.	S	T	G	.	G	E	N	6	33	50
URA3043	P, X, R	14	2	E	A	G	A	.	A	.	.	.	.	.	.	7	12	20
URA4203	P, X, R, T, E, C	14	1.6	E	A	G	A	.	A	.	.	.	.	.	.	7	12	20
URA5128	P	14	0.5	E	A	G	A	.	A	.	.	.	.	.	.	7	12	20
URA3699	P, T, E, C	19F	0.5	E	.	G	.	A	A	A	.	.	G	K	N	37	33	83
URA5450	P, T, E, C	24F	0.5	.	A	G	.	.	S	.	.	.	.	.	.	4	12	17

[a] The main motifs in the transpeptidase domain in PBP2B are 391SVVK, 448SSN, and 619KTG; in PBP2X are 337STMK, 395SSN, and 547KSG; and in PBP1A are 370STMK, 428SRN, and 557KTG. The amino acid positions of strain R6 were used as reference. The positions within or close to conserved motifs in the PBPs are shown. The dots indicate homologous amino acids. DM, dimerization domain; TP, transpeptidase domain; ORF, signifies amino acid substitutions encoded in the open reading frame; PASTA, penicillin-binding protein and serine/threonine kinase-associated domain; TG, transglycosylase domain; S, susceptible; T, tetracycline; E, erythromycin; C, clindamycin; F, chloramphenicol; P, penicillin; X, cefotaxime; R, ceftriaxone; ND, not determined.

[b] MIC values are against penicillin (μg/ml); strains were considered penicillin susceptible for MICs ≤0.06 μg/ml, intermediate for MICs of 0.1 to 1 μg/ml, and resistant for MICs ≥1.6 μg/ml.

[c] Total number of amino acid substitutions in PBP2B, PBP2X, and PBP1A.

TABLE 1—*Continued*

Amino acid substitutions in PBP2X at position:																No. of amino acid substitutions in PBP2X:			
338	339	343	346	347	364	369	371	384	394	400	546	550	552	595	605	DM	TP	PASTA	ORF
T	M	M	A	A	L	A	I	R	H	M	L	T	Q	Y	N				
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	1
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	2	0	2
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	1
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	2
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	1
.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	0	1	0	2
.	.	T	.	.	.	V	.	.	L	.	.	.	.	.	.	16	12	3	37
.	.	.	.	.	.	.	.	G	.	.	.	.	E	.	.	0	13	3	26
A	.	T	.	.	.	V	.	G	.	.	.	.	.	.	.	3	8	1	17
.	.	T	.	.	.	.	.	G	.	.	.	.	E	.	.	0	10	0	10
A	.	T	.	S	.	V	.	G	.	.	.	.	.	.	.	1	9	4	19
.	.	.	.	.	.	.	.	G	.	.	.	.	E	.	.	2	24	5	44
.	.	T	.	.	.	.	.	G	.	.	.	.	E	.	.	4	23	15	54
.	.	.	.	.	.	.	.	.	.	.	.	.	E	.	.	1	21	2	27
A	.	T	S	S	F	.	T	G	.	.	V	.	.	.	T	2	37	1	45
A	.	T	S	S	S	V	T	G	.	.	.	.	.	.	.	3	28	20	65
A	.	T	S	S	F	.	T	G	.	.	V	.	.	.	T	3	34	18	70
A	F	.	S	S	F	.	T	G	.	T	V	A	.	.	T	2	37	21	74
A	.	T	S	S	F	.	T	G	.	.	V	.	.	.	T	8	35	21	79
A	F	.	S	S	F	V	T	G	.	T	V	.	.	F	T	3	42	23	82
A	.	T	S	S	F	.	T	G	.	.	V	.	.	.	T	2	37	21	73
A	.	T	S	S	F	.	T	G	.	.	V	.	.	.	T	3	37	21	75
A	.	T	S	S	F	.	T	G	.	.	V	.	.	.	T	3	37	21	75
A	.	T	S	S	F	.	T	G	.	.	.	.	.	.	.	18	32	20	104
.	.	.	.	.	.	.	.	G	.	.	.	.	E	.	.	1	20	3	25

mutation (4/17) close to the KTG motif was previously associated with chromosomal DNA from a *Streptococcus mitis* isolate (6). Close to the SSN motif of PBP2B, the Thr451Ala (15/17) mutation associated with β-lactam resistance (5) was found associated with the Glu481Gly (16/17) mutation. Both these substitutions had also been observed in France, South Africa, and Japan (7, 9, 10). These mutations were also associated with a Thr494Ser/Ala (16/17) substitution, making the three the major mutations related to β-lactam resistance in PBP2B (2). Met400Thr at PBP2X was found in two strains resistant to cefotaxime. These mutations had been described as being implicated in the development of β-lactam resistance (1, 2). The mutation Gln552Glu, which participates in an alternative mechanism of resistance (8), was found in 10 strains. The Ile371Thr mutation (10/17) is associated with the conformational change of a loop in the entrance of the active-site cavity of PBP2X (1). We also found four insert sequences in PBP1A: 680APTTS681 (1/17), 680SSSTSQ681 (1/17), and 710NQNQ711 (2/17).

Amino acid sequences from penicillin-intermediate strains showed higher divergence than those from highly resistant strains for PBP2B (d = 0.0573, standard error [SE], 0.0052; and d = 0.0463, SE, 0.0053, respectively), PBP2X (d = 0.084, SE, 0.0067; and d = 0.0435, SE, 0.0044, respectively), and PBP1A (d = 0.0735, SE, 0.0059; and d = 0.0527, SE, 0.0054, respectively); also, the greatest diversity of serotypes was found in isolates with intermediate resistance (Table 1). This might be attributed to the higher clonality of resistant clones than of intermediate clones (12) or to the recombination events which may be the origin of the new PBP alleles found in the study (11).

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TABLE 1—*Continued*

Amino acid substitutions in PBP1A at position:												No. of amino acid substitutions in PBP1A:			Total amino acid substitutions[c]
371	388	432	546	574	575	576	577	606	609	611	612	TG	TP	ORF	
T	E	P	N	T	S	Q	F	L	N	L	T				
.	D	.	.	.	.	.	.	.	.	.	.	1	2	8	9
.	D	.	.	.	.	.	.	.	.	.	.	1	2	7	9
.	D	.	.	.	.	.	.	.	.	.	.	1	2	8	10
.	D	.	.	.	.	.	.	.	.	.	.	1	2	7	11
.	D	.	.	.	.	.	.	.	.	.	.	4	1	10	13
.	D	.	.	.	.	.	.	.	.	.	.	1	1	9	14
.	D	.	.	.	.	.	.	.	.	.	.	1	4	56	97
.	D	.	.	.	.	.	.	.	.	.	.	2	3	11	37
.	D	.	.	.	.	.	.	.	.	.	.	1	1	7	42
.	D	.	.	.	.	.	.	.	.	.	.	1	1	7	34
.	D	.	.	.	.	.	.	.	.	.	.	1	2	8	40
.	D	.	.	N	T	G	Y	.	D	.	.	1	11	19	100
.	D	.	.	.	.	.	.	.	.	.	.	2	2	9	103
.	D	.	.	.	.	.	.	.	.	.	.	3	28	43	86
A	D	T	G	N	T	G	Y	I	D	F	L	2	44	60	119
.	D	.	G	N	T	G	Y	V	D	.	.	13	44	90	173
S	D	T	G	N	T	G	Y	I	D	F	L	12	43	80	200
S	D	T	G	N	T	G	Y	I	D	F	L	1	47	60	148
A	D	T	G	N	T	G	Y	I	D	F	L	2	44	52	168
A	D	T	G	N	T	G	Y	I	D	F	L	2	37	52	184
A	D	T	G	N	T	G	Y	I	D	F	L	2	47	55	148
A	D	T	G	N	T	G	Y	I	D	F	L	2	36	43	138
A	D	T	G	N	T	G	Y	I	D	F	L	2	37	52	147
A	D	T	G	N	T	G	Y	I	D	F	Y	1	42	70	257
A	D	T	G	N	T	G	Y	I	D	F	Y	1	42	70	112

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