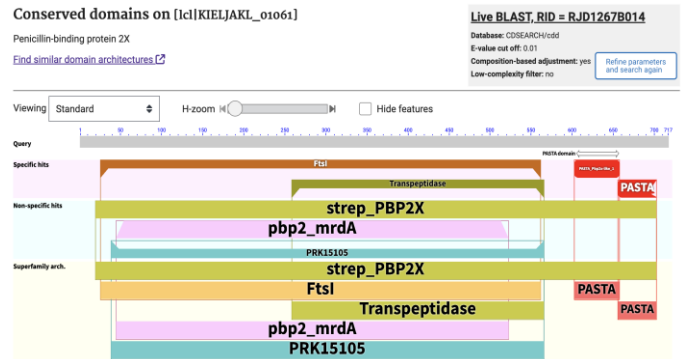


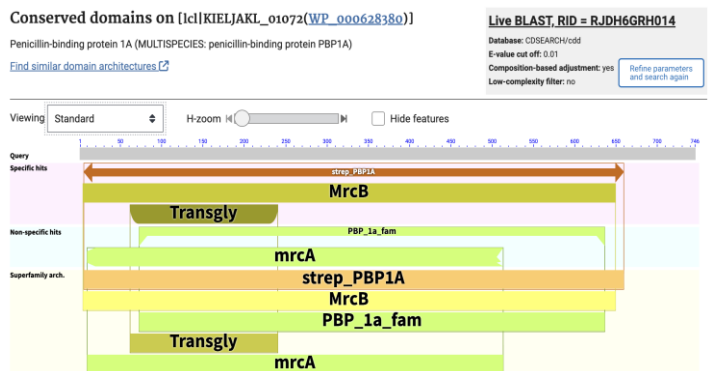
Figure S1. BLASTp alignment of penicillin-binding protein sequences (PBP2X, PBP1A, and PBP2B) of the reference genome *Streptococcus agalactiae* 2603V/R (Accession no. AE009948.1) and the genome of the penicillin non-susceptible isolate reported in this study and graphic summary of conserved domains. (a) sequence alignment and conserved domains of PBP2X; (b) sequence alignment and conserved domains of PBP1A; (c) sequence alignment and conserved domains of PBP2B.

(a)	Score	Expect	Method	Identities	Positives	Gaps
	1457 bits(3772)	0.0	Compositional matrix adjust.	714/717(99%)	716/717(99%)	0/717(0%)
Query Sbjct	1 36		MILTLTFFFIINFIIVITGDSKFGVNLSEAKKVVQSMVTQAKRGTIYDRNGNPIAE	60	95	
Query Sbjct	61 96		DATTYSLYAIISKNYTTATGKLYVQPSQYKEVASILENLGKKNLVLKQLNKKLFQV	120	155	
Query Sbjct	121 156		SFGSSGSLSYTKMADIKKTEKSDIKIGFSTSPGRIYPNGIFASQFIGTLPQDDGGG	180	215	
Query Sbjct	181 216		KKLVGNTGLEAANKVLSGTDGKVTYKDRSGNVLLGTATTERRAVNGKDIYTTLSEPIQ	240	275	
Query Sbjct	241 276		TVLETQMDVFAETKGFASATVWNAKTGEILATSQRPTNPSTLKGVDKKNLGTYNLL	300	335	
Query Sbjct	301 336		YDNFFPEPSTMKVMTLASIDSKHFNSTEVNSAQYIADAVIRDWDVNEGLSSGSYMTF	360	395	
Query Sbjct	361 396		PQGFHSSNVGMVTLQKMGDKMLNLSKFKGYPTFRGMLHESGGLFPSDNEVTIAMS	420	455	
Query Sbjct	421 456		SFGQIGIVTQVQLRAFTSISNDGVMLOPQFISSIDPNTGTSRTARKEVVGKPSKEAA	480	515	
Query Sbjct	481 516		SKTRDYMVTGTDPYGTLYAAGAPVIQVGNQSVAVKSGTAQIAQEGGGYLGKNDIN	540	575	
Query Sbjct	541 576		SVVAMVPSNPDPFIMYVTIQQSEKFSITFKVDVNPVLEQATAMKETILKPLNDSEHQ	600	635	
Query Sbjct	601 636		KYKLSKIVGNPHGVAEELRNVLQPIILGNQSKVSKVRKANLAENEQLLVLTNKL	660	695	
Query Sbjct	661 696		ELPDYMGWSKANVEQFAKWTGIKVYKGTSGKVRKQSIDVGSINKIKIKITIGD	717	752	



(b)

Score	Expect	Method	Identities	Positives	Gaps
1509 bits(3908)	0.0	Compositional matrix adjust.	743/750(99%)	744/750(99%)	4/750(0%)
Query 1	MITIKKESVIKLLKYAFGIIMGFIIITIVIGLLFAYVSRSPKLDQALKSVNSSLVD	60			
Sbjct 1	A.....	60			
Query 61	GNKKLIADLGSEKRESVADSIPNLNVAITSIEDKRFKRGVDIYRILGAAMHNLVSS	120			
Sbjct 61		120			
Query 121	NTQGGSLDQQLIKLAYFSTNKSQTLKRKSEVWLALQMERKYKEILTIFYINKVYM	180			
Sbjct 121		180			
Query 181	NGNYGMRTAKSYFGDKLKLISIAQLLAGIPQAPTQYDPYNPESAQTRNNTVLQMY	240			
Sbjct 181		240			
Query 241	QDKNISKKYEQAVATPVTGDKLKKQSTYPKYMDNLYKQVISEVKQTKGDIPTAGLK	300			
Sbjct 241		300			
Query 301	VYTNINTDAQQLQIYINDSYIAYPNNELQIASTINDATNGKVIQALGGRHQENISFG	360			
Sbjct 301		360			
Query 361	TNQSLVTRDWSGTMKPIAYAPADISGVYNSGTQSLNDSVYYPGTSQLYDWDQYMG	420			
Sbjct 361		420			
Query 421	WMSQTAIQSSRNVPVRALEAAGLDEAKSFLEKGIYYPENMYSNAISSNSSDAKYG	480			
Sbjct 421		480			
Query 481	ASSEKMAAASAFANGGYTKPYQVNIKEFSQGTNDTYAASGRAMKETTAYMTDMLKT	540			
Sbjct 481		540			
Query 541	VLFTGTGTAALPGVAQAGKTGTSNYTEDELAKEATTGIYNSAVGTMAPDENFVGYSK	600			
Sbjct 541		600			
Query 601	YTMAIWTGYNRLTPLYGSQLDIATEVYRAMSYLTGGYADNTMPGLYRSGSYLING	660			
Sbjct 601		660			
Query 661	TTTTGTYSYVYKNYQNSGQSSSSSTSEKQKEDNTANDANSSQVETPNNGAT	720			
Sbjct 661		720			
Query 721	TPNNSQTVPGTGH-----GNGNSNTAPNGN	746			
Sbjct 721	GNGN...N...V....	750			



(c)

Score	Expect	Method	Identities	Positives	Gaps
1386 bits(3587)	0.0	Compositional matrix adjust.	678/680(99%)	679/680(99%)	0/680(0%)
Query 1	MNRKKRYRLTVKKQNASIPRLNLLFFIIVLLFTVLILRLQMQIGQSFYMKKLTALTS	60			
Sbjct 2	L.....	61			
Query 61	YTVKESKARGQIFDAKGVAVENDERPTVAFSRGNISQSIKELANKLSHYITLTVAS	120			
Sbjct 62	V.....	121			
Query 121	SDRAKRDYLLADKANYYKVESLPDSKRYDKFGNHLAESTVYANAVAAVPVSAINSEDE	180			
Sbjct 122		181			
Query 181	LKVVALFNQMAVPTFGSVKSLTSELSDQIKKLADKELLGISVTSNHRKKGTSLTS	240			
Sbjct 182		241			
Query 241	DILGTISTEKAGLPREEVKYLLKGVSLNDRVGTSYLEKQYEDDLQGRQIRKVVNKKG	300			
Sbjct 242		301			
Query 301	KVVSQNIQEGKSGRNLLKLTIDLNQNKVESILKQYYSSELSSGRASFEGYVAIAEPS	360			
Sbjct 302		361			
Query 361	TGKVLAMAGLKNHNLVDDSLGTIAKNFTPGSVVKGATLSSGWNKVLRGNEVLVDQEI	420			
Sbjct 362		421			
Query 421	ANIRSWFTRGLTPIISAAQALEYSNTYMQVALRLMGQDNTGDALTRGQYQEAAMLRK	480			
Sbjct 422		481			
Query 481	TYGEYGLGVSTGLDPESEGYVPGKYSGLTLMESFGQDAYTPMQLGQYISTIANNGNR	540			
Sbjct 482		541			
Query 541	LAPHVSDIYEGNSNKAFLVRSITPKTLNKIAISDQELAIQEGFNVNWSGSGYATG	600			
Sbjct 542		601			
Query 601	TSMRGNVTIISGKTGAETFAKNVNGQTVSYNLNIAIYDNRKIAVAVMYPHTTDTTK	660			
Sbjct 602		661			
Query 661	SHQLVARDMIDQYISQFTGQ	680			
Sbjct 662		681			

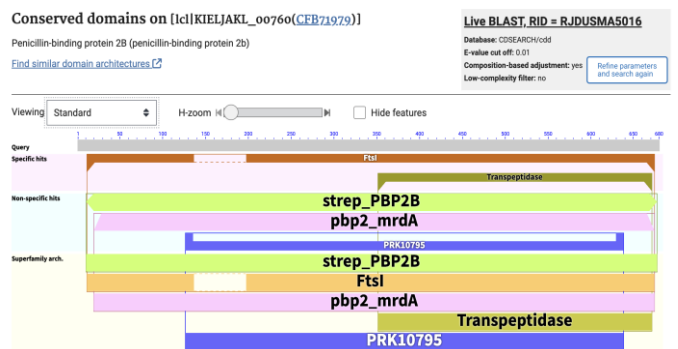


Figure S2. Temporal distribution of serotypes (A) and clonal complexes (B) among consecutively collected maternal GBS isolates from a single maternity in Brazil, 2008–2024. (A) Distribution of serotypes according to year of isolation (B) Distribution of clonal complexes according to year of isolation. *Indicates statistical significance ($P < 0.05$).

