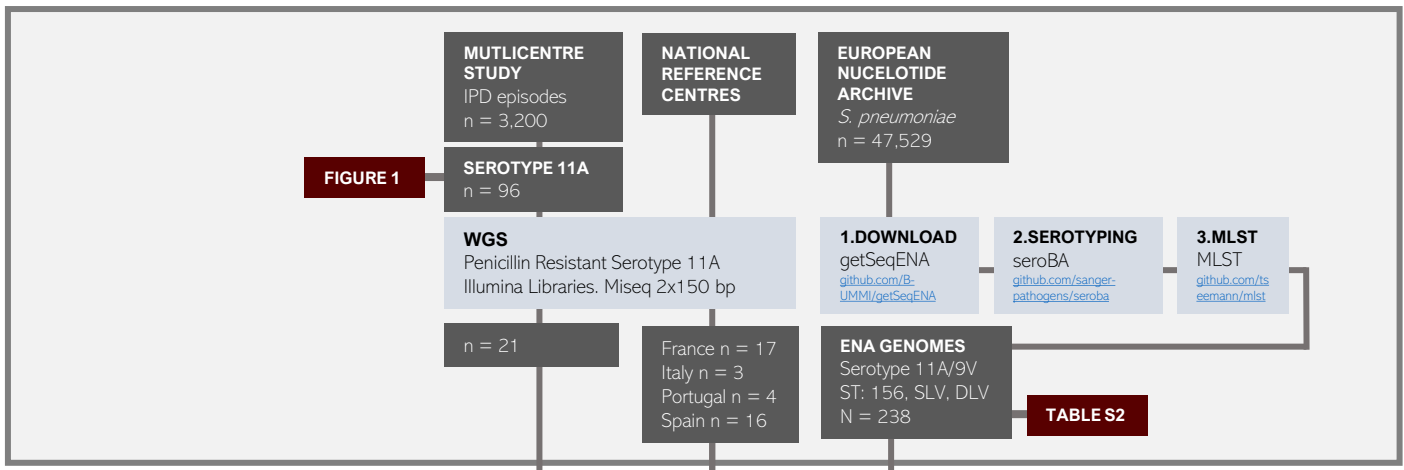


"This supplementary material is hosted by Eurosurveillance as supporting information alongside the article **"Two multi-fragment recombination events resulted in the β -lactam-resistant serotype 11A-ST6521 related to Spain^{9V}-ST156 pneumococcal clone spreading in south-western Europe, 2008 to 2016"** on behalf of the authors who remain responsible for the accuracy and appropriateness of the content. The same standards for ethics, copyright, attributions and permissions as for the article apply. Eurosurveillance is not responsible for the maintenance of any links or email addresses provided therein."

Supplementary Figure S1. Study design and Bioinformatic analysis diagram.

Schematic representation of flowchart used in this paper. Genomes from the previous multicentric study, genomes from National Reference centres and genomes downloaded from ENA 29-10-2018 are represented. All figures, supplementary material as well programs and pipelines used in bioinformatic analysis are also specificity in the diagram.



1.MOLECULAR ANTIBIOTIC RESISTANCE MECHANISM

1.GENE MUTATIONS
 Geneious R9
pbp1a, pbp2x, pbp2b, parC, parE, gyrA, folA and *folP* from R6 genome (NC_003098)

2.ACQUIRED RESISTANCE
 Abricate
 CARD and ResFinder
<https://github.com/tseemann/abricate>

FIGURE S3 **TABLE S3**

STUDY GENOMES
 Penicillin Resistant Serotype 11A
 N = 61

TABLE S1

FIGURE S2

1.ASSEMBLY
 INNUca
github.com/B-UMMI/INNUca

2.ANNOTATION
 PROKKA
github.com/tseemann/prokka

ALL GENOMES
 ENA genomes
 Study genomes
 N = 299

2.PHYLOGENETIC ANALYSIS

1.CORE-GENOME SNPs TREE
 Parsnp from Harvest suite
 Reference strain: ERR2681167
 PMID 25410596

2.VISUALIZATION
 Microreact
 PMID 28348833

FIGURE 2A

3.CAPSULAR OPERON ANALYSIS

REGION 1 – 3 ANALYSIS
 Geneious R9
 Region 1 (*pbp2x* to *dexB*)
 Region 2 (capsular locus)
 Region 3 (*aliA* to *pbp1a*)

REFERENCE GENOMES IDENTITY
 BLASTn
blast.ncbi.nlm.nih.gov/Blast.cgi

FIGURE 2B **TABLE S4**

SELECTED GENOMES
 n = 120

LINEAGE 1 ST156 n = 14 9V n = 4 11A n = 10	LINEAGE 2 ST166 n = 38 9V n = 2 11A n = 36	LINEAGE 3 ST838/6521 n = 68 9V n = 13 11A n = 55
--	--	--

4.IDENTIFICATION OF RECOMBINATION REGIONS

1.CORE-SNP ALIGNMENT BY READS MAPPING
 Snippy
github.com/tseemann/snippy

2.RECOMBINANT REGIONS DETECTION BY DENSISTY BASE SUBSTITUTION
 Gubbins
github.com/sanger-pathogens/gubbins

3.VISUALIZATION
 Phandango
 PMID

FIGURE 3

4.ACQUIRED FRAGMENT ANALYS
 Geneious R9
 Region A-D
 Region 1-7

5.REFERENCE GENOMES IDENTITY
 BLASTn
blast.ncbi.nlm.nih.gov/Blast.cgi

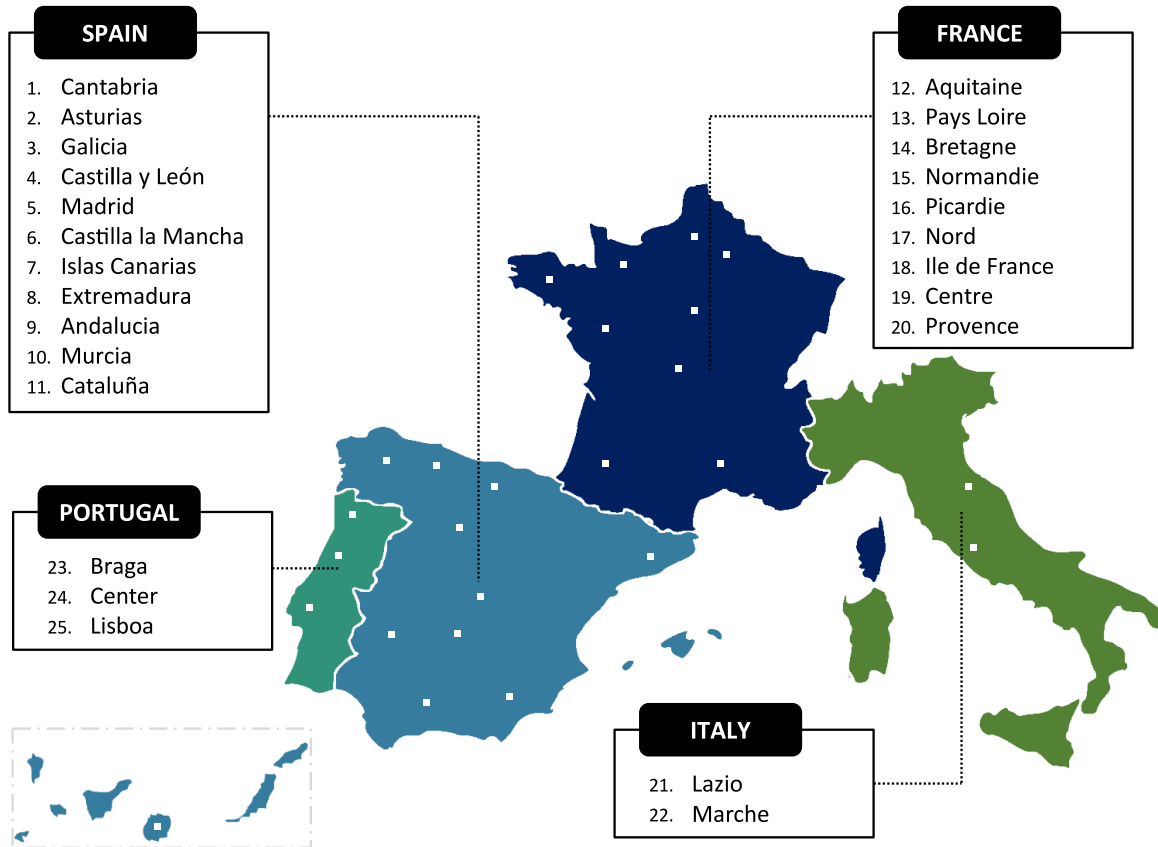
TABLE S5

5.ACCESSORY AND CORE GENOME

1.GENE PRESENCE/ABSENCE
 Roary
 Region 1 (*pbp2x* to *dexB*)
 Region 2 (capsular locus)
 Region 3 (*aliA* to *pbp1a*)
github.com/sanger-pathogens/Roary

2.GENE PRESENCE/ABSENCE ASSOCIATION BY ST
 Scoary
github.com/AdmiralenOla/Scoary

Supplementary Figure S2. Geographical distribution of strains subjected to whole genome sequencing. The map shows the region where strains were isolated. A total of 61 strains from 25 regions in 4 countries were included in this study.



Supplementary Table S1. Demographic, antibiotic MIC and WGS-derivate metadata.

- (a) Underlined and boldface ID indicate previously published strains
- (b) SP: Spain, IT; Italy, FR; France, PT; Portugal.
- (c) Capsular structures are the ones described in Figure 3

Supplementary Table S2. European Nucleotide Archive available genomes. *S. pneumoniae* genomes downloaded on 10-29-2018 belonging to serotype 9V or 11A with ST156, ST166, ST838 or ST6521.

Supplementary Table S3. Protein Alleles. The table summarizes the Pbp2X, Pbp1A, Pbp2B, DHFR and DHPS protein sequences of each strain compared to the R6 reference strain. Polymorphic sites associated with β -lactam resistance are highlighted with blue.

Supplementary Table S4. Amino acid sequence variation in the capsular operon. Amino acid changes relative to the capsular operon of strain 980/60-9V for serotype 9V strains and relative to the capsular operon of strain 1813/39-11A for serotype 11A strains.

Supplementary Table S5. Detailed description of the ORFs present in the recombination regions (core-genome and capsular locus). For each recombinational region in Figure 3 the putative genes are described. For each ORF, the locus tag of the genome-like recipient as well the putative protein function is specified. An approximate coordinates of the close genome 9V-ST156 (4041STDY6836167) are indicated as well the recombinational fragment size. Capsular locus genes are marked in grey, genes where the recombination could have occurred are coloured in light-blue. Those sequences which presented higher identity with the 4041STDY6836167 genome inside the recombinational regions are marked in yellow. Finally, regions inside recombinant fragment which are not related with the genome donor or genome recipient are marked in orange.