belong to the clonal complex (CC) CC130. In Spain, within this CC, the most common sequence type and *spa* type are ST1945 and t843, respectively.[5,8] In our patient, the initial PCR and the immunochromatographic technique that detects the presence of PBP2a identified the isolate as MSSA. However, after carrying out the antibiogram, resistance was confirmed by specific PCR and sequencing, also demonstrating the presence of the *mecC* gene integrated into the *SCCmec* XI cassette chromosome. Furthermore, through multilocus sequence typing (MLST), it was catalogued within CC130, and through molecular typing by PCR and subsequent sequencing of the *spa* type, a new *spa* type registered as t20888, within ST1945, was detected.

In conclusion, this was a case of invasive infection by *mecC* MRSA with a new *spa* type and with a fatal outcome in which the patient met the risk factors for infection by this microorganism: close contact with livestock, underlying disease, advanced age and nasal colonisation. In microbiology laboratories, it is important to carry out a manual antibiogram along with the use of rapid molecular techniques, since mechanisms not included in the molecular targets of commercial PCRs may be identified. This is essential for choosing the most appropriate antibiotic therapy.

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High frequency of *Streptococcus pneumoniae* serotype 3 in negative pleural fluid cultures from paediatric samples obtained in the Madrid region from 2018 to 2022, detected by direct identification using PCR-reverse-hybridization strip-based assay

Elevada frecuencia del serotipo 3 de Streptococcus pneumoniae *detectado mediante una técnica de PCR e hibridación reversa, en muestras pediátricas de líquido pleural con cultivo negativo, obtenidas en la Comunidad de Madrid entre 2018 y 2022*

The first pneumococcal conjugate vaccine introduced in 2006 in the paediatric immunization calendar of the Madrid region was the 7-valent (PCV7), which was substituted in 2010 by the 13-valent pneumococcal conjugate vaccine (PCV13). In 2012, PCV13 was withdrawn from the Madrid paediatric public immunization programme, being prescribed privately. In 2015, the PCV13 was re-introduced at the Spanish national paediatric public vaccine scheme.[1] The Quellung reaction is the gold standard for pneumococcal serotyping. This method requires strains isolated in culture. However, in samples as parapneumonic effusion (PPE) and pleural empyema (PE), commonly grouped as PPE/PE,[2] the bacterial growing is fastidious and the availability of colonies may be difficult. The objective of this work was to identify the not growing *Streptococcus pneumoniae* serotypes (SPNGST) causing pleural infection in children, in which the standard microbiological culture shows negative results.

Thirty-five PFS from paediatric patients (aged six months to 8 years; mean 3.4 years, standard deviation 2.1) with negative culture results, obtained between September 2018 and December 2022, were processed for the detection of the α-fucosidase gene using a real time PCR method (*Streptococcus pneumoniae* alpha-fucosidase gene Genesig® Advanced Kit; Primerdesign Ltd, United Kingdom). Positive α-fucosidase samples were subsequently tested by a PCR reverse-hybridization strip-based assay (S. PneumoStrip test; Operon S.A., Zaragoza, Spain) that allows serotype identification.

The α-fucosidase real-time PCR method was positive in 30 of the 35 samples studied (85.7%). The PCR reverse-hybridization assay showed positive results for the *lytA*, *cpsA* and *ply* genes in 28 of the 30 samples (93.3%). The serotypes detected by this technique in these 28 samples were twenty-one serotype 3 (75%), two serotype 8 (7.1%), one serotype 19A (3.6%), one serotype 1 (3.6%), one serotype 10A (3.6%), one 9A/9V (3.6%) and one 33F/33A (3.6%). Table 1 shows the distribution of PCR SPNGST detected in PFS according to time. Most serotype 3 cases occurred in 2022 (17/21). The only one PCV13 covered serotype 19A was identified in 2018. The only one PCV13 serotype 1 case was detected in 2020. The number of serotype 3 cases detected in PFS after 2020 was significantly

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Table 1
Distribution of not-growing *Streptococcus pneumoniae* serotypes detected by PCR in pleural fluid samples according to the year the sample was obtained.

Year	Number of samples	Positive real-time PCR for the α-fucosidase-gene	Positive PCR hybridization for *lytA*, *cpsA*, and *ply* genes	Serotypes detected by PCR hybridization						
				3	19A	8	10A	1	9A/9V	33F/33A
2018	4	3	3	1	1					1
2019	9	6	4	2		1			1	
2020	3	3	3	1		1		1		
2021	1	1	1				1			
2022	18	17	17	17						
2018–2022	35	30	28	21	1	2	1	1	1	1

higher (Fisher exact test $p < 0.01$) than the rest of the serotypes. Among the twenty-one children infected by serotype 3, one had received one dose of PCV13, another one two doses, fifteen children received three doses, one child four doses, and in three this data was unknown. The two cases caused by serotype 19A received two PCV13 doses and the case due to serotype 1 received four.

Although the number of cases included is small and this is not a systematic epidemiological study, the elevate frequency of detected SPNGST 3 in PFS is worrying. Serotype 3 is a well-identified cause of pleural PPE/PE in children and these cases are increasing in the PCV13 era.[2–4] We do not have a definitive explanation for the high number of cases of serotype 3 in 2022. The synergism between Influenza virus and *S. pneumoniae*[5] would to have influenced in this fact. During 2022, the number of influenza cases in the region of Madrid was higher than in the previous season and affected children to a great extent.[6]

Despite the fact that the use of PCV13 in children has led to a substantial reduction of IPD in Spanish children[7] and pneumococcal PPE/PE (especially associated to the drop of serotype 1),[8] the immunogenicity and the effectiveness against each of the 13 serotypes of the vaccine seems to be non-homogeneous.[3,4] The immunogenic activity has been described lower for serotype 3 than for other PCV13 serotypes,[9] and the individual vaccine protection against this serotype is also lower.[10] Moreover, the sensitivity of culture seems to be particularly very low for this serotype. Thus, serotype 3 prevalence data in pleural infection may be underestimated when surveillance is only based in culture and conventional phenotypic serotyping techniques. The routine implementation of PCR procedures in order to identify the serotype involved in culture-negative specimens provides additional information and can improve substantially the microbiological investigation of *Streptococcus pneumoniae* pleural infections. This approach can support epidemiological surveillance and monitoring of immunization plans.[4]

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