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To cite this version:

Yves Gillet, Anne Tristan, Jean-Philippe Rasigade, Mitra Saadatian-Elahi, Coralie Bouchiat, et al.. Prognostic factors of severe community-acquired staphylococcal pneumonia in France. European Respiratory Journal, In press. hal-03276134

HAL Id: hal-03276134

https://hal.science/hal-03276134v1

Submitted on 1 Jul 2021

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Prognostic factors of severe community-acquired staphylococcal pneumonia in France

Yves Gillet[1,2], Anne Tristan[2*], Jean-Philippe Rasigade[2*], Mitra Saadatian-Elahi[3], Coralie Bouchiat[2], Michele Bes[2], Oana Dumitrescu[2], Marie Leloire[2], Céline Dupieux[2], Frédéric Laurent[2], Gérard Lina[2], Jerome Etienne[2], Philippe Vanhems[3], Laurent Argaud[4], Francois Vandenesch[2], and the PVL pneumonia study group

A complete list of the "PVL pneumonia study group" is listed at the end of this article

* these authors equally contributed to the work

Authors affiliations:

1, Paediatric Emergency & Intensive Care Unit, Hospices Civils de Lyon, Lyon, France

2, French Reference Centre for Staphylococci, Institute for Infectious Agents, Hospices Civils de Lyon, Lyon, France

3, Infection Control and Epidemiology Department, Hospices Civils de Lyon, Lyon, France

4, Medical Intensive Care Unit, Hospices Civils de Lyon, Lyon, France (L.A.)

Corresponding author.

Francois Vandenesch, MD, PhD, Professor

Infectious Agents Institute

Hopital de la Croix Rousse

103, Bd de la Croix-Rousse, 69004 Lyon, France

email: francois.vandenesch@univ-lyon1.fr, tel: +33 4 72 00 37 03

Alternate corresponding author:

Yves Gillet, email: yves.gillet@chu-lyon.fr, tel: +33 4 27 85 56 42

Take home message.

In severe community-acquired staphylococcal pneumonia, Panton-Valentine leukocidin is associated with pleuropneumonia in toddlers and, in olders patients, with increased mortality independent of methicillin resistance and other virulence factors.

Abstract. (250)

Purpose. *Staphylococcus aureus* causes severe forms of community-acquired pneumonia (CAP), namely staphylococcal pleuropneumonia in young children and staphylococcal necrotising pneumonia in older patients. Methicillin resistance, the Panton-Valentine leukocidin (PVL) toxin, as well as less specific factors have been associated with poor outcome in severe CAP, but their respective roles are unclear.

Methods

A prospective multicentre cohort study of severe staphylococcal CAP was conducted in 77 paediatric and adult intensive care units in France between January 2011 and December 2016. After age-clustering, risk factors for mortality, including pre-existing conditions, clinical presentation, laboratory features, strain genetic lineage, PVL, other virulence factors, and methicillin resistance were assessed using univariate and multivariable Cox and LASSO regressions.

Results

Of 163 included patients, aged one month to 87 years, 85 (52.1%) had PVL-positive CAP; there were 20 (12.3%) patients aged <3 years (hereafter "toddlers"), among whom 19 (95%) had PVL-positive CAP. The features of PVL-positive CAP in toddlers matched with the historical description of staphylococcal pleuropneumonia, with a lower mortality (n=3/19, 15%) compared to PVL-positive CAP in older patients (n=31/66, 47%). Mortality in older patients was independently predicted by PVL-positivity (hazard ratio 1.81, 95% CI, 1.03 to 3.17) and methicillin resistance (2.37, 95% CI 1.29 to 4.34) independently from *S. aureus* lineages and the presence of other virulence determinants.

Conclusion

PVL was associated with staphylococcal pleuropneumonia in toddlers and was a risk factor for mortality in older patients with severe CAP, independently of methicillin resistance, *S. aureus* genetic background and other virulence factors.

Key words. Community-acquired pneumonia; *Staphylococcus aureus*; Panton Valentine leucocidin, Sepsis; Severity; Mortality

Plain Language summary

Pneumonia due to *Staphylococcus aureus* is rare but severe and is often associated with the production of a rarely encountered toxin, the Panton-Valentine leucocidin (PVL). This toxin is known to be associated with staphylococcal pleuropneumonia in young children and with necrotising pneumonia in adults but its role in severity, independently from other severity-associated factors, remains controversial. To address this issue, a prospective cohort study of patients with severe community-acquired staphylococcal pneumonia (CAP) admitted to an intensive care unit (ICU) was performed in France between January 2011 and December 2016. CAP was diagnosed using the previously described definition, and clinical and laboratory data were recorded at admission to ICU as well as during stay, with calculation of age-adapted severity scores. The causative isolate was fully characterised by the French national reference centre for staphylococci for genetic background and major virulence factors. One hundred and sixty-three patients were retained for analysis, of whom 52.1% were infected by a PVL-positive strain. Age-clustering found that patients aged <3 years (n=20) were almost all (95%) infected by a PVL-positive isolate, presented with symptoms distinct from those of older patients, and experienced lower mortality. In patients aged ≥3 years, potential predictors of mortality were investigated using multivariable Cox proportional hazards models. Mortality was significantly higher in the PVL-positive group and was independently predicted by PVL-positivity and methicillin resistance. The previously known effects of PVL (namely haemoptysis and leukopenia) were independent predictors of death regardless of *S.aureus* genetic background and the presence of other virulence determinants. This supports a model whereby PVL-induced respiratory epithelial necrosis (leading to haemoptysis) and leukopenia (due to the leukotoxic properties of PVL) mediate the link between PVL and severity, and not a mere association between PVL and severity in staphylococcal CAP.

Introduction

Staphylococcal necrotising pneumonia caused by *Staphylococcus aureus* was first described in 1919 in adults during the influenza pandemic [1]. It is associated with airway haemorrhage, epithelial necrosis, and a high fatality rate in otherwise healthy patients [2]. It was seldom reported during the XXth century [3, 4] until the epidemiological and pathophysiological links with Panton-Valentine Leucocidin (PVL) were discovered at the turn of the century [2, 5]. Subsequent reports confirmed the high fatality rates (40-50%) of PVL-associated pneumonia in adults [6–9].

Independently of staphylococcal necrotising pneumonia, staphylococcal pleuropneumonia in young children was described in the late 1950s and 1960s as a specific clinical entity characterised by round-shaped lung infiltrations evolving towards bullous lesions, purulent pleural effusion, and a lack of airway haemorrhage or epithelial necrosis [10, 11]. Recent case series of pleuropneumonia involving PVL-positive community-acquired *S. aureus* [12, 13] pointed to a possible relationship between pleuropneumonia and PVL; mortality was below 5% in these series.

The striking differences between pleuropneumonia and necrotising pneumonia, both associated with PVL but exhibiting contrasting mortality rates, may have contributed to controversies regarding the impact of PVL on the severity of staphylococcal pneumonia [14–16]. In this context, whether age affects both the clinical picture and the outcome of community-acquired PVL-positive staphylococcal pneumonia, which factors are associated with severity and whether PVL is an independent factor of severity, are questions that remain to be answered and which are addressed herein.

Patients and methods

Ethics.

The regional ethics committee approved the study (number: A11-39). Written informed consent was obtained from all patients or their parents.

Study design and participants.

This is a prospective, multicentre, observational cohort of patients with *S. aureus* community-acquired pneumonia (CAP) requiring intensive care. All French adult and paediatric intensive care units (ICU) were contacted via the main learned medical societies (*Société de Réanimation de Langue Française,* ca 200 adult ICU; and *Groupe Francophone de Réanimation et d'Urgence Pédiatrique* of the *Société Française de Pédiatrie,* 41 paediatric ICU) and invited to participate. Patients with *S. aureus* CAP (see criteria in supplementary methods) and admitted to a participating ICU were included between January 2011 and December 2016. Exclusion criteria were HIV-positive status, hospital-acquired pneumonia, or admission to hospital in the past 3 months. Clinical, laboratory, and therapeutic data were collected prospectively at admission to ICU and on day 1, 3, and 7. Severity was evaluated using age-adapted severity scores (see details in the supplementary methods).

Microbiology.

The causative staphylococcal isolates were transmitted to the French National Reference Centre for Staphylococci for full characterisation and DNA array genotyping (supplementary methods) [17].

Statistical analysis.

We determined whether the previous descriptions of different PVL-associated diseases, namely pleuropneumonia in children and staphylococcal necrotising pneumonia in adults, correlated with identifiable age groups in patients with severe CAP. To this aim, patterns in patient age distribution were detected using a clustering procedure (supplementary figure S1). Following this, disease presentation and outcome in toddlers (<3 years of age, as determined by the age clustering procedure) were compared to that found in older patients. The comparison of PVL-positive and PVL-negative cases was restricted to older patients (≥3 years of age) because 95% of toddlers had PVL-positive CAP. Between-group comparisons used Fisher's exact test or Student's t-test, as appropriate. Possibly non-linear changes of mortality rates in function of patient age were visualised using kernel density estimation with bootstrap confidence intervals (supplementary methods). Potential predictors of mortality were investigated using univariate and multivariable Cox proportional hazards models. To avoid bias due to row-wise deletion of cases containing missing data in multivariate models [18], all missing data were imputed prior to analysis using a non-linear, random forest-based multiple imputation technique. Imputation uncertainty was accounted for by replacing binary factors (such as, for example, leukopenia) by a probability estimate between 0 (absence) and 1 (presence).

To further examine the relationship between microbiological characteristics and outcome, we assessed whether the isolate's lineage and the presence of virulence factors, as determined using DNA arrays, predicted mortality and major clinical characteristics at admission, namely, rash, haemoptysis and leukopenia. These analyses were restricted to adult patients. All models included the Charlson score as a covariate to account for the diversity of age and baseline health in the cohort. Models with death as the response variable used Cox regression, with coefficients reported as hazard ratios. Models with rash, haemoptysis or leukopenia as the response variable used logistic regression, with coefficients reported as odds ratios. In a

first series of models, each outcome was regressed on the clonal complex, treated as a categorical variable, to identify associations of the bacterial lineage with clinical presentation. Given the strong genetic diversity in our collection, lineages with less than 10 isolates were pooled into an 'Other' category before regression analysis. In a second series of models, we examined a near-exhaustive array of virulence determinants for associations with death, rash, haemoptysis and leukopenia. A subset of the 332 loci and alleles, as determined using DNA arrays, was selected *a priori* based on their plausible role in virulence (e.g. excluding antimicrobial resistance determinants other than *mec*A) and non-redundancy (e.g. excluding the *luk*F component of *pvl* which is virtually always present with the *luk*S component; supplementary table S8). The alleles defining *agr* groups I to IV were also included, but not the lineage (clonal complex) which would be redundant with the genotypic profile. Variables present or absent in less than 10 isolates were excluded. A final set of 28 non-constant genotypic determinants were included in the final analyses (Supplementary Table S8). Determinants were coded as 0 when absent and 1 when present. Ambiguous DNA array results (missing data, 0.8% of results) were imputed using the random forest procedure described above. Given the large number of determinants, we used sparse regression (LASSO) as implemented in the *glmnet* R package, to identify the determinants retained in sparse models [19]. Briefly, LASSO models are built sequentially by modifying a L1 penalisation constraint such that more penalised models include fewer predictors. The best-fitting models (using death, rash, haemoptysis or leukopenia as the response variable) were selected using leave-one-out cross-validation.

Analyses were conducted using R software v3.6.0 (R Core Team [2019]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Anonymised data and software code required to reproduce the results are available at github.com/rasigadelab/severecap.

Results

Clustering analysis of patient age identifies specific patterns in PVL-positive and PVL-negative *S. aureus* pneumonia. A total of 228 patients were eligible. After removal of those who did not comply with the inclusion criteria, those who did not provide consent, and those with missing CRF, 163 patients were included (supplementary figure S1). The included patients were hospitalised in 77 ICUs and aged from one month to 87 years. Eighty-five patients (52.1%) were infected by a PVL-positive *S. aureus*. Clustering by age of patients with PVL-positive CAP identified 2 non-overlapping clusters below and above 3 years of age (supplementary figure S2). In patients with PVL-negative CAP, we identified 2 overlapping clusters centred at 28 and 60 years of age. Almost all cases in patients aged <3 years (hereafter "toddlers") were PVL-positive while PVL positivity in older patients was evenly distributed according to age (supplementary figure S2). In contrast, PVL-negative CAP was exceptional in younger patients (only one case <15 years of age) and its prevalence increased with patient age, peaking at 60 years. Hence, toddlers accounted for 12.3% of all severe staphylococcal CAP in our cohort, and 22.3% of PVL-positive cases. The microbiological and clinical features of CAP differed markedly between toddlers and older patients (supplementary table S1). PVL positivity was more frequent in toddlers, more strongly so than methicillin resistance (n=7/20 vs. 23/143, odds ratio 2.8, 95% CI 0.85 to 8.6). Additional details on the specificities observed in toddlers are given in the supplementary results.

Mortality according to age. Mortality increased with age, reaching a plateau at ~30 years in PVL-positive cases. Age-dependent increase of mortality was more linear in PVL-negative cases (supplementary figure 3). Mortality was lower in toddlers than older patients (n=3/20 vs. 52/143, odds ratio 0.31, 95% CI 0.06 to 1.15). In toddlers with PVL-positive CAP

(supplementary figure 3 c-d), most cases occurred before 12 months of age and were non-lethal, while mortality increased after 1 year of age.

Since only 1 toddler had PVL-negative CAP, we could not compare the outcomes of PVL-positive and -negative CAP in this group. Further comparisons according to PVL positivity and analysis of severity factors were restricted to patients aged ≥3 years to avoid the interpretation bias that would have resulted from the pooling of such markedly different patients (namely, toddlers and older patients) in a single analysis.

PVL is associated with specific symptoms and is a severity factor in patients aged ≥3 years.

Among the 143 patients aged ≥3 years, 66 (46.2%) had PVL-positive CAP. Compared to PVL-negative CAP, patients with PVL-positive CAP were younger and less likely to have an underlying condition (table 1). At admission, they had more frequently airway haemorrhage and cutaneous eruption or rash evoking immune system reaction. PVL-positive CAP patients were also more likely to present with septic shock, leukopenia, elevated blood lactate and SOFA severity score, and to require extra corporeal membrane oxygenation (ECMO).

Appropriate initial antibiotic treatment, defined as ≥1 drug active against the corresponding strain, was equally likely in PVL-positive and PVL-negative CAP. However, the initial treatment was less frequently appropriate in MRSA cases (odds ratio 0.027, 95% CI 0.006 to 0.097). See the supplementary results for additional information on antibiotic treatment.

Most of the differences observed between patients with PVL-positive and -negative CAP at admission persisted during the first week in ICU (table 2 & 3). The higher SOFA score at admission persisted at day 1, as did the higher median blood lactate. Temperature >39°C, airway haemorrhage, and rash remained significantly more common in PVL-positive cases, who received more frequently inhaled nitric oxide and required ECMO support.

Survival.

We used survival analysis to examine the influence of microbiological and clinical factors on lethal outcome in the 143 patients aged ≥3 years. Kaplan-Meier survival curves suggested that PVL-positivity and methicillin resistance contributed additively to mortality (Fig. 2); median survival time was 1 day (interquartile range, IQR 0 to 7 days) for PVL-positive cases and 7 days (IQR 3 to 14 days; $p = 0.02$, Mann-Whitney *U*-test) for PVL-negative cases. Among the 91 survivors, length of ICU stay was longer in those with PVL-positive CAP (median time until discharge, 39 days, IQR 26 to 68 days) than in those with PVL-negative CAP (29 days, IQR 20 to 42 days; $p = 0.01$, Mann-Whitney *U*-test).

We then used bivariate and multivariable Cox proportional hazards models to identify potential independent risk factors for mortality (table 4). Predictors were included in the models based on their expected relevance to disease outcome. These included, in addition to PVL and methicillin resistance: baseline patient characteristics, namely sex and the Charlson comorbidity score; characteristics reflecting: (1) severity upon admission, namely the SOFA score, haemoptysis, leukopenia and blood lactates, (2) inflammatory reaction, namely rash and blood procalcitonin, (3) a risk factor for staphylococcal superinfection of the lung, namely a flu-like illness; and the appropriateness and expected toxin-suppressing activity of the antibiotics received (see the supplementary results for details). In the best-fitting multivariable model, the independent predictors of death were methicillin resistance, haemoptysis, rash, leukopenia, elevated blood lactates, and the absence of a flu-like illness (supplementary table S3).

Interestingly, PVL positivity significantly predicted death in bivariate analysis (table 4) while the best-fitting multivariable model included previously recognised severity factors in PVL-

positive lung infection, namely haemoptysis and leukopenia [20], but not PVL itself. The exclusion of PVL, but not its clinical consequences, from the final model is suggestive of a chain of consequence between PVL, severity factors and death, where the severity factors mediate the effect of PVL on death [21]. To verify this interpretation, we constructed an additional multivariable Cox regression model including only microbiological and baseline predictors, but not the potential consequences of PVL such as treatment or severity factors. In this model, PVL positivity predicted death (adjusted hazard ratio 1.81, 95% CI, 1.04 to 3.17) independent of methicillin resistance, sex, Charlson score and a flu-like illness. Thus, PVL positivity was a risk factor of mortality independent of other baseline factors. See the supplementary results and figure S4 for additional data on severity-associated features and on interactions between predictors of mortality.

Contribution of the strain genetic background and other virulence factors to severity.

Although PVL and methicillin resistance have been repeatedly associated with poor outcome in previous studies, it is still debated whether they exert a direct effect on outcome or rather act as surrogate markers for more virulent lineages. To examine this issue, we tested whether mortality and major severity factors at admission, namely, rash, haemoptysis and leukopenia, could be predicted by the isolate's lineage (supplementary table S2) and virulence gene content, independently of the Charlson comorbidity score.

In models using lineage as a predictor (table 5), the Charlson score predicted leukopenia, but not death, rash or haemoptysis, while the isolate's lineage contributed to predict rash, possibly leukopenia ($p = 0.06$, analysis of deviance Chi^2 test), but not death or haemoptysis. Substantial associations between lineage and outcome were found, namely between CC121 and a rash, and between CC152, CC398 and leukopenia (table 5). In an additional, mixed-effect Cox regression including one random intercept per lineage, both *mec*A (hazard ratio

2.25, 95% CI, 1.18 to 4.27) and *pvl* (hazard ratio 1.98, 95% CI, 1.08 to 3.62) were independent predictors of death. Collectively, these results indicate that the *S. aureus* lineage *per se* does not predict death independently of *mec*A and *pvl*, which favours a direct role of these determinants rather than an indirect role as lineage markers. However, other clinical characteristics such as rash or leukopenia were influenced by the strain's lineage, suggesting a potential role of other virulence factors.

To further decipher the relationships between virulence and CAP outcome, we examined 28 virulence determinants for associations with death, rash, haemoptysis, and leukopenia (see Supplementary Figure S5 for correlation between the selected predictors). The best-fitting LASSO regression models for each outcome, selected using leave-one-out cross-validation, are reported in table 6 (see supplementary figure S5 and supplementary tables S4 to S7 for details of LASSO models). This analysis confirmed that both *mec*A and *pvl* were independent predictors of death, even when all other virulence markers were considered. The *pvl* was also an independent predictor of haemoptysis and leukopenia, but not rash. Rash was mainly predicted by an *agr*IV background, in line with the association of rash with lineage CC121 of the *agr*IV background (table 5). Of note, PVL remained among the top-ranking predictors not included in the best-fitting model (Supplementary Table S5).

The LASSO models of haemoptysis and leukopenia yielded additional insights into the interplay of virulence factors with CAP presentation. In addition to PVL, *edin*B (encoding the epidermal differentiation inhibitor) was associated with both conditions, and the staphylococcal enterotoxin A gene (*seA*) gene predicted leukopenia.

Discussion

The present results improve our understanding of the role of PVL in different clinical presentation and severity of staphylococcal pneumonia. In addition, we identify the role of other severity-associated factors including bacteriological ones, and their link with PVL.

PVL-positive CAP and its severity was not evenly distributed according to age. PVL-negative CAP was virtually absent in toddlers and mortality was low. Many infectious processes vary in terms of presentation between young and older subjects, but the differences we observed exceed the expected variations due to age alone. For instance, radiological findings in pneumonia are usually similar at all ages [22], however, we observed substantial radiological differences, notably regarding pleural effusion, pneumothorax and unilateral involvement that were all more frequent in toddlers. It was also unexpected to observe a lower mortality in very young children compared to young adults without underlying conditions. The lower severity associated with PVL in toddlers could be due to: (i) immunological immaturity, considering that local inflammation plays a central role in PVL-induced lesions [23], (ii) passive protection by maternal antibodies, (iii) the route of infection – the bullous lesions in toddlers point towards the involvement of the supporting tissue, suggesting a haematogenous route of infection as opposed to a likely inhalation route in adolescent and adults leading to direct necrosis of the respiratory epithelium. The observed specific symptoms and lower severity in toddlers might reflect a combination of one or more of the factors described above, but also other yet to be identified features (see additional comments in the supplement). Thus, despite similarities in terms of site of infection and bacteriology, staphylococcal CAP in young children (i.e. pleuropneumonia) and staphylococcal necrotising pneumonia in adults should be considered as two distinct entities.

As we uncovered major differences according to age in PVL-positive staphylococcal pneumonia, the lack of consideration of age-specificity of the disease severity may at least

partially explain some conflicting results reported in the literature [14, 24]. Considering that PVL-negative staphylococcal pneumonia is almost absent in the youngest patients, toddlers should be excluded from analysis to assess the role of PVL in severity.

We observed that PVL was associated with specific symptoms in staphylococcal pneumonia of adolescents and adults. Previously described features strongly associated with PVL [2, 7] were observed herein among patients aged ≥3 years: PVL-positive *S. aureus* pneumonia occurred mainly in younger people without underlying condition and was associated with cutaneous rash, airway haemorrhage, and leukopenia. However, logistic regression revealed that rash was most strongly predicted by the *agrI*V-CC121 genetic background, independently of PVL or the presence of superantigens. The basis of the association of PVL with rash appears elusive although rash as a symptom remains a relevant marker of PVL-positive CAP.

Airway haemorrhage is a consequence of respiratory epithelial necrosis, as described in lung autopsies [1, 2]. Haemorrhage is an indirect effect of PVL since the presence of polymorphonuclear (PMN) cells in the lung is required for PVL-induced necrotic lesions of the epithelium [25]. PMN cell influx to the lung may be generated by two non-exclusive mechanisms: a preceding viral infection producing chemoattractant for neutrophils by epithelial cells [25], and PVL-mediated inflammasome activation (releasing IL-1β), leading to PMN cell recruitment and activation [26]. Interestingly, this inflammatory pathway is amplified by the release of pathogen- and damage associated molecular patterns from dying neutrophils, possibly explaining the fulminant nature of PVL-associated CAP [27]. Of note, the PSMalpha3, beta-, gamma-, delta hemolysins, and LukDE have been shown to synergise with PVL to amplify IL-1β release, indicating that these factors cooperate with PVL to trigger inflammation [26]. However, among the factors listed above, LukDE is the only variable genotypic determinant and there was no direct association with haemoptysis in the LASSO model. Conversely, the LASSO model revealed that beside PVL, *edin*B was the second

genotypic marker associated with haemoptysis, a feature not described previously. Edin-B elicits large transcellular tunnels in endothelial cells (macroapertures) inducing a loss of barrier function and providing direct access of the endothelium basement membrane to *S. aureus*. In mouse models Edin-B promotes the translocation of *S. aureus* to the bloodstream during the course of pneumonia [28]. How these properties translate into haemoptysis remains to be explored.

We show that leukopenia is another major feature associated with PVL and death. The relationship between leukopenia and PVL has nowadays a mechanistic explanation after the identification of C5aR on myeloid cells as the receptor for lukS-PV [29]. Beside PVL, other determinants contributed to leukopenia such as CC152 and CC398 lineage, Edin-B and SEA. This later association is novel in humans; it potentially results from extravasation of cells in a Vbeta-unrestricted manner as demonstrated experimentally in rabbits for another major superantigen, the TSST-1 [30].

One objective of the present study was to assess the link between PVL and severity, considering that such an association has been controversial since the first description of PVL-associated pneumonia in 2002 [2]. The frequency of PVL in severe CAP in adults herein contrasts with the low frequency of PVL (<5%) in the staphylococcal carriage population in France [17] and this striking difference constitutes the initial evidence for an association of PVL with severity. We observed that PVL, especially but not exclusively when associated with methicillin resistance, was an independent factor of mortality in staphylococcal CAP even when other virulence markers were considered, outweighing protective factors such as young age and the absence of underlying conditions. The present study indicates that PVL is associated with non-specific severity markers of infection such as elevated lactate or presence of septic shock at admission and absence of reduction in lactate between admission and day 1. All these factors are associated with a higher mortality in sepsis, irrespective of the bacteria

involved [31, 32], and their higher frequency in the PVL-positive cases confirms greater severity in such patients. Surrogate markers of respiratory failure, such as the need for nitric oxide and for ECMO, were more frequent in PVL-positive cases, which also indicates greater severity in accordance with recent studies [33, 34]. The major symptoms associated with PVL (i.e. airway haemorrhage and leukopenia) were associated with mortality in the multivariable prediction model (Table 4), strongly suggesting that these factors mediate the link between PVL and death.

Since its first description [2], PVL-positive *S. aureus* pneumonia has been reported worldwide [34–37]. A particular situation is observed in the USA where the description of necrotising pneumonia coincided with the emergence of the PVL-positive CA-MRSA USA300 lineage [4, 38]. PVL has been also associated with the CA-MRSA ST-80 lineage in Europe [39]. However, only 11 and five strains of the present study belong to these clones, respectively, and PVL was distributed within a large diversity of genetic background (i.e. 13 CCs) with a majority of MSSA, ruling out a possible clonal bias associated with PVL-positive cases. The lack of genetic diversity of CA-MRSA in the US may have initially led to confusion in the understanding of the determinant of severity associated with staphylococcal CAP. Thus, methicillin resistance was thought to be the prominent determinant for severity, presumably by inducing a delay in initiation of appropriate antibiotics, in line with our observations. However, this higher mortality in MRSA was present both in PVL-positive and PVL-negative-pneumonia patients.

Mortality increased above 60 years (markedly in PVL-negative cases), which is anticipated for bacterial pneumonia; however, mortality reached a plateau in PVL-positive at ~30 years, an observation for which we have only hypothetical and non-exhaustive explanations. Mortality results from a balance of protective and deleterious factors. Protection is likely conferred by a gradual acquisition of toxin neutralising- and opsonising-antibodies against

S. aureus. Conversely, enhanced severity may be caused by a highly reactive innate immune system (typically lacking in toddlers). Alternatively, the age-dependent acquisition of auto-antibodies against cytokines and chemokines involved in *S. aureus* response (IL-1b, TNF-a, MIP-1a, GMCSF, IL-17A, IFN-g [40]) may impair protective immune responses in a subset of young adults, mimicking the immunosuppression associated with autoantibodies against type I IFNs in patients with severe SARS-CoV2 [41].

We acknowledge limitations of the present study, most notably the observational design whereby all participating centres were encouraged to enrol patients fulfilling the inclusion criteria. We cannot exclude under-reporting, and a higher reporting of the most severe cases may have occurred, but this is unlikely to have affected directly the comparison between PVL-positive and PVL-negative cases. The number of missing data were limited and exhaustivity was reached for all major parameters. Furthermore, the study was restricted to France but covers almost entirely the French territory with 77 participating centres, thus limiting possible local epidemiological bias.

In conclusion, the present study demonstrates the association of PVL with two distinct facets of staphylococcal CAP with marked differences between toddlers and adolescents/adults regarding clinical presentation and outcome. In toddlers, PVL appears to be prominent in staphylococcal pneumonia, presentation matches with pleuropneumonia, and standard of care in modern ICU appears to be sufficient for favourable outcome. In contrast, PVL-positive CAP in adolescents and adults remains extremely severe despite aggressive management; it deserves further research to develop tailored therapeutic approaches.

Acknowledgments

We thank the technicians of the French Reference Centre for Staphylococci for their contribution in characterizing *S. aureus* isolates. We thank Vincent Cottin, Etienne Javouhey and Thomas Henry for fruitful discussions. Finally, we thank Philip Robinson (DRCI, Hospices Civils de Lyon) for copy-editing the manuscript.

The PVL pneumonia study group. Olivier Baldesi, Nathalie Brieu, Aix-En-Provence; Gérald Boussicault, Marie Kempf, Carole Lemarie, Angers; Valérie Charbonneau, Arnaud Desachy, Caroline Garandeau, François Pettinelli, Angouleme; Stéphane Bland, David Bougon, Annecy; Anne-Marie Colin-Gorski, Laurence Courdavault, Hervé Mentec, Agnès Scanvic Argenteuil; Marie-Pierre Lacomme, Jean-Pierre Zanini, Aubertvilliers; Alexandre Avenel, Fabrice Bertrand, Elodie Colin, Huong Porcheret, Aulnay-Sous-Bois; Laurence Delapierre, Cécile Jensen, Avignon; David Leyssene, Jérôme Pillot, Bayonne; Émilie Benabid, Jack Richecoeur, Beaumont Sur Oise; Katy Jeannot, Anaïs Potron, Olivier Schulze, Besancon; Magali Anastay, Juliette Chabrol, Bethune; Christophe Clec'h, Mathilde Lescat, Bobigny Avicenne; Henri De Montclos, Xavier Tchenio, Bourg-En-Bresse; Frédéric Brun, Philippe Crova, Charlotte Tellini, Bourgoin-Jallieu; Bianca Podac, Martial Delorme, Chalon Sur Saone; Emmanuelle Lavalard, Salem Ould Zein, Chalons En Champagne; Marion Levast, Matteo Miquet, Chambery; Olivier Gontier, Abdelkader Ouchenir, Oana Zamfir, Chartres; Geneviève Barjon, Jean-Philippe Edmond , Compiegne; Guillaume Chevrel, Sabina Djouhri, Philippe Cormier, Corbeil-Essones; Said Aberrane, Rusel Leon, Creteil; Julien Bador, Pierre-Emmanuel Charles, Catherine Neuwirth, Jean-Pierre Quenot, Dijon; Caroll Barthalay-Le Risbe, Annick Bosseray, Barbara Colombe, Armelle Gayot, Christine Recule, Sébastien Thomas, Pascale Verger, Grenoble; Sylvaine Bastian, Pascale Piednoir, Guadeloupe Pointe A Pitre ; Ahmed-Said Laggoune, Odile Sevin, Gueret; Henri Faure, Karine Grenet, Philippe Martinais, Jossigny; Laurent Bellec, Olivier Belmonte, Marie Lagrange-Xelot, La Reunion

Saint-Denis; Emmanuel Antok Julien Cousty, Sandrine Picot, La Reunion Saint-Pierre; Hélène Biessy, Alexandre Herbland, La Rochelle; Céline Ramanantsoa, Patrice Tirot, Le Mans; François Fourrier, Nadine Lemaitre, Lille; Elodie Couve Deacon, Deborah Postil, Philippe Vignon, Limoges; Pierre-Eric Danin, Oana Dumitrescu, Arnaud Friggeri, Elisabeth Hodille, Lyon Chls; Arnaud Francois, Lionel Liron, Lyon Tonkin; Bertrand Delannoy, Coralie Bouchiat, Céline Dupieux-Chabert, Frédéric Laurent, Sylvestre Tigaud, Grégoire Wallon, Lyon Croix Rousse; Laurent Argaud, Coralie Bouchiat, Martin Cour, Romain Hernu, Julien Illinger, François Vandenesch, Lyon Hôpital Edouard Herriot; Etienne Javouhey, Yves Gillet, Anne Tristan, Lyon HFME; Matthias Jacquet-Lagreze, Laure Olivier-Soubirou, Lyon Louis Pradel ; Patrick Gerome, Floriane Klack, Christophe Lions, Fabrice Petitjeans, Marc Puidupin, Marie-Pierre Otto, Lyon Hia Desgenettes; Anne Carta, Marc Houvenaeghel, Marignane Clinique Générale; Grégory Dubourg, Nadim Cassir, Frédérique Gouriet, Philippe Minodier, Claire Nicaise, Laurent Papazian, Marseille Aphm; Jean-Louis Ferge, Claude Olive, Rafaelle Theodose, Martinique Fort De France; Abdourahim Chamouine, Louis Collet, Philippe Durasnel, Thierry Benoit-Cattin, Mayotte; Françoise Crepet, Mélanie Crozet, Montbrison; Patrick Marthelet, Gilles Rival, Montelimar; Helene Jean Pierre, Camille Maury, Montpellier; Philippe Linval, Aina Raobison, Moulin Yzeure; Thierry Conroy, Nicolas Ducrocq, Serge Le Tacon, Pierre Perez, Cynthia Pianetti, Nancy ; Michaël Afanetti, Nicolas Clement, Nicolas Degand, Jean Dellamonica, Sophie Leotard, Nice; Christelle Guillet-Caruba, Pierre Trouiller, Paris Hôpital Antoine Béclère ; Faten El Sayed, Xavier Repesse, Valérie Sivadon-Tardy, Nathalie Soismier, Valérie Soussan-Banini, Paris Hôpital Ambroise Paré; Etienne De Montmollin, Etienne Ruppe, Paris Hôpital Bichat-Claude Bernard ; Loic De Pontual, Isabelle Poilane, Paris Hôpital Jean-Verdier ; Rishma Amarsy Guerle, Joaquim Mateo, Paris Hôpital Lariboisière ; Mikael Alves, Jacques Tankovic, Paris Hôpital St Antoine; Jean-Luc Donay, David Schnell, Paris Hôpital St Louis; Mouna Doufair, Muriel

Fartoukh, Antoine Parrot, Tai Pham, Charlotte Verdet, Sophie Vimont-Billarant, Paris Hôpital Tenon, Alexandra Aubry, Jean Chastre, Yannick Costa, Delphine Desbois, Sylvie Dubourdieu, Guillaume Hekimian, Antoine Landivier, Emilie Lafeuille, Ania Nieszkowska, Florence Richardin, Maxime Thouvenin, Paris Pitié Salpétrière; Stéphane Bonacorsi, Chantal Chaplain, Stéphane Dauger, Mathie Lorrot, Patricia Mariani-Kurkdjian, Paris Robert Debré; Chantal Chaplain, Vincent Ioos, Nathalie Memain, Bruno Verdiere, Paris Saint-Denis; Philippe Badia, Aude Bourrouillou, Gisèle Mourin, Cristel Voinot, Pau; Pierre-Guy Durand, Philippe Gueudet, Perpignan; Caroline Piau-Couapel, Samer Kayal, Pierre Tattevin, Fabrice Uhel, Rennes ; Pascal Beuret, Michelle Boyer, Roanne; Quentin Blanc, Jean-Claude Colombani, Romans Sur Isere; Anne Vachee, Clément Vanbaelinghem, Roubaix; Kevin Guernon, Jean-Louis Pons, Christophe Girault, Ludovic Lemee, Rouen; Anne Carricajo, Florence Grattard, Richard Jospe, Pierre Lafond, Stephanie Rapin, Emmanuelle Rolland, Saint-Etienne; Anne-Marie Camin-Ravenne, Thierry Dulac, Alain Le Coustumier, Tarbes; Maryse Archambaud, Jean-Marie Conil, Toulouse Rangueil; Kildie Barrial, Patrice Fernandez, Valence; Julien Huntzinger, Nathalie Prades, Vannes; Fabrice Bruneel, Eric Farfour, Versailles, Hôpital Mignot; Jamal Bouhaddouch, Kevin Chaulier, Elodie Dorangeon, Camille Piffaut, Villefranche Sur Saone; Philippe Labadie, Bruno Soullie, Villenave D'ornon Hia Robert Picqué.

Author contributions.

Concept and design: Gillet, Argaud, Vandenesch; Acquisition, analysis or interpretation of the data: Gillet,Tristan, Saadatian-Elahi, Bouchiat, Dumitrescu, Leloire, Dupieux, Laurent, Lina, Etienne, Vanhems, Argaud, Vandenesch; Statistical analysis: Rasigade, Vanhems; Drafting of the manuscript: Gillet, Tristan, Rasigade, Vandenesch; Critical revision of the manuscript for

important intellectual content: all authors; Obtained funding: Vandenesch; Administrative, technical of material support: Bes, Dupieux; Study Supervision: Saadatian-Elahi, Tristan

Competing interests.

The authors declare that they have no competing interests

Support statement.

The study was funded by French Ministry of Health under the Programme Hospitalier de Recherche Clinique 2011 (Grant number 2010-A01132-37). This report presents independent research funded by the Ministry of Health; the views and opinions expressed in this publication are those of the authors and do not necessarily reflect those of the Ministry of Health.

Tables

Table 1: Clinical and laboratory characteristics at admission according to PVL status in patients ≥3 years of age. (percentage are calculated among those with data)

	PVL-positive *S.aureus*		**PVL-negative *S.aureus***		
	(n=66)	**missing**	**(n=77)**	**missing**	**p value**
Mean delay in days between onset and ICU admission (SD)	3.39 (3.74)	0	4.64 (4.80)	4	0.092
Sex (F/M)	*31/35*	*0*	*27/50*	*0*	*0.173*
Median age in years (IQR)	*47 (28.7-60.2)*	*0*	*58 (48-67)*	*0*	*<0.001*
Children aged < 18 years	*6 (9.1%)*	*0*	*1 (1%)*	*0*	*0.049*
MRSA	*14 (21.2%)*	*0*	*9 (11.7%)*	*0*	*0.170*
Absence of underlying condition°	*39 (59.1%)*	*0*	*22 (28.6%)*	*0*	*<0.001*
Mean Charlson's score (SD)	*1.18 (0.18)*	*0*	*2.14 (0.22)*	*0*	*0.001*
Temperature > 39°C	35 (53%)	0	29 (38.2%)	1	0.092
Airway hemorrhage	29 (43.9%)	0	18 (23.4%)	0	0.012
Toxin-mediated rash	14 (21.2%)	0	5 (6.6%)	1	0.013
Bilateral pneumonia	46 (70.8%)	1	52 (68.4%)	1	0.855
Signif. pleural effusion°°	3 (4.5%)	0	1 (1.4%)	3	0.343
Leucocytes (G/l), med (IQR)	4.60 (1.4-15)	1	9.34 (5-15.8)	3	0.011
Leukopenia < 3G/L	28 (43.1%)	1	14 (18.9%)	3	0.003
Median blood lactate in mg/l (IQR)	3.95 (2.3-5.5)	8	2.4 (1.7-4.6)	9	0.015
Median CRP in mg/l (IQR)	327.5 (191.2-396.7)	26°°°	251.6 (127-351)	18°°°	0.041
Median procalcitonin in µg/l) (IQR)	137.5 (51.7-488.5)	40°°°	57 (8-200)	58°°°	0.032
Appropriate antibiotics	47 (75.8%)	4	62 (80.5%)	0	0.539

MRSA: Methicillin resistant *Staphylococcus aureus*; CRP: C-Reactive Protein
°underlying conditions include: Malignancies, Tobacco smoking,Alcohol abuse, Diabetes and BMI >30
°°needing drainage
°°°Inflammatory markers (CRP or Procalcitonin) are missing for 14 patients (7 in each group)

Table 2: Clinical and laboratory characteristics during stay according to PVL status in patients ≥3 years of age. (percentage are calculated among those with data)

	PVL-positive *S.aureus*		**PVL-negative *S.aureus***		
	(n=66)	**missing**	**(n=77)**	**missing**	**p value**
Temperature > 39°C	44 (75.9%)	8	44 (58.7%)	2	0.043
Airway hemorrhage	35 (59.3%)	7	28 (37.3%)	2	0.015
Toxin-mediated rash	15 (25.4%)	7	8 (10.7%)	2	0.037
Bilateral pneumonia	45 (78.9%)	9	61 (83.6%)	4	0.505
Signif. pleural effusion°	10 (16.4%)	5	10 (13.7%)	4	0.808
Median highest CRP in mg/L, (IQR)	352 (230-447)	38	228 (106.0-347)	38	0.017
Median highest procalcitonin in µg/L (IQR)	91 (28.5-375)	37	35.4 (3.3-98.6)	47	0.032

CRP: C-Reactive Protein,
NOTE: needing drainage

Table 3: Severity scores and markers at admission and during evolution according to PVL status in patients ≥3 years of age. (percentage are calculated among those with data)

	PVL-positive *S.aureus*		PVL-negative *S.aureus*		
	n=66	**missing**	**n=77**	**missing**	**p value**
Admission					
Mean SOFA score (SD)	8.9 (5.68)	2	6.9 (5.01)	8	0.004
Septic shock	31 (48.4%)	4	22 (30.1)	2	0.035
ARDS	20 (32.8%)	7	16 (22.9)	5	0.352
Severe ARDS	12 (19.7%)	7	10 (14.3)	5	0.485
Invasive ventilation	32 (48.5%)	0	31 (42.5)	4	0.499
ECMO	11 (16.9%)	1	3 (4.2)	5	0.022
Day 1					
Mean SOFA score (SD)	10.9 (5.96)	14	8.8 (5.25)	8	0.040
Septic shock	31 (54.4%)	9	28 (40.6%)	8	0.152
ARDS, n (%)	27 (50%)	12	32 (50%)	13	1
Severe ARDS, n (%)	21 (38.9%)	12	19 (29.7%)	13	0.332
Death, n (%)	7 (10.6%)	0	4 (5.2%)	0	<0.001
Mean lactate variation°(SD)	0.62 (4.38)	19	- 0.69 (2.10)	14	0.063
Day 3					
Mean SOFA score (SD)	10.6 (6.51)	24	8.1 (5.34)	8	0.045
Cumulative death°°(%)	17 (25.8%)	0	7 (9.1%)	0	<0.001
Day 7					
Mean SOFA score (SD)	8 (6.84)	29	6.8 (5.70)	16	0.347
Cumulative death°°	23 (34.8%)	0	10 (13%)	0	<0.001
During whole ICU stay					
Invasive ventilation	56 (84.8%)	0	60 (81.1%)	3	0.655
Inhaled NO	21 (33.3%)	3	11 (15.3%)	5	0.016
ECMO	20 (32.3%)	4	8 (11%)	4	0.003
Death	31 (47.0%)	0	21 (27.3%)	0	0.023

NOTE : **SOFA : Sequential Organ Failure Assesment, ARDS : Acute Respiratory Distres Syndrome, ECMO : Extra Corporal Membrane Oxygenation**
° lactate variation between Day 0 and Day one,
°° From Day 0 to day 3 or day 7,

Table 4: Cox regression analysis of predictors of death in patients ≥3 years with *S. aureus* pneumonia, including clinical predictors at admission and microbiological predictors.

	Hazard ratio (95% confidence interval)		
Predictor	**Bivariate models**	**Full model**	**Best-fitting model**
Charlson comorbidity score (per point)	1.05 (0.91 to 1.21)	1.03 (0.85 to 1.26)	-
Male sex (95%CI)	0.68 (0.39 to 1.17)	0.95 (0.51 to 1.78)	-
PVL (95%CI)	1.95 (1.12 to 3.41)	0.87 (0.42 to 1.83)	-
Methicillin resistance (95%CI)	2.57 (1.41 to 4.69)	3.86 (1.34 to 11.16)	2.87 (1.53 to 5.40)
SOFA score (95%CI)	1.11 (1.05 to 1.16)	1.03 (0.96 to 1.11)	-
Flu-like illness (95%CI)	0.71 (0.41 to 1.23)	0.32 (0.15 to 0.68)	0.32 (0.17 to 0.60)
Hemoptysis (95%CI)	2.62 (1.52 to 4.52)	1.87 (0.98 to 3.54)	2.15 (1.17 to 3.93)
Rash (95%CI)	2.17 (1.12 to 4.23)	2.59 (1.22 to 5.48)	2.52 (1.24 to 5.12)
Leukopenia < 3G/L (95%CI)	3.45 (2.00 to 5.97)	2.38 (1.19 to 4.76)	2.38 (1.23 to 4.58)
Procalcitonin (per 2-fold increase) (95%CI)	1.40 (1.21 to 1.63)	1.05 (0.88 to 1.24)	-
Lactates (per 2-fold increase) (95%CI)	2.40 (1.85 to 3.12)	2.23 (1.52 to 3.28)	2.64 (1.95 to 3.57)
Adapted antimicrobial therapy (95%CI)	0.82 (0.44 to 1.53)	1.54 (0.52 to 4.59)	-
Antitoxin therapy (95%CI)	2.67 (1.48 to 4.81)	1.29 (0.63 to 2.65)	-

NOTE. Bivariate models were independent Cox proportional hazards models, one per predictor. The full multivariable model included all predictors (likelihood ratio test, $P < 0.001$; Akaike information criterion [AIC), 420 with 13 degrees of freedom). The best-fitting model was obtained using a stepwise procedure, starting from the full model, and minimizing the AIC (409 with 6 degrees of freedom). Confidence interval widths were not corrected for test multiplicity.

Table 5. Association of *S. aureus* lineage with death, rash, hemoptysis and leukopenia in 143 adult patients with severe CAP.

	Regression coefficient (95% CI)			
Predictor	**Death (HR)**	**Rash (OR)**	**Haemoptysis (OR)**	**Leukopenia (OR)**
Charlson's score	1.11 (0.95 to 1.29)	0.90 (0.61 to 1.32)	0.97 (0.78 to 1.21)	0.80 (0.62 to 1.04)
CC121	1.71 (0.66 to 4.42)	24.19 (4.85 to 120.48)	0.68 (0.16 to 2.90)	0.90 (0.16 to 4.97)
CC152	2.55 (1.07 to 6.09)	0.47 (0.05 to 4.61)	3.30 (0.96 to 11.29)	7.84 (2.06 to 29.81)
CC30	0.69 (0.25 to 1.89)	0.87 (0.15 to 4.95)	0.68 (0.21 to 2.23)	1.17 (0.31 to 4.40)
CC398	1.07 (0.39 to 2.94)	0.62 (0.07 to 5.62)	1.04 (0.30 to 3.58)	4.47 (1.22 to 16.34)
CC5	0.38 (0.09 to 1.66)	-	0.56 (0.14 to 2.33)	0.79 (0.15 to 4.31)
CC8	1.03 (0.34 to 3.11)	-	0.53 (0.10 to 2.88)	2.62 (0.52 to 13.15)
CC80	1.59 (0.58 to 4.35)	0.94 (0.10 to 9.13)	1.39 (0.34 to 5.68)	3.47 (0.77 to 15.58)
Predictor	**Analysis of deviance, Chi² test**			
Charlson's score	p = 0.54	p = 0.45	p = 0.32	p = 0.02
Lineage (CC)	p = 0.15	p < 0.001	p = 0.35	p = 0.01

NOTE. CCs with sample size <10 were pooled and used as the reference category. Death prediction model used multivariable Cox regression, with coefficients reported as hazard ratios (HRs). Other models used multivariable logistic regression, with coefficients reported as odds ratios (ORs). '-', unresolved coefficient due to small sample size.

TABLE 6. Association of *S. aureus* virulence determinants with death, rash, haemoptysis and leukopenia in 143 adult patients with severe CAP.

	Coefficient in best-fitting LASSO model			
Predictor	**Death (HR)**	**Rash (OR)**	**Haemoptysis (OR)**	**Leukopenia (OR)**
Charlson score (per point)	-	-	-	0.87
*mec*A	1.89	-	-	-
*agr*I	-	-	-	1.52
*agr*III	-	-	-	0.79
*agr*IV	-	13.99	-	-
*luk*S-PV	1.23	-	1.15	2.52
*edin*B	-	-	2.27	1.60
cap5	-	*0.63*	-	*1.78*
etD	-	-	-	*2.32*
sea	-	-	-	2.05
egc	-	-	-	0.92
*luk*D	-	-	-	0.53
*ica*C	0.81	-	-	-
cna	-	1.31	-	-
eap	0.86	-	-	-
*sdr*D	0.99	-	-	-

NOTE. Models with LASSO (L1) penalty based on Cox (death) or logistic (rash, haemoptysis, leukopenia) regression. The best-fitting model minimizes prediction error in leave-one-out cross-validation. In all models, 49 non-constant virulence factors as well as the Charlson comorbidity score were considered as potential predictors. Note that LASSO models do not define confidence intervals. '-', predictor not included in model. HR, hazard ratio; OR, odds ratio.

Figures legend

figure 1: Mortality of patients with *S. aureus* community-acquired pneumonia (CAP) according to age and the presence of the Panton-Valentine leukocidin (PVL). Panels a and c present density estimates of the occurrence of pneumonia in all patients of the considered group (green lines) and in deceased patients (red lines). Panels b and d present estimates of mortality rate. Coloured areas present bootstrap-based 95% confidence bands of the estimates. Analyses were conducted separately for patients with PVL-positive CAP (a and b) and PVL-negative CAP (c and d).

figure 2: Survival of 143 patients ≥3 years of age with *S. aureus* pneumonia. Survival after ICU admission according to whether the causative strain was methicillin-resistant (MRSA) or -susceptible (MSSA) and whether it harboured the PVL toxin are shown. '+' marks denote censored (discharged) patients. Log-rank test, p=0.0007.

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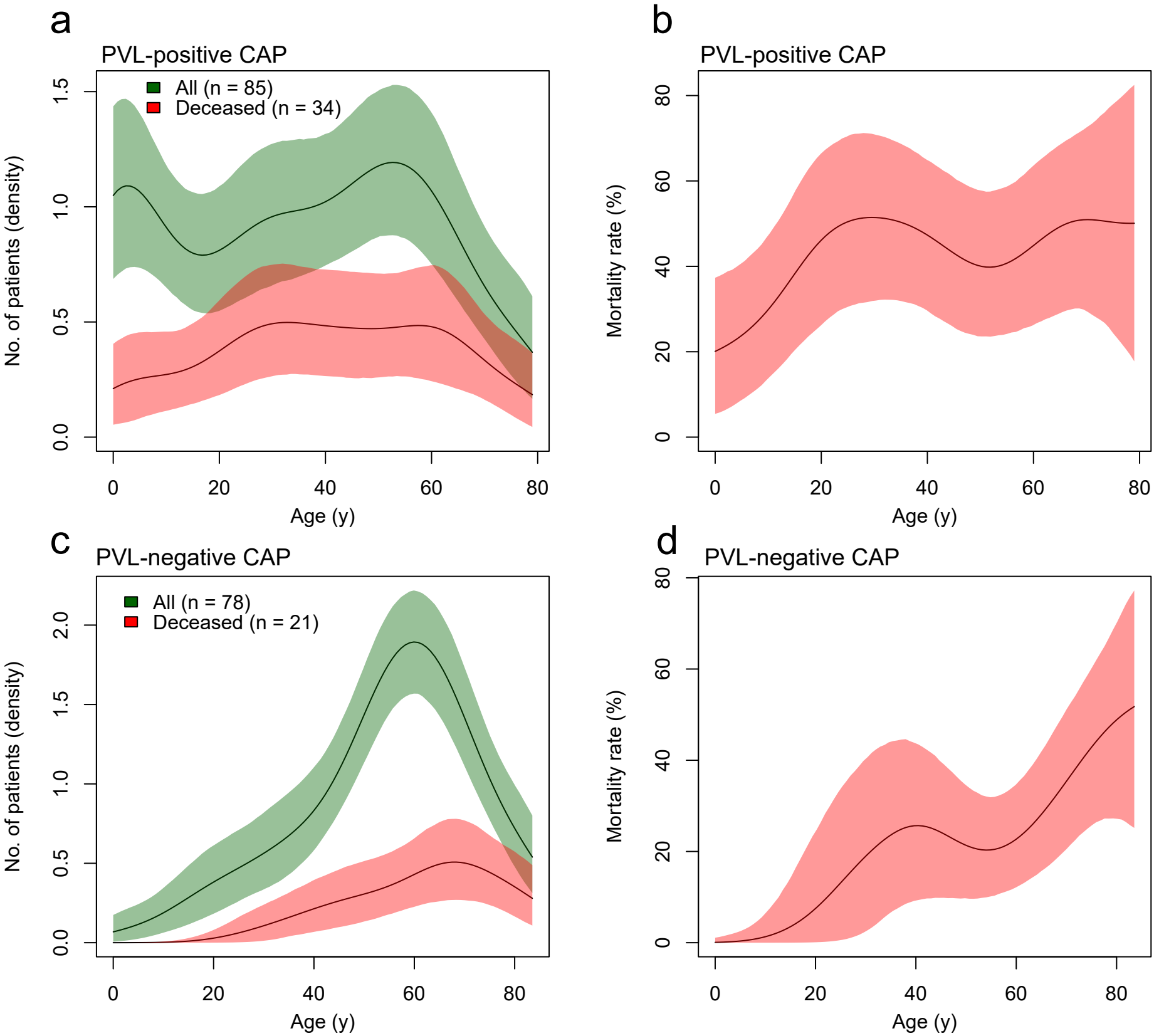
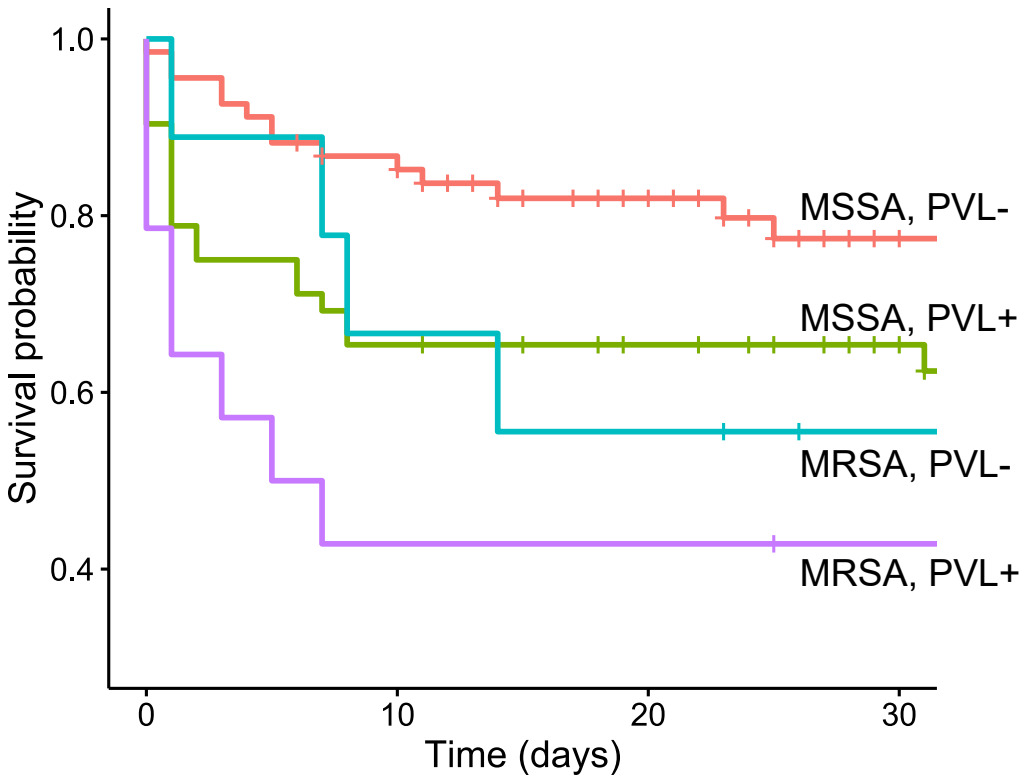

a
PVL-positive CAP
All (n = 85)
Deceased (n = 34)
No. of patients (density)
0.0
0.5
1.0
1.5
0
20
40
60
80
Age (y)
b
PVL-positive CAP
Mortality rate (%)
0
20
40
60
80
Age (y)
c
PVL-negative CAP
All (n = 78)
Deceased (n = 21)
No. of patients (density)
0.0
0.5
1.0
1.5
2.0
Age (y)
d
PVL-negative CAP
Mortality rate (%)
Age (y)


1.0
0.8
0.6
0.4
Survival probability
0
10
20
30
Time (days)
MSSA, PVL-
MSSA, PVL+
MRSA, PVL-
MRSA, PVL+

Supplementary Material

Prognostic Factors of Severe Staphylococcal Pneumonia in France

Yves Gillet[1,2], Anne Tristan[2], Jean-Philippe Rasigade[2], Mitra Saadatian-Elahi[3], Coralie Bouchiat[2], Michele Bes[2], Oana Dumitrescu[2], Marie Leloire[2], Céline Dupieux[2], Frédéric Laurent[2], Gérard Lina[2], Jerome Etienne[2], Philippe Vanhems[3], Laurent Argaud[4], Francois Vandenesch[2], and the PVL pneumonia study group

A complete list of the "PVL pneumonia study group" is listed at the end of the article

Authors affiliations:

1, Paediatric Emergency & Intensive Care Unit, Hospices Civils de Lyon, Lyon, France

2, French Reference Centre for Staphylococci, Institute for Infectious Agents, Hospices Civils de Lyon, Lyon, France

3, Infection Control and Epidemiology Department, Hospices Civils de Lyon, Lyon, France

4, Medical Intensive Care Unit, Hospices Civils de Lyon, Lyon, France (L.A.)

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References

Supplementary Methods

Case definition and criteria for inclusion and exclusion

Staphylococcal pneumonia

Patients who fulfilled the following criteria were included: 1) clinical signs of lung infection (fever and cough or expectoration or abnormal auscultation) associated with radiological signs (new pulmonary infiltrate or pleural effusion), 2) >1 positive microbiological assessment of *S. aureus* involvement (blood culture or pleural fluid positive for *S. aureus*, $>10^3$ CFU/mL *S. aureus* in protected distal bronchial brush, $>10^4$ CFU/mL *S. aureus* in bronchio-alveolar lavage, $\geq 10^6$ CFU/mL *S. aureus* in pure culture in sputum or tracheal aspirate), 3) admission to an intensive care unit (ICU).

Sepsis, septic shock, and Acute Respiratory Distress Syndrome (ARDS)

Since all data required were collected, sepsis and septic shock could be classified according to the third international consensus definition on sepsis and septic shock [1] despite the publication of these definitions after the onset of the study. Likewise, ARDS was classified as light, moderate, or severe according to the "Berlin definition" for ARDS [2].

Age-adapted severity scores

Organ dysfunction was graded at admission, and at day 1, 3, and 7 according to the Pediatric Logistic Organ Dysfunction (PELOD) score for children <18 years and the Sequential Organ Failure Assessment (SOFA) score for adults. To compare the number of organ dysfunctions between paediatric and adult patients, organ dysfunction was considered as present when the corresponding score was above 0.

Study size determination

The target for study size determination was the detection of a 2-fold relative risk of death in PVL-positive severe CAP compared with PVL-negative in a 2-tailed Chi-squared test with 80% power and 0.05 significance threshold. Based on initial estimates of mortality of 50% and 25% in PVL-positive

and -negative CAP, respectively, the per-group sample size required to reject the null hypothesis of equal mortality was n ≥ 58 patients per group.

List of variables

The clinical, laboratory, and microbiological variables were recorded for each included patient through a standardised case report form. The reported variables were:

Patient characteristics

- Age /sex
- Body weight and Body Mass Index (BMI) for adults
- Underlying conditions including immunosuppression, alcohol abuse, and smoking
- Charlson's score
- Personal and familial history of furuncles or other staphylococcal infection
- Flu-like syndrome in the preceding days

Microbiological data

- *Staphylococcus aureus* lineage,
- PVL status,
- Methicillin resistance

Clinical, radiological, and treatment data

The following clinical data were fully assessed at ICU admission (day 0) as well as at day 1, 3, and 7:

- Fever >39°C
- Producing cough and/or purulent expectoration
- Airway haemorrhage,
- Cutaneous rash,

- Therapeutic procedures including ventilator support, prone ventilation, nitric oxide inhalation, need for curare, pleural drainage, antibiotics, and need for inotropic support.
- Radiological features with Murray's score
- Severity score (PELOD for children <18 years and SOFA for adults) with detailed organ dysfunction according to score.

Laboratory data

The following laboratory data were assessed at admission and at day one: total leucocytes and PMN cell count, blood lactate level with change in lactate level between day 0 and day 1, CRP and/or PCT value. If a laboratory value was measured more than once in the corresponding day, the worse value was retained.

Analysis of antibiotic treatment

Due to the observational design of the study, antibiotic treatment regimen varied considerably between patients (>10 different drugs, variously associated, used for initial treatment). Therefore, the potential role of antibiotics in the evolution could not be assessed precisely, nor the effects of potential associations. We could only determine whether at least one drug recommended for treatment of pneumonia was active *in vitro* according to the antibiogram of the corresponding strain ("appropriateness" of the treatment) and whether a drug with toxin-suppressive properties was used. An antibiotic was considered to have toxin-suppressive properties if an inhibition of PVL production *in vitro*, even at sub-inhibitory concentration, was demonstrated, as previously described [3]. Hence, linezolid, clindamycin and rifampicin were considered as toxin-suppressive treatment, irrespectively of the potentially associated molecule.

Statistical analysis of age-dependent distribution of PVL-positivity and mortality

Evolution of pneumonia occurrence and mortality with age was investigated using bootstrapped density estimation [4]. Univariate Gaussian Kernel Density Estimation (KDE) was used to estimate the number of patients with pneumonia per year of age, as well as the number of patients who died. The mortality rate at each age was derived from these estimates. For each estimate, 95% confidence intervals (95% CIs) were obtained from 10,000 bootstrap replicates and represented as confidence bands. The analysis was conducted with a KDE bandwidth of 8 years. An additional analysis was conducted for toddlers (<3 years of age) with PVL-positive *S. aureus*, using a KDE bandwidth of 3 months.

Genotyping of *Staphylococcus aureus* strains

Bacterial DNA was extracted using commercial extraction kits (Qiagen, Courtaboeuf, France) according to the manufacturer's recommended protocol. The *S. aureus* microarray genotyping kit StaphyType DNA array (Alere Technologies GmbH, Jena, Germany) used, as well as related procedures and protocols, have been previously described in detail [5]. Briefly, the method uses multiplex PCR covering 332 alleles corresponding to 180 genes. The assignation of isolates to MLST CCs was determined by comparing whole-array hybridization profiles to previously MLST-typed reference strains in a dedicated database as described elsewhere [5]. The genetic diversity of *S. aureus* isolates at the clonal complex (CC) level was estimated using Simpson's diversity index, representing the probability that two random isolates in a collection belong to different groups. To account for sample size variation between groups, the asymptotic Simpson index was used and 95% CIs were computed using a bootstrap procedure as described elsewhere [6].

Additional results and comments

Bacteriology

The causative strains belonged to 22 Clonal Complexes (CC; table S2); the overall diversity was 91.9% (95% CI, 91.3 to 93.3%). The population structures of PVL-positive and PVL-negative isolates differed markedly at the CC level ($p<0.001$; Fisher's exact test); PVL-positive isolates belonged mostly to CC30, 80 and 121, and PVL-negative isolates to CC398, 30 and 8. Of note, among the PVL-positive MRSA (21 strains), 11 European CC80 clones and 5 USA300 were observed. Diversity was not significantly different between PVL-positive (88.2%; 95%CI, 87.2 to 91.0%) and PVL-negative isolates (89.9%; 95%CI, 88.8 to 92.8%). Among PVL-positive isolates, there was no significant difference in population structure between toddlers and older patients ($p=0.41$).

Interactions between predictors of mortality in multivariable Cox regression

Multicollinearity and interactions between clinical predictors can harm the robustness and bias the interpretation of multivariable models. To investigate the patterns of interactions between potential predictors of mortality, we constructed 13 additional multivariable models by removing, in each model, exactly one predictor from the full multivariable model. We examined how removing one predictor impacted the coefficients of other predictors by recording the differences between the coefficient Z-score in the full model and in the reduced model (figure S4). This revealed potential interactions of interest based on their magnitude and/or biological relevance. Interactions with a large magnitude included:

(1) the SOFA score and blood lactate level, where the SOFA score strongly predicted death only when ignoring blood lactate;
(2) methicillin resistance and the appropriateness of the antibiotics received, where an appropriate therapy moderately predicted survival only when ignoring methicillin resistance.

Interactions with a smaller amplitude bearing a potential biological relevance included:

(1) a flu-like illness and leukopenia, considering that the leukopenia is more likely to represent a severity factor if it results from the inflammatory response to *S. aureus* than from a viral infection.

(2) PVL and a toxin-suppressive chemotherapy, considering that the expected protective effect of toxin-suppressive antibiotics might be maximal in PVL-positive CAP patients.

We examined the impact of these 4 potential interactions by augmenting the full multivariable model with the appropriate interaction terms, performing the stepwise selection procedure (table S3). The model with interactions revealed several features compared to the full model without interactions shown in table 4. First, the SOFA score was retained only when taking the interaction with blood lactate into account. Second, the interaction between flu-like illness and a leukopenia was retained in the final model. Finally, an antitoxin therapy, independent of whether the CAP was PVL-positive or PVL-negative, was retained as a predictor of mortality, although with a wide uncertainty margin.

These data suggest that a flu-like illness appears as a protective factor in patients with leukopenia. Apart from PVL-induced leukopenia, other causes (*i.e.* viral-induced) may be associated with staphylococcal pneumonia and we show herein that preceding influenza-like illness appeared to be protective of severity but only if leukopenia is added as a covariate. One possible explanation may be that, in case of preceding viral-induced leukopenia, the local PMN recruitment may be limited, thus restricting the necrotic effects of PVL. Conversely, in the absence of pre-existing leukopenia, PMN recruitment and degranulation should lead to maximal necrotic effects. This model is supported by experimental data in the rabbit pneumonia model: neutropenic rabbits exposed to a high concentration of PVL developed less intense pulmonary oedema or necrotic lesions as compared to non-neutropenic rabbits [7].

The possible association of an antitoxin therapy with mortality was intriguing at first. However, this association exhibited positive interactions with several other severity factors (figure S4), namely, haemoptysis, leukopenia, and elevated blood lactate. This suggests that antitoxin therapy was preferentially prescribed in the more severe cases and that its role in predicting mortality was more indirect, by capturing otherwise unmeasured factors of severity at admission, than causal.

Specificities of staphylococcal pneumonia in patient aged <3 years (toddlers)

Comparison between toddlers and older patients are summarised in table S1. Features significantly less frequently observed in toddlers were underlying condition (p<0.001), documented *Influenza* infection (p=0.026), haemoptysis (p=0.002), and bilateral involvement (p<0.001). Conversely, pneumonia was more frequently associated with a pleural effusion needing drainage (p<0.001) and pneumothorax (p<0.001). A familial history of furuncles was more frequent in toddlers (p=0.002). No significant difference regarding the values of inflammatory markers (*i.e.* leukocyte count, C reactive protein,) neither at admission nor during hospitalisation, was observed but among PVL-positive patients, toddlers had significantly lower procalcitonin levels at admission (p=0.048). Respiratory failure (p=0.043) and invasive ventilation (p<0.001) were less frequently reported in toddlers. The mean number of organ dysfunctions assessed by age-adapted severity scores was lower in toddlers from admission to day 3, and the mortality rate in toddlers was also significantly lower (p=0.004).

Clinical presentation in toddlers is remarkably different from the symptoms observed in older patients: in toddlers, PVL-positive *S. aureus* pneumonia presented as a pleuropneumonia with pleural effusion or pneumothorax needing drainage in most cases. Progression towards ARDS and features associated with PVL-positive *S. aureus* pneumonia in older patients, such as airway haemorrhage and leukopenia, were only exceptionally observed in toddlers and, despite the very young age, severity was lower. The mean number of organ dysfunctions was lower in toddlers and the mortality in this population was by far lower than in older patients with PVL-positive *S. aureus* CAP. This clinical presentation is consistent with the published descriptions of staphylococcal pneumonia (SPP) in young children which were described as round-shaped multifocal bilateral pneumonia, often associated with pleural effusion. For instance, Carrillo-Marquez *et al*. found a median age of 0.9 months among 117 children aged <18 years and hospitalised for staphylococcal pneumonia (74% community-acquired) during an 8-year period in Texas [8]; 95.5% of cases were due to PPSA and clinical features were very close to those described herein: pleural effusion was common (89.5% of patients), needing drainage in most cases, severity was mild, and mortality <1%. Similar results were observed in France and in Greece

where, respectively, 13/13 and 22/25 strains isolated from children's pneumonia were PVL-positive *S. aureus*. Patients were characterised by young age, high frequency of pleural effusion, and low mortality [9, 10].

The factors determining the difference between staphylococcal pneumonia in toddlers and older patients is a matter of speculation. Bacterial factors are unlikely as lineage distribution of PVL-positive *S. aureus* was similar between the 2 age groups. Host response may be involved considering that local inflammation plays a central role in PVL-induced lesions, as demonstrated by Diep *et al.* in a rabbit model [7]. This lung inflammation is notably a consequence of PVL-mediated inflammasome activation, leading to polymorphonuclear (PMN) cell recruitment and activation [11]. Recruited cells are killed by PVL, resulting in uncontrolled protease release leading to necrosis [11, 12]. In toddlers, it is possible that these effectors are less potent due to immunological immaturity in accordance with the lower PCT level observed in PVL-positive *S. aureus* toddlers than in PVL-positive *S. aureus* adults herein.

Another hypothesis involves passive protection by maternal antibodies. Since a familial history of furuncles was reported in nearly a quarter of toddler cases, their mothers are likely to have developed neutralising antibodies, the transmission of which may attenuate the systemic effects of PVL in the infant. After the clearance of maternal antibodies, repeated encounters of PVL-positive *S. aureus* by the toddler in the household might help elicit a protective immune response against PVL before pneumonia onset. Further analyses of PVL-positive *S. aureus* prevalence and serological tests in the mothers of infected toddlers could help test this hypothesis.

The route of infection may also be involved as PVL-positive *S. aureus* pneumonia of the young adult is characterised by extensive necrotic lesions of respiratory epithelium [13], indicating inhalation as the route of infection. Conversely, the bullous lesions in toddlers point towards the involvement of the supporting tissue, suggesting a haematogenous route of infection. The observed specific symptoms and lower severity in toddlers might reflect a combination of one or more of the above described factors but also other yet to be identified features.

Supplementary table S1. Characteristics of the patients according to age (percentages are calculated among those with data.)

	Age <3 years		Age ≥3 years		p
	n=20	**missing**	**n=143**	**missing**	
PVL +,	19 (95%)	0	58 (41%)	0	<0.001
MRSA,	7 (35%)	0	23 (16%)	0	0.048
Absence of underlying disease*	20 (100%)	0	65 (46%)	0	<0.001
Familial history of furuncles,	4 (23%)	3	1/93 (1%)	47	0.002
Mean delay before ICU admission(SD)	3.15 days (3.31)	0	4.05 days (4.36)	4	0.377
Documented Influenza infection	2 (10%)	0	47 (33%)	1	0.026
Bilateral pneumonia,	7 (37%)	1	106 (82%)	13	<0.001
Significant pleural effusion^	13 (68%)	1	20 (15%)	9	<0.001
Pneumothorax	8 (42%)	1	9 (7%)	12	<0.001
Haemoptysis	2 (10%)	1	63 (47%)	9	0.002
Mean leucocytes at admission(SD)	16.3 G/L (12.9)	1	12.4 G/L (18.1)	4	0.359
Mean CRP at admission in mg/L(SD)	216.1 (132.5)	1	280.1 (148.9)	44	0.083
Mean N° of organ dysfunctions at Day 0(SD)	1.4 (1.8)	0	2.9 (1.7)	0	0.001
Mean N° of organ dysfunctions at Day 1(SD)	1.4 (1.8)	0	3.4 (1.8)	2°	<0.001
Mean N° of organ dysfunction at Day 3 (SD)	0.7 (0.2)	1°°	3 (0.3)	24°°	<0.001
Respiratory failure at admission	9 (45%)	0	99 (69%)	0	0.043
Invasive ventilation	8 (42%)	1	116 (83%)	3	<0.001
Mortality, n (%)	3(15%)	0	52 (36%)	0	0.045
Mortality among PVL+	3 (16%)	0	31 (47%)	0	0.017

PVL, Panton Valentine leucocidin; MRSA, methicillin resistant *Staphylococcus aureus*; ICU, intensive care unit; SD, standard deviation; CRP, C reactive protein.
N°: Number
* Underlying diseases include Malignancies, Tobacco smoking, Alcohol abuse, Diabetes and BMI >30
^ needing drainage
° missing due to death before the end of Day 0
°° missing due to death before Day 2

Supplementary table S2. Clonal complex and resistance according to PVL status and patient age

Clonal complex	**PVL-positive *S.aureus* (n=85)**		**PVL-negative *S.aureus* (n=81)**		**TOTAL n=166[a]**
	MSSA <3 / ≥3 years	**MRSA <3 / ≥3 years**	**MSSA <3 / ≥3 years**	**MRSA <3 / ≥3 years**	
CC 30	5/9		1/12		27
CC 152	1/16				17
CC 8		2/3b	0/5	0/6	16
CC 398	0/2		0/14		16
CC 5	1/2	1/0	0/11	0/1	16
CC 80	0/2	3/8c			13
CC 121	1/10	0/1		0/1	13
CC 15	2/4		0/5		11
CC 1	2/4		0/2	0/1	9
CC45			0/8		8
CC 88		1/2			3
CC 188			0/3		3
CC 97			0/1	0/1	2
CC 7			0/2		2
CC 12			0/2		2
CC 22	0/1		0/1		2
CC 6			0/1		1
CC 20			0/1		1
CC 25			0/1		1
CC 93	0/1				1
CC 96			0/1		1
CC 1153	0/1				1

CC, clonal complex, PVL, Panton Valentine leucocidin ; MSSA, methicillin susceptible *Staphylococcus aureus;* MRSA, methicillin resistant *Staphylococcus aureus*; CA, community-acquired
[a] 163 patients but 166 isolates (2 different isolates for 3 patients).
[b] all 5 belong to CA-MRSA USA300lineage.
[c] all 11 belong to CA-MRSA European ST80 lineage

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Supplementary table S3. Cox regression analysis of predictors of death in patients older than 3 years with *S. aureus* pneumonia, including interaction terms between predictors as suspected in pairwise interaction analysis.

Predictor	Hazard ratio (95% confidence interval)	
	Full model	**Best-fitting model**
Charlson's comorbidity score (per point)	1.00 (0.82 to 1.22)	-
Male sex	0.96 (0.50 to 1.84)	-
PVL	0.86 (0.40 to 1.89)	-
Methicillin resistance	3.12 (0.68 to 14.34)	3.25 (1.70 to 6.21)
SOFA score	1.26 (1.02 to 1.56)	1.26 (1.04 to 1.51)
Flu-like illness	0.54 (0.21 to 1.38)	0.54 (0.22 to 1.35)
Haemoptysis	2.25 (1.14 to 4.47)	2.50 (1.31 to 4.77)
Rash	1.94 (0.90 to 4.22)	2.01 (0.98 to 4.14)
Leukopenia	3.42 (1.28 to 9.17)	3.17 (1.34 to 7.51)
Blood procalcitonin (per 2-fold increase)	1.06 (0.89 to 1.26)	-
Blood lactates (per 2-fold increase)	4.98 (1.88 to 13.19)	5.35 (2.18 to 13.15)
Antitoxin therapy	1.10 (0.25 to 4.95)	1.86 (0.94 to 3.69)
Adapted antimicrobial therapy	1.05 (0.27 to 4.13)	-
Adapted antimicrobial therapy and methicillin resistance	1.61 (0.25 to 10.34)	-
SOFA score times blood lactates	0.92 (0.85 to 1.00)	0.92 (0.86 to 0.99)
Flu-like illness and leukopenia	0.34 (0.08 to 1.39)	0.36 (0.10 to 1.28)
Antitoxin therapy and PVL	1.94 (0.33 to 11.53)	-

NOTE. The full multivariable model included all predictors (likelihood ratio test, $P < 0.001$; Akaike information criterion (AIC), 421 with 17 degrees of freedom). The best-fitting model was obtained using a stepwise procedure, starting from the full model and minimizing the AIC (408 with 10 degrees of freedom). Confidence interval widths were not corrected for test multiplicity. Abreviations: PVL, Panton-Valentine leukocidin; SOFA score, sequential organ failure assessment score.

Supplementary Table S4. Ranking of 28 *S.aureus* virulence determinants by inclusion order in LASSO Cox regression survival analysis in 143 adult patients with severe CAP.

Predictor	Model size	AICc	BIC	Coefficient
mecA	1	0.446	3.380	0.107
lukS-PV	2	0.213	6.053	0.007
edinB	4	1.495	13.056	0.003
icaC	4	1.495	13.056	-0.041
eap	4	-0.068	11.494	-0.021
sdrD	5	-1.903	12.473	-0.015
sasG	6	-1.124	16.035	-0.031
agrIV	7	-0.455	19.455	0.045
charlson	8	-0.667	21.961	0.003
sea	10	2.632	30.594	0.022
egc	10	2.632	30.594	-0.022
agrIII	12	4.682	37.836	-0.042
fnbB	12	4.682	37.836	-0.044
chp	13	3.209	38.904	0.060
sdrC	14	4.405	42.603	0.000
splA	17	9.864	55.336	0.006
cna	17	9.864	55.336	0.038
agrI	18	8.753	56.568	-0.018
hlgC	18	6.756	54.571	0.027
sak	19	8.531	58.647	0.026
bbp	20	10.436	62.808	0.018
lukD	21	12.435	67.018	-0.022
aur	22	14.545	71.294	-0.004
lukE	23	14.112	72.980	-0.014
cap5	25	19.651	82.611	-0.004
cap8	25	19.651	82.611	0.000
agrII	26	22.465	87.395	0.000
etD	-	-	-	-
fib	-	-	-	-

NOTE. Model size is the number of predictors in the most parsimonious model including the predictor. Coefficient shown is from the most parsimonious model, which is not necessarily the best-fitting one. AICc, Akaike information criterion with small sample correction. BIC, Bayes information criterion. In both criteria, lower values correspond to better fit. BIC is more conservative (favors more parsimonious models) compared to AICc. '-', predictor not included in model.

Supplementary Table S5. Ranking of 28 *S.aureus* virulence determinants by inclusion order in LASSO logistic regression of rash at admission in 143 adult patients with severe CAP.

Predictor	Model size	AICc	BIC	Coefficient
agrIV	1	-4.379	-1.452	0.453
cap5	3	-19.500	-10.806	-0.023
cap8	3	-19.500	-10.806	0.000
cna	4	-19.022	-7.490	0.003
lukS-PV	5	-20.959	-6.621	0.043
agrI	6	-20.904	-3.791	-0.012
bbp	6	-19.472	-2.360	-0.071
chp	9	-15.793	9.446	-0.035
eap	9	-15.793	9.446	0.011
fnbB	10	-13.989	13.890	0.006
egc	11	-12.224	18.259	-0.056
charlson	12	-11.945	21.107	-0.004
agrII	12	-10.612	22.439	0.071
lukE	13	-10.691	24.891	-0.073
sak	15	-6.964	33.563	0.104
splA	15	-6.964	33.563	-0.088
mecA	17	-4.329	40.984	0.033
sea	18	-7.033	40.611	-0.005
lukD	19	-5.920	44.011	-0.045
aur	20	-3.682	48.492	0.046
etD	21	-1.720	52.653	0.011
sasG	21	-2.351	52.021	0.014
hlgC	23	0.873	59.502	0.059
edinB	23	1.107	59.735	0.004
sdrD	23	2.955	61.583	-0.081
fib	25	6.484	69.172	0.039
agrIII	-	-	-	-
icaC	-	-	-	-
sdrC	-	-	-	-

NOTE. Model size is the number of predictors in the most parsimonious model including the predictor. Coefficient shown is from the most parsimonious model, which is not necessarily the best-fitting one. AICc, Akaike information criterion with small sample correction. BIC, Bayes information criterion. In both criteria, lower values correspond to better fit. BIC is more conservative (favors more parsimonious models) compared to AICc. '-', predictor not included in model.

Supplementary Table S6. Ranking of 28 *S.aureus* virulence determinants by inclusion order in LASSO logistic regression of hemoptysis at admission in 143 adult patients with severe CAP.

Predictor	Model size	AICc	BIC	Coefficient
edinB	1	-0.095	2.839	0.136
lukS-PV	2	-4.840	1.000	0.018
egc	3	-5.325	3.391	-0.031
agrII	4	-4.932	6.630	0.016
sea	5	-3.589	10.788	0.058
mecA	9	4.222	29.534	-0.058
lukE	9	4.222	29.534	0.033
sak	9	4.222	29.534	0.006
cap5	9	4.222	29.534	0.029
bbp	10	3.137	31.099	-0.028
chp	11	4.810	35.386	0.014
eap	12	5.781	38.935	0.052
cap8	14	8.704	46.903	-0.008
sdrC	14	8.704	46.903	-0.008
icaC	16	13.273	56.361	-0.002
sasG	16	13.273	56.361	-0.006
fib	17	15.312	60.785	-0.022
sdrD	18	17.304	65.119	0.013
fnbB	19	19.390	69.505	0.014
agrI	20	20.455	72.827	-0.018
aur	20	19.639	72.010	-0.028
charlson	21	22.153	76.736	0.000
agrIII	22	24.565	81.315	-0.007
splA	23	27.249	86.117	0.005
hlgC	24	29.642	90.581	0.023
lukD	25	32.491	95.451	0.006
cna	26	34.984	99.914	-0.002
agrIV	27	37.813	104.662	-0.001
etD	-	-	-	-

NOTE. Model size is the number of predictors in the most parsimonious model including the predictor. Coefficient shown is from the most parsimonious model, which is not necessarily the best-fitting one. AICc, Akaike information criterion with small sample correction. BIC, Bayes information criterion. In both criteria, lower values correspond to better fit. BIC is more conservative (favors more parsimonious models) compared to AICc. '-', predictor not included in model.

Supplementary Table S7. Ranking of 28 *S.aureus* virulence determinants by inclusion order in LASSO logistic regression of leukopenia at admission in 143 adult patients with severe CAP.

Predictor	Model size	AICc	BIC	Coefficient
edinB	1	-0.826	2.079	0.163
icaC	2	-5.734	0.046	-0.004
sdrC	3	-4.859	3.767	0.000
lukS-PV	6	-0.933	16.038	0.021
lukD	6	-0.933	16.038	-0.054
cap5	6	-0.933	16.038	0.021
cap8	6	-0.933	16.038	0.000
charlson	7	-3.236	16.450	-0.010
agrI	8	1.128	23.496	0.040
sea	9	-6.192	18.822	0.076
egc	9	-6.192	18.822	-0.003
etD	10	-6.172	21.454	0.038
agrIII	11	-7.620	22.581	-0.063
mecA	12	-11.628	21.109	-0.016
splA	12	-9.185	23.553	0.093
bbp	12	-10.895	21.843	0.016
agrII	15	-5.397	34.717	0.000
sak	15	-5.397	34.717	0.009
fib	16	-5.250	37.242	-0.018
sasG	17	-2.923	41.905	0.005
chp	18	-0.859	46.262	-0.005
fnbB	19	1.558	50.926	-0.036
sdrD	20	3.270	54.840	-0.008
agrIV	21	5.924	59.651	-0.009
lukE	23	10.954	68.847	0.007
eap	24	13.686	73.587	0.000
cna	25	16.649	78.506	-0.005
hlgC	26	19.535	83.295	-0.002
aur	26	19.605	83.366	0.000

NOTE. Model size is the number of predictors in the most parsimonious model including the predictor. Coefficient shown is from the most parsimonious model, which is not necessarily the best-fitting one. AICc, Akaike information criterion with small sample correction. BIC, Bayes information criterion. In both criteria, lower values correspond to better fit. BIC is more conservative (favors more parsimonious models) compared to AICc.

Supplementary Table S8. Targets of DNA array included in survival models.

Locus in StaphyType DNA array	Considered for inclusion	Included in analysis
rrnD1 (S. aureus)		
gapA		
katA		
coA	+	
nuc1		
spa	+	
sbi	+	
sarA		
saeS		
vraS		
agrI (total)	+	+
agrB-I		
agrC-I		
agrD-I		
agrII (total)	+	+
agrB-II		
agrC-II		
agrD-II		
agrIII (total)	+	+
agrB-III		
agrC-III		
agrD-III		
agrIV (total)	+	+
agrB-IV		
agrC-IV		
hld	+	
mecA	+	+
delta_mecR		
ugpQ		
ccrA-1		
ccrB-1		
plsSCC (COL)		
Q9XB68-dcs		
ccrA-2		
ccrB-2		
kdpA-SCC		
kdpB-SCC		
kdpC-SCC		
kdpD-SCC		
kdpE-SCC		

mecI		
mecR		
xylR		
ccrA-3		
ccrB-3		
merA		
merB		
ccrAA (MRSAZH47)_probe 1		
ccrAA (MRSAZH47)_probe 2		
ccrC (85-2082)		
ccrA-4		
ccrB-4		
mecA-M10 = mecC		
blaZ-SCCmec XI = (blaZ-M10)		
blaZ		
blaI		
blaR		
erm(A)		
erm(B)		
erm(C)		
lnu(A)		
msr(A)		
mef(A)		
mph(C)		
vat(A)		
vat(B)		
vga(A)		
vga(A) (BM 3327)		
vgB(A)		
aacA-aphD		
aadD		
aphA3		
sat		
dfrS1		
far1		
fusC (Q6GD50)		
mupA		
tet(K)		
tet(M)		
cat (total)		
cat (pC221)		
cat (pc223)		
cat (pMC524/pC194)		
cat (pSBK203R)		
cfr		

fexA		
fosB		
fosB (plasmid)		
qacA		
qacC (total)		
qacC (consensus)		
qacC (equine)		
qacC (SA5)		
qacC (Ssap)		
qacC (ST94)		
sdrM		
vanA		
vanB		
vanZ		
tst1 (consensus)	+	
tst1 ("human" allele)		
tst1 ("bovine" allele)		
sea	+	+
sea (320E)		
sea (N315) / sep	+	
seb	+	
sec	+	
sed	+	
see	+	
seg		
seh	+	
sei		
sej		
sek	+	
sel		
selm		
seln (consensus)		
seln (other than RF122)		
selo		
egc (total)	+	+
seq		
ser		
selu		
ORF CM14_ probe1		
ORF CM14_ probe2		
lukF		
lukS	+	
lukS (ST22+ST45)		
hlgA	+	+
lukF-PV		

lukS-PV	+	+
lukF-PV (P83)		
lukM		
lukD	+	+
lukE	+	+
lukX		
lukY		
lukY (ST30+ST45)		
hl		
hla	+	
hlIII (consensus)		
hlIII (other than RF122)		
hlb_probe 1		
hlb_probe 2		
hlb_probe 3		
un-disrupted hlb	+	
sak	+	+
chp	+	+
scn	+	
etA	+	
etB	+	
etD	+	+
edinA	+	
edinB	+	+
edinC	+	
arcA-SCC		
arcB-SCC		
arcC-SCC		
arcD-SCC		
aur (consensus)	+	+
aur (other than MRSA252)		
aur (MRSA252)		
splA	+	+
splB		
splE		
sspA	+	
sspB	+	
sspP (consensus)	+	
sspP (other than ST93)		
setC		
ssl01/set6_probe1_11		
ssl01/set6_probe2_11		
ssl01/set6_probe1_12		
ssl01/set6_probe2_12		
ssl01/set6_probe4_11		

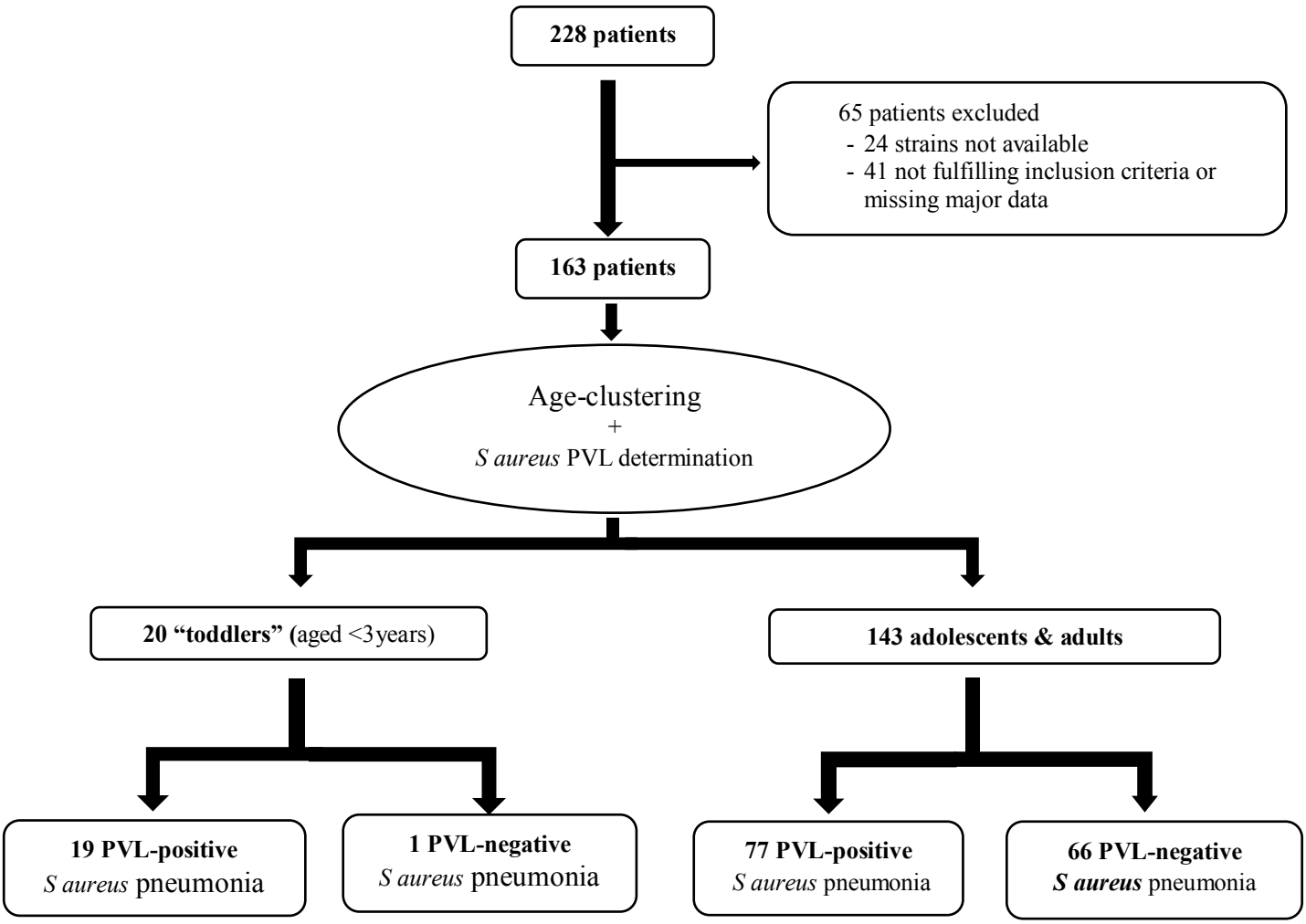
ssl01/set6_probeRF122		
ssl01/set6 (COL)		
ssl01/set6 (Mu50+N315)		
ssl01/set6 (MW2+MSSA476)		
ssl01/set6 (MRSA252)		
ssl01/set6 (RF122)		
ssl01/set6 (other alleles)		
ssl02/set7		
ssl02/set7 (MRSA252)		
ssl03/set8_probe 1		
ssl03/set8_probe 2		
ssl03/set8 (MRSA252, SAR0424)		
ssl04/set9		
ssl04/set9 (MRSA252, SAR0425)		
ssl05/set3_probe 1		
ssl05/set3 (RF122, probe-611)		
ssl05/set3_probe 2 (612)		
ssl05/set3 (MRSA252)		
ssl06/set21		
ssl06 (NCTC8325+MW2)		
ssl07/set1		
ssl07/set1 (MRSA252)		
ssl07/set1 (AF188836)		
ssl08/set12_probe 1		
ssl08/set12_probe 2		
ssl09/set5_probe 1		
ssl09/set5_probe 2		
ssl09/set5 (MRSA252)		
ssl10/set4		
ssl10 (RF122)		
ssl10/set4 (MRSA252)		
ssl11/set2 (COL)		
ssl11+set2(Mu50+N315)		
ssl11+set2(MW2+MSSA476)		
ssl11/set2 (MRSA252)		
setB3		
setB3 (MRSA252)		
setB2		
setB2 (MRSA252)		
setB1		
cap 1 (total)	+	
capH1		
capJ1		
capK1		
cap 5 (total)	+	+

capH5		
capJ5		
capK5		
cap 8 (total)	+	+
capH8		
capI8		
capJ8		
capK8		
icaA	+	
icaC	+	+
icaD	+	
bap	+	
bbp (total)	+	+
bbp (consensus)		
bbp (COL+MW2)		
bbp (MRSA252)		
bbp (Mu50)		
bbp (RF122)		
bbp (ST45)		
clfA (total)	+	
clfA (consensus)		
clfA (COL+RF122)		
clfA (MRSA252)		
clfA (Mu50+MW2)		
clfB (total)	+	
clfB (consensus)		
clfB (COL+Mu50)		
clfB (MW2)		
clfB (RF122)		
cna	+	+
ebh (consensus)	+	
ebpS (total)	+	
ebpS_probe 612		
ebpS_probe 614		
ebpS (01-1111)		
ebpS (COL)		
eno	+	
fib	+	+
fib (MRSA252)		
fnbA (total)	+	
fnbA (consensus)		
fnbA (COL)		
fnbA (MRSA252)		
fnbA (Mu50+MW2)		
fnbA (RF122)		

fnbB (total)	+	+
fnbB (COL)		
fnbB (COL+Mu50+MW2)		
fnbB (Mu50)		
fnbB (MW2)		
fnbB (ST15)		
fnbB (ST45-2)		
map (total) (aka eap)	+	+
map (COL)		
map (MRSA252)		
map (Mu50+MW2)		
sasG (total)	+	+
sasG (COL+Mu50)		
sasG (MW2)		
sasG (other than MRSA252+RF122)		
sdrC (total)	+	+
sdrC (consensus)		
sdrC (B1)		
sdrC (COL)		
sdrC (Mu50)		
sdrC (MW2+MRSA252+RF122)		
sdrC (other than MRSA252+RF122)		
sdrD (total)	+	+
sdrD (consensus)		
sdrD (COL+MW2)		
sdrD (Mu50)		
sdrD (other)		
vwb (total)	+	
vwb (consensus)		
vwb (COL+MW2)		
vwb (MRSA252)		
vwb (Mu50)		
vwb (RF122)		
isaB		
isaB (MRSA252)		
mprF (COL+MW2)		
mprF (Mu50+MRSA252)		
isdA (consensus)	+	
isdA (MRSA252)		
isdA (other than MRSA252)		
lmrP (other than RF122)_probe1		
lmrP (other than RF122)_probe2		
lmrP (RF122)_probe1		
lmrP (RF122)_probe2		
hsdS1 (RF122)		

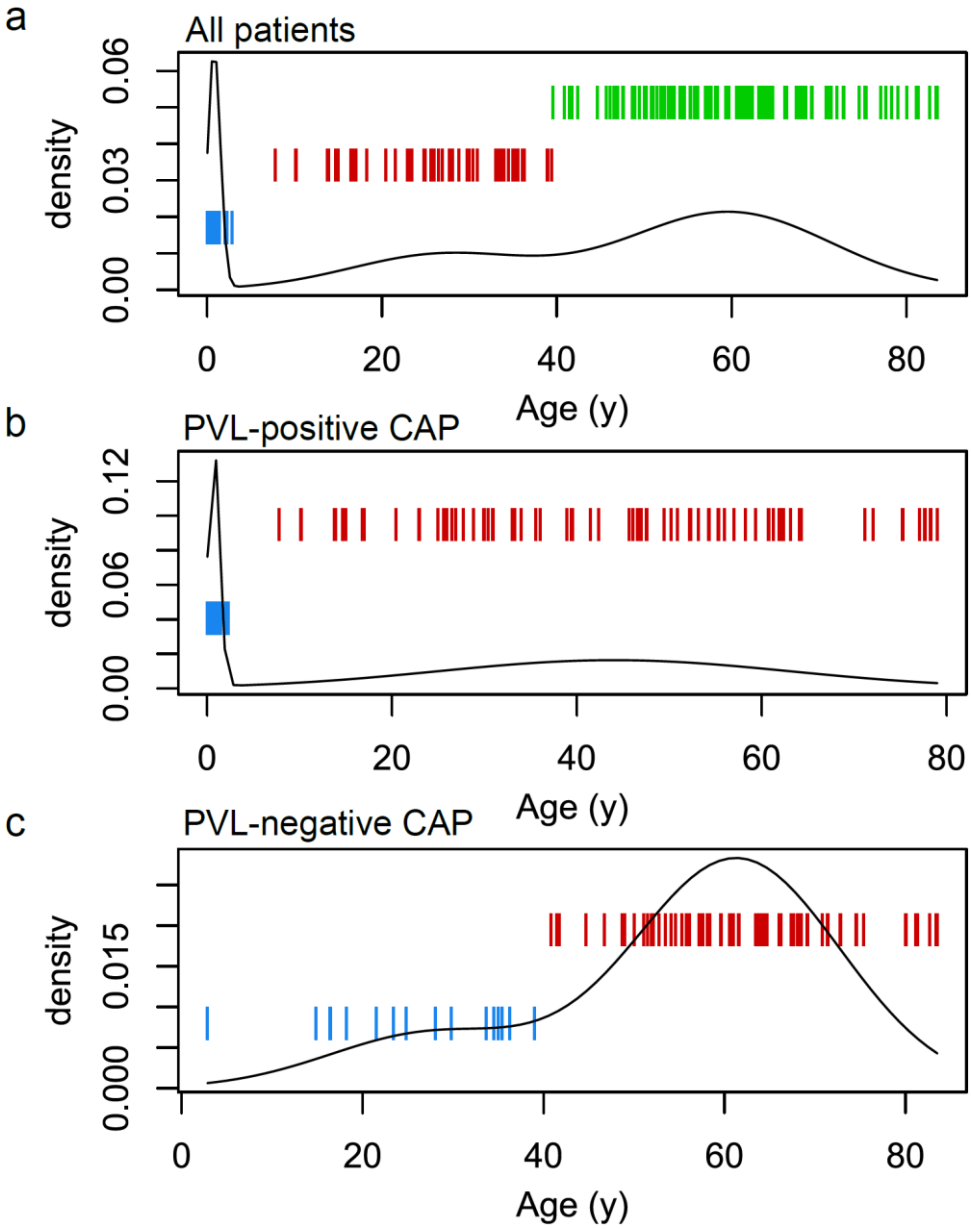
hsdS2 (Mu50+N315+COL+USA300+NCTC8325)		
hsdS2 (MW2+MSSA476)		
hsdS2 (RF122)		
hsdS2 (MRSA252)		
hsdS3 (all other than RF122+ MRSA252)		
hsdS3 (COL+USA300+NCTC8325+MW2+MSSA476+RF122)		
hsdS3 (Mu50+N315)		
hsdS3 (CC51+ MRSA252)		
hsdS3 (MRSA252)		
hsdSx (CC25)		
hsdSx (CC15)		
hsdSx (etd)		
Q2FXC0		
Q2YUB3		
Q7A4X2		
hysA1 (MRSA252)		
hysA1 (MRSA252+RF122) and/or hysA2 (consensus)		
hysA1 (MRSA252+RF122) and/or hysA2 (COL+USA300)		
hysA2 (all other than MRSA252)		
hysA2 (COL+USA300+NCTC8325)		
hysA2 (MRSA252)		

Supplementary figure S1: Flow chart.

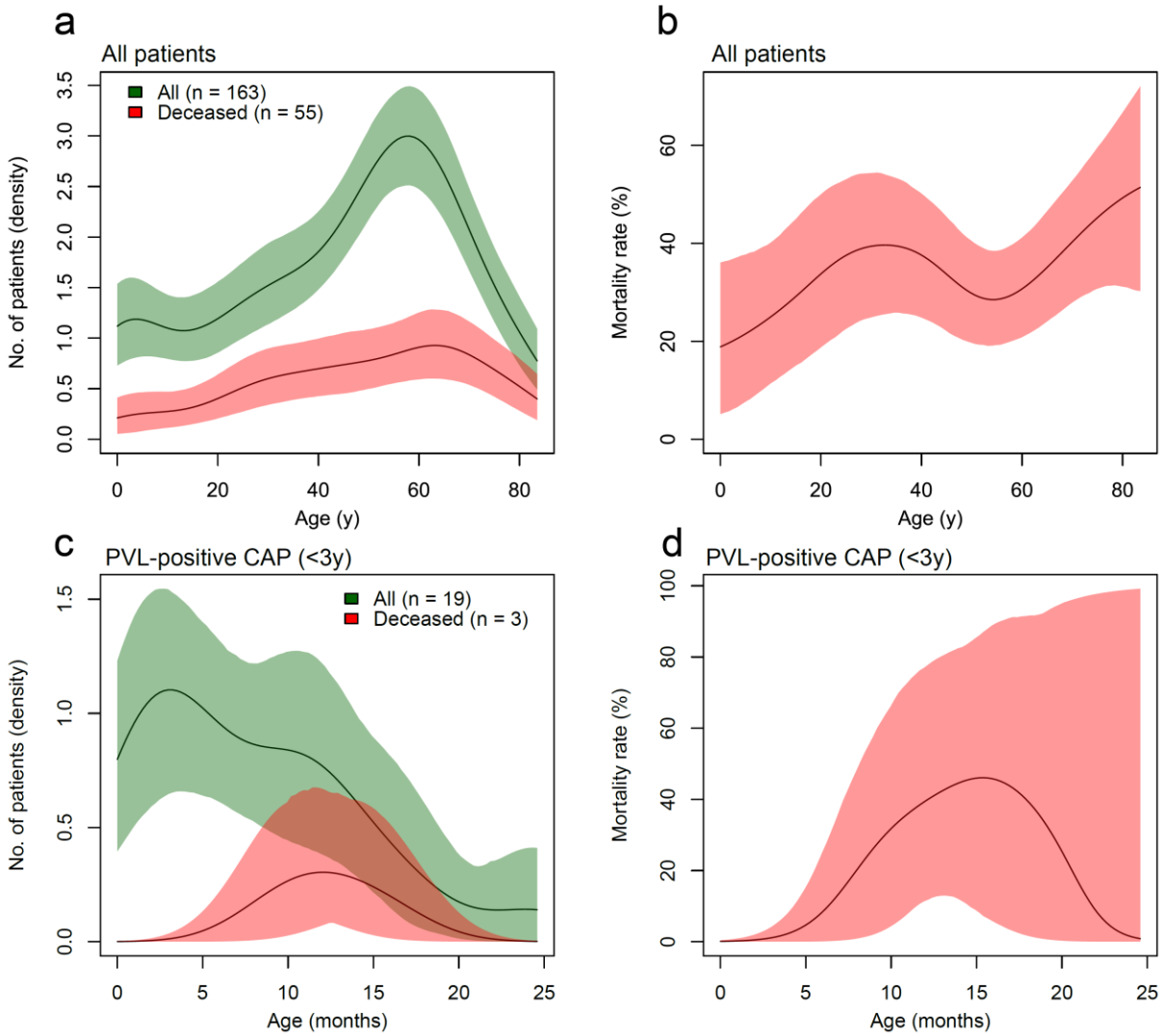


Age clustering was assessed as described in supplementary Methods. Of note, among the 41 excluded patients for which strain characteristics were available, there were two toddlers bearing a PVL-positive strains and 39 older patients of which 51% were PVL-positive. Overall, this distribution reflects that of the population retained for analysis

Supplementary figure S2: Age distribution in patients with *S. aureus* pneumonia depends on PVL presence. Shown are density curves of patients based on best-fitting Gaussian clusters in all patients (a; three clusters), PVL-positive pneumonia patients (b; two clusters) and PVL-negative pneumonia patients (c; two clusters). Vertical marks denote individual patients and colour denote cluster assignment.



Supplementary figure S3. Age distribution and age-dependent mortality in patients with *S. aureus* community-acquired pneumonia (CAP). Panels a and c present density estimates of the occurrence of pneumonia in all patients of the considered group (green lines) and in deceased patients (red lines). Panels b and d present estimates of mortality rate. Coloured areas present bootstrap-based 95% confidence bands of the estimates. Analyses were conducted separately for all patients (a and b) and for patients <3 years of age (toddlers) with PVL-positive CAP (c and d).



Supplementary figure S4. Pairwise interaction analysis between predictors of mortality in multivariable Cox regression. Panel A shows the Z-scores of coefficients (in rows) obtained by removing exactly 1 other coefficient (in columns) from the full multivariable model. Z-scores in the reduced model represent the direction and strength of the association of the predictor in rows after exclusion of the predictor in column. Panel B shows the difference between Z-scores in the reduced model and in the full model. Pairs of predictors with positive Z-score difference exhibit a negative (or antagonistic) interaction while a negative Z-score difference suggests a positive (or synergistic) interaction.

A

Predictor excluded from the full model

	Full model	Charlson comorbidity score (per point)	Male sex	PVL	Methicillin resistance	SOFA score	Flu-like illness	Haemoptysis	Rash	Leukopenia	Blood procalcitonin (per 2-fold increase)	Blood lactates (per 2-fold increase)	Adapted antimicrobial therapy	Antitoxin therapy
Charlson comorbidity score (per point)	0.33		0.31	0.54	0.98	0.29	1.33	0.47	0.46	0.20	0.35	0.56	0.45	0.38
Male sex	-0.15	-0.08		-0.12	0.09	-0.20	-0.41	-0.07	-0.40	-0.17	-0.11	-0.70	-0.09	-0.32
PVL	-0.35	-0.55	-0.34		0.23	-0.37	-0.51	0.01	-0.01	-0.25	-0.33	0.09	-0.29	-0.28
Methicillin resistance	2.50	2.68	2.49	2.49		2.51	2.99	2.39	2.10	2.27	2.61	2.23	3.03	2.63
SOFA score	0.91	0.90	0.92	0.92	0.93		0.48	1.10	0.87	0.55	1.12	2.99	0.94	0.86
Flu-like illness	-2.94	-3.19	-2.98	-2.96	-3.30	-2.85		-2.99	-3.14	-2.55	-2.89	-3.02	-3.22	-2.94
Haemoptysis	1.91	1.94	1.91	1.87	1.77	2.01	2.02		2.19	2.63	2.04	1.30	1.91	2.00
Rash	2.49	2.52	2.53	2.47	2.04	2.48	2.79	2.76		2.47	2.58	1.45	2.40	2.47
Leukopenia	2.45	2.43	2.45	2.43	2.20	2.34	1.89	3.05	2.41		2.55	3.00	2.42	2.69
Blood procalcitonin (per 2-fold increase)	0.54	0.55	0.53	0.53	0.93	0.83	-0.12	0.87	0.79	0.87		1.50	0.63	0.54
Blood lactates (per 2-fold increase)	4.10	4.11	4.17	4.08	3.96	4.90	4.17	3.93	3.68	4.41	4.31		4.12	4.18
Adapted antimicrobial therapy	0.77	0.83	0.76	0.74	-1.70	0.80	1.51	0.78	0.50	0.71	0.84	0.91		0.95
Antitoxin therapy	0.70	0.72	0.76	0.67	1.05	0.63	0.64	0.91	0.63	1.32	0.70	1.10	0.90	

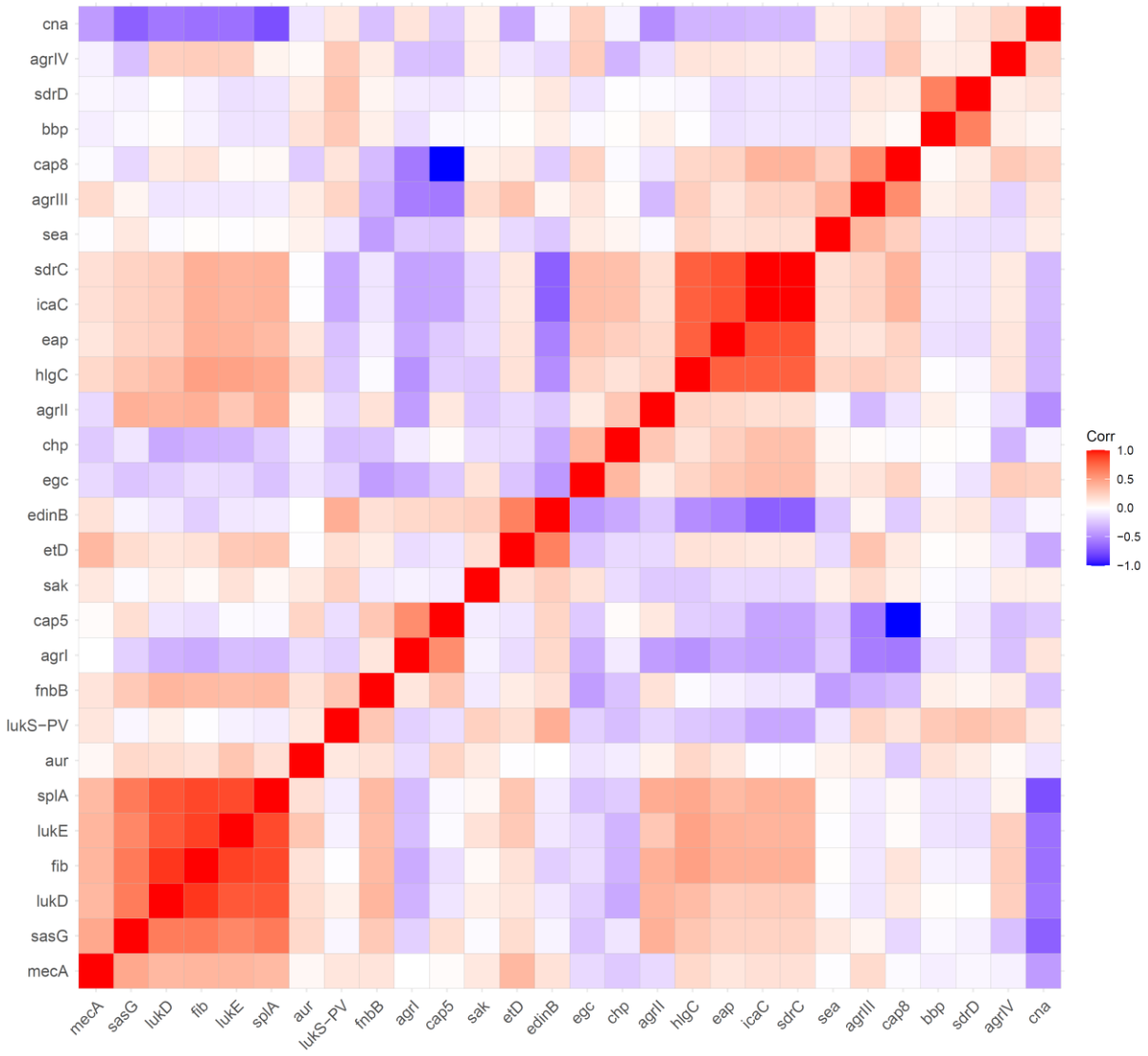
Z-score of coefficient

B

	Full model	Charlson comorbidity score (per point)	Male sex	PVL	Methicillin resistance	SOFA score	Flu-like illness	Haemoptysis	Rash	Leukopenia	Blood procalcitonin (per 2-fold increase)	Blood lactates (per 2-fold increase)	Adapted antimicrobial therapy	Antitoxin therapy
Charlson comorbidity score (per point)	0.33		-0.02	0.21	0.65	-0.04	1.00	0.14	0.13	-0.13	0.02	0.23	0.12	0.05
Male sex	-0.15	0.06		0.03	0.23	-0.05	-0.26	0.08	-0.25	-0.03	0.04	-0.55	0.06	-0.17
PVL	-0.35	-0.20	0.01		0.59	-0.01	-0.15	0.37	0.34	0.11	0.02	0.45	0.07	0.07
Methicillin resistance	2.50	0.18	0.00	-0.01		0.01	0.49	-0.11	-0.39	-0.23	0.11	-0.27	0.53	0.13
SOFA score	0.91	-0.01	0.01	0.01	0.01		-0.44	0.19	-0.04	-0.36	0.20	2.08	0.03	-0.05
Flu-like illness	-2.94	-0.24	-0.04	-0.02	-0.36	0.09		-0.05	-0.20	0.39	0.06	-0.08	-0.28	0.01
Haemoptysis	1.91	0.03	0.00	-0.04	-0.14	0.10	0.11		0.29	0.73	0.13	-0.60	0.00	0.09
Rash	2.49	0.03	0.04	-0.02	-0.45	-0.01	0.30	0.27		-0.03	0.09	-1.04	-0.09	-0.02
Leukopenia	2.45	-0.01	0.00	-0.01	-0.25	-0.11	-0.56	0.61	-0.04		0.10	0.56	-0.02	0.24
Blood procalcitonin (per 2-fold increase)	0.54	0.01	-0.01	-0.01	0.39	0.29	-0.66	0.33	0.25	0.33		0.96	0.09	0.00
Blood lactates (per 2-fold increase)	4.10	0.02	0.07	-0.01	-0.14	0.81	0.07	-0.17	-0.42	0.31	0.21		0.03	0.09
Adapted antimicrobial therapy	0.77	0.06	-0.01	-0.03	-2.47	0.03	0.73	0.00	-0.28	-0.07	0.06	0.14		0.17
Antitoxin therapy	0.70	0.02	0.06	-0.03	0.35	-0.07	-0.06	0.21	-0.07	0.62	0.00	0.40	0.20	

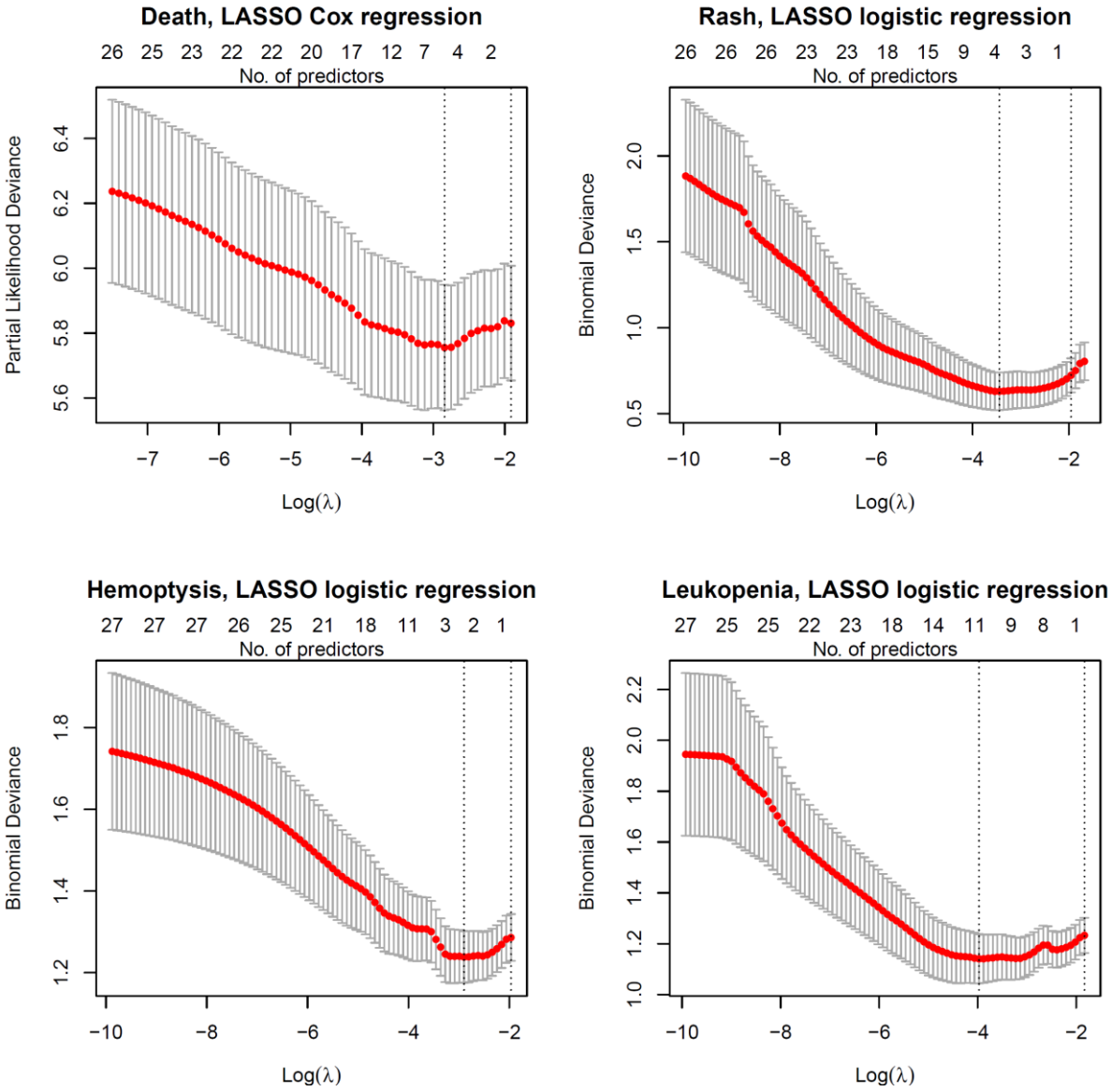
Z-score difference compared to the full model

Supplementary Figure S5. Heatmap of Spearman correlations between 28 genetic determinants in 143 *S. aureus* isolates from severe CAP patients.



Supplementary Figure S6. Leave-one-out cross-validation profiles of LASSO regression models of death (Cox regression), rash, hemoptysis and leukopenia (logistic regression).

Each panel shows the leave-one-out deviance and standard deviation (error bar) as a function of the LASSO penalty parameter λ, where larger penalty favors more parsimonious models. In each panel, vertical dashed lines identify the penalty with minimal leave-one-out deviance (left line), corresponding to the best-fitting models shown in Table 6; and the penalty with deviance equal to the minimal deviance plus one standard deviation (right line), corresponding to more parsimonious but weakly informative models that were not considered further.



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