Staphylococcus aureus Ventilator-Associated Pneumonia: A Study of Bacterio-Epidemiological Profile and Virulence Factors

Lamia Tilouche[1,2] · Refka Ben Dhia[1,2] · Sameh Boughattas[1,2] · Soumaya Ketata[1,2] · Olfa Bouallegue[1,3] · Cherifa Chaouch[1,2] · Noureddine Boujaafar[1,2]

Abstract

Ventilator-associated pneumonia (VAP) represents a major cause of nosocomial infections in the intensive care units in which *Staphylococcus aureus* is frequently involved. Better knowledge of this pathogen is required in order to enhance the patient's treatment and care. In this article, we studied the bacteriological profile and virulence factors of *S. aureus*-related VAP on a 3-year period. We included a collection of *S. aureus* strains ($n = 35$) isolated from respiratory samples from patients diagnosed with VAP in the intensive care units. We studied the bacteriological aspects and we searched for the presence of virulence factors (*SpA, FnbpA, Hla,* and *PVL genes*) in the strains, and we also studied the clinical and biological aspects of the infections. The average age of our patients was of 36 years and they were predominantly males (*sex ratio* = 3.37). A severe head trauma or a history of coma was noted in 73.43% of the patients. The average duration of ventilation was 29 days. Among the studied strains, five were Methicillin-resistant *S. aureus* of which three expressed the *mecA* gene. Overall, the Hla gene was detected in 85.7% of the strains and it was more prevalent in Methicillin-susceptible than Methicillin-resistant strains (93.3% versus 40%; $P = 0.014$). *FnbpA, Spa,* and *PVL* genes were detected, respectively, in 80%, 45.7%, and 20% of the strains. Therefore, our studied strains were essentially associated with the production of Hla and FnbpA genes. It is, however, important to elucidate their expression in order to establish their role in the VAP pathogenesis.

Introduction

Hospital-acquired pneumonia is a growing concern in the healthcare system, because of its impact on the prognosis of the initial condition and the significant cost of its management. In intensive care units, among intubated and mechanically ventilated patients, ventilator-associated pneumonia (VAP) is usually linked to high mortality and morbidity rates [1].

Staphylococcus aureus is frequently implicated in these infections and usually complicates them due to its important virulence factors and its resistance to antibiotics [2]. In this article, we conducted a retrospective study in order to establish the clinico-epidemiological profile of *S. aureus*-related VAP in intensive care units (ICU), study the bacteriological characteristics, elucidate the virulence factors of the isolated strains, and to compare our results to the literature data.

Materials and Methods

Collected Strains and Bacterial Culture

Our study, conducted on a three-year period (from 2017 to 2019) involved all non-redundant strains of *S. aureus* isolated in our laboratory from different types of respiratory samples (sputum, bronchoalveolar lavage, and protected specimen brush) in patients diagnosed with VAP in the surgical ICU of our hospital.

For sputum, after fluidification (in equal quantities of secretions and thinning agent), a dilution to 10^{-4} is seeded on 5% sheep blood agar and chocolate blood agar supplemented with PolyVitaleX, using a calibrated handle of 10 μL, and submitted for culture on Wilkins Chalgren anaerobe broth.

✉ Refka Ben Dhia
refkabendhia@gmail.com

1 Department of microbiology, Sahloul University Teaching Hospital, Route de la ceinture, H.Sousse, 4011 Monastir, Tunisia

2 Faculty of Pharmacy of Monastir, University of Monastir, Monastir, Tunisia

3 Faculty of medicine Ibn El-Jazzar of Sousse, University of Sousse, Sousse, Tunisia

The media are examined after 24–48 h of incubation. One colony represents 10^6 CFU/mL (dilution factor $= 10^{-4}$). The threshold is 10^7 CFU/mL. After homogenization, the bronchoalveolar lavage and the protected specimen brush are directly submitted to culture on the same media without dilution using a calibrated handle of 10 µL. One colony represents 10^2 CFU/mL. The threshold is of 10^4 CFU/mL for the bronchoalveolar lavage and 10^3 CFU/mL for the protected specimen brush.

Study Population

Demographic data (age, gender, and underlying diseases), clinical data (duration of mechanical ventilation, clinical criteria of VAP, antibiotic therapy, and outcomes of patients), and biological data [C-reactive protein (CRP), procalcitonin (PCT), and leukocyte count] were collected by consulting medical files.

Microbiology

The identification of *S. aureus* was done using Gram staining and phenotypic tests among catalase production, rapid agglutination test with latex particles (Pastorex™ Staph-Plus BIORAD®), and the tube coagulase method using rabbit plasma (BIORAD®, France).

Antibiotic susceptibility testing of *S. aureus* was performed with the disk diffusion method using Mueller–Hinton agar base or with the Vitek2® system (BioMerieuxInc. Marcy l'Etoile, Lyon, France) using the AST-P580 card. The results of the zone diameters or the minimum inhibitory concentrations (MIC) were interpreted according to the standards issued by the Antibiotic Committee of the French Microbiology Society (CASFM) guidelines of the year corresponding to the sampling date. For methicillin-resistant *Staphylococcus aureus* (MRSA) strains, the determination of CMI of glycopeptides (Vancomycin and Teicoplanin) was conducted using the broth microdilution.

Molecular Analysis

The studied strains were screened for the presence of the genes expressed by the following virulence factors: staphylococcal protein A (*SpA*), fibronectin A binding protein (*FnbpA*), alpha hemolysin (*Hla*), and Panton–Valentine leukocidin (*PVL*). The search of the *mecA* gene, which encodes for an additional penicillin binding protein (PLP2a) with a low affinity for beta-lactams, was also performed. The DNA extraction was done using a fresh culture, by thermal lysis or by Jena Bioscience® Genomic DNA Purification Kit. The amplification was carried out by PCR simplex to demonstrate the different genes' presence. The primers used and the amplification conditions are summarized in Tables 1 and 2. The products of the PCR were revealed by electrophoresis on 1% agarose gel.

Statistical Analysis

The statistical study of our series was carried out using the IBM®SPSS®Statistics 21.0 software. *P* values below 0.05 were considered significant.

Results

Clinical and Biological Features

Patients diagnosed with *S. aureus* VAP were predominantly male (sex ratio = 3.37) with an average age of 36 years. Their age distribution is detailed in Fig. 1 and their main antecedents are summarized in Table 3. The duration of ventilation ranged from 4 to 90 days with an average of 29 days. A prolonged hospital stay has been reported in all patients with an average of 36.26 days and ranging from 9 to 145 days. The most frequently described signs were as follows: fever ($n = 34$), worsening gas exchange (SaO_2

Table 1 Primers used for each gene

Targeted gene	Primers	Product size (bp)
Pvl	F: 5′ATCATTAGGTAAAATGTCTGGACATGATCCA-3′	433
	R: 5′-GCATCAASTGTATTGGATAGCAAAAGC-3′	
FnbpA	F: 5′-CATAAATTGGGGAGCAGCATCA-3′	128
	R: 5′-ATCAGCAGCTGAATTCCCATT-3′	
Hla	F: 5′-CTGATTACTATCCAAGAAATTCGATTG-3′	209
	R: 5′-CTTTCCAGCCTACTTTTTTATCAGT-3′	
SpA	F: 5′-ATCTGGTGGCGTAACACCTG-3′	350
	R: 5′-CGCTGCACCTAACGCTAATG-3′	
mecA	F: 5′-GGGATCATAGCGTCATTATTC-3′	527
	R: 5′-AACGATTGTGACACGATAGCC-3′	

Table 2 Conditions for amplifying simplex PCRs

Steps	Simplex PCR *FnbpA*	Simplex PCR *PVL*	Simplex PCR *Hla*	Simplex PCR *spA*	Simplex PCR *mecA*
Initial denaturation	1 min at 94 °C	5 min at 94 °C	7 min at 94 °C	2 min at 94 °C	5 min at 94 °C
Number of cycles	30	30	35	35	30
Denaturation	45 s at 94 °C	30 s at 94 °C	1 min at 94 °C	30 s at 94 °C	30 s at 94 °C
Hybridization	45 s at 55 °C	30 s at 55 °C	1 min at 65 °C	30 s at 60 °C	30 s at 56 °C
Elongation	1 min at 72 °C	1 min at 72 °C	1 min at 72 °C	1 min at 72 °C	1 min at 72 °C
Final elongation	7 min at 72 °C	5 min at 72 °C	7 min at 72 °C	5 min at 72 °C	10 min at 72 °C
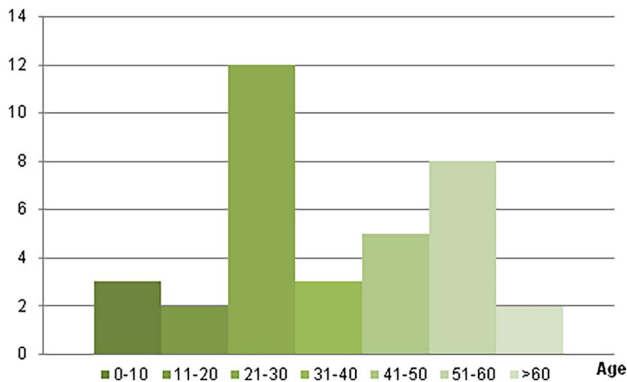
Fig. 1 Distribution of patients by age groups

Table 3 History of patients with *S. aureus* pneumonia

Patient's history	Number and percentage of patients
Mechanical ventilation	35 (100%)
Intubation period > 3 days	35 (100%)
Ventilation period > 7 days	35 (100%)
Prolonged hospitalization	34 (97.14%)
Tracheal orthosis (tracheostomy, tracheal intubation)	34 (97.14%)
Coma or head trauma	25 (71.43%)
Recent chirurgery (abdominal; thoracic)	7 (20%)
Swallowing disorder	5 (14.3%)
Tobacco	4 (11.4%)
Diabetes	4 (11.4%)
Chronic respiratory disease (COPD; cystic fibrosis)	3 (8.6%)
Neurological disease	3 (8.6%)
Chronic renal failure	1 (2.9%)
Substance addiction	1 (2.9%)
Alcohol	1 (2.9%)
Cardiovascular disease	0
Chronic hepatic failure	0
S. aureus nasal colonization	0

$\downarrow$; O$_2$ requirement $\uparrow$) ($n = 20$), and new onset of purulent tracheal secretions ($n = 20$). The median level of leukocytosis was 14.000 cells/mm^3. Those of CRP and PCT were, respectively, 191 mg/L and 3.49 ng/mL. An empirical antibiotic therapy was prescribed for 64.57% patients (24/35) from whom 66.66% (16/24) were placed on an association of antibiotics. Empirical treatment targeting MRSA was only prescribed in five cases. The average duration of antibiotic therapy was 10.5 days, with a minimum of 6 days, and a maximum of 15 days. Overall, patients had an optimal outcome in 80% of the cases.

Bacteriological Data

From 2017 to 2019 a total of 322 bacterial strains were isolated from respiratory samples taken from patients diagnosed with VAP in the surgical ICU of our hospital. Among the latter, only 35 non-redundant strains of *S. aureus* ($n = 35$) were collected. Their distribution over time was as follows: 11 isolates in 2017 ($n = 107$), 10 isolates in 2018 ($n = 100$), and 14 isolates in 2019 ($n = 115$).

Respiratory samples were mainly collected using the protected specimen brush method (91.42%) followed by sputum (5.7%) and bronchoalveolar lavage (2.9%). Monomicrobial pure culture of *S. aureus* was obtained in 77.1% ($n = 27$) of the cases. Antibiotic susceptibilities of the isolated strains of *S. aureus* are presented in Table 4. Among them 91.4% ($n = 32$) were resistant to Penicillin G, with 14.3% ($n = 5$) resistant to Oxacillin. On the other hand, 22.9% ($n = 8$) strains were resistant to Tetracycline and 2.9% ($n = 1$) resistant to Rifampicin and Fosfomycin.

Molecular Analysis

All the screened genes were present with a variable frequency as illustrated in Fig. 2: 85.7% ($n = 30$) and 80% ($n = 28$) of the isolates of *S. aureus* carried the *Hla* and the *FnbpA* genes, respectively. The *Hla* gene was more prevalent in methicillin-susceptible *S. aureus* (MSSA) than in MRSA [93.33% ($n = 28$) versus 40% ($n = 2$); $P = 0.014$] (Table 5). However, only 20% ($n = 7$) of the strains were *PVL* producers. The corresponding patients presented only with

Table 4 Study of antibiotic susceptibility for the 35 isolated strains of *S. aureus*

Antibiotics	Number (*n*) and Percentage of strains	
	Susceptible	Resistant
Penicillin G	8.6% (3)	91.4% (32)
Oxacillin	85.7% (30)	14.3% (5)
Cefoxitin	88.6% (30)	14.3% (5)
Erythromycin	100% (35)	0
Lincomycin	100% (35)	0
Pristinamycin	100% (35)	0
Ofloxacin	94.3% (33)	5.7% (2)
Tetracycline	77.1% (27)	22.9% (8)
Vancomycin	100% (35)	0
Teicoplanin	100% (35)	0
Trimethoprim–Sulfameth-oxazole	100% (35)	0
Fosfomycin	97.1% (34)	2.9% (1)
Chloramphenicol	100% (35)	0
Fusidic acid	100% (35)	0
Rifampicin	97.1% (34)	2.9% (1)
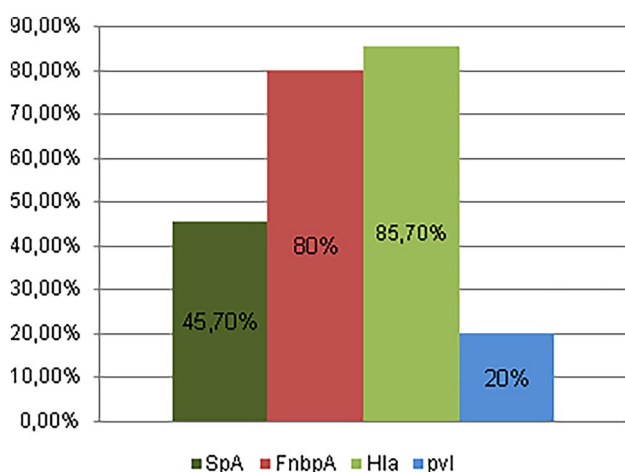
Fig. 2 Prevalence of virulence genes in *Staphylococcus aureus* strains

Table 5 Prevalence of virulence genes based on Methicillin resistance

Gene	Distribution of isolates		*P*
	MRSA (*n* = 5)	MSSA (*n* = 30)	
SpA (*n* = 16)	3 (60%)	13 (43.3%)	0.489
FnbpA (*n* = 28)	4 (80%)	24 (80%)	0.744
Hla (*n* = 30)	2 (40%)	28 (93.33%)	0.014
pvl (*n* = 7)	2 (40%)	5 (16.7%)	0.256

MRSA Methicillin-resistant *Staphylococcus aureus*, *MSSA* Methicillin-susceptible *Staphylococcus aureus*

fever with normal leukocyte count, six out of these seven cases had a good outcome. In addition, the genotypic study revealed that among the five strains resistant to Oxacillin according to the phenotypic susceptibility testing methods; only two isolates of *S. aureus* carried the *mecA* gene.

Discussion

Risk factors for hospital-acquired pneumonia (HAP) may be intrinsic due to the differences in host factors or extrinsic, such as diagnostic procedures or medical interventions undergone in the ICU. Inspecting the literature, we noted that an elderly age seems to be a predisposing factor for HAP caused by *S. aureus.* However, gender is not considered to be a risk factor [3]. In our study, *S. aureus*-related VAP affected a young population (average age = 36 years) with only two patients over 60 years. A male predominance with a gender ratio of 3.37 was also noted. Our result is comparable to those reported by an American, Korean, and a Canadian multicenter study with male percentages of 61%, 65.3%, and 67.1%, respectively [4–6]. This could be explained by the fact that within intensive care units, there is usually an uneven distribution of patients in favor of the male sex [7–9].

Other risk factors predisposing patients to the development of HAP can be related to the severity of their underlying diseases; for instance, coma and head trauma injuries play an important role in the selection and colonization by microorganisms, such as *S. aureus* [2]. In our study, 71.43% of patients had an antecedent of coma or a severe head trauma. A similar result was reported by Pujo*t et al.,* while Rello *et al.* had shown that coma is the only reported risk factor for *S. aureus*-related VAP in a multivariable study [10, 11]. In neurosurgical patients, the high frequency of colonization with *S. aureus* could be linked to the prolonged use of paralytic sedative, and the treatment by hyperventilation and corticosteroids, and these factors may also contribute to the alteration of the airway defenses of these patients [12, 13].

The incidence of MRSA in HAP varies by department, hospital, and country. High rates were reported in Germany (37%), the United States (54%), Asia, and Latin America (78%) [14].

As reported in previous studies, a prolonged hospital stay, the presence of intubation, and mechanical ventilation are also considered as risk factors for the development of MRSA HAP. [10, 11, 15]. 97.14% of our patients had a long hospital stay with an average of 36.26 days. All patients were intubated and mechanically ventilated with an average duration of 29 days, and extremes ranging from 4 to 90 days. Thus, surprisingly, in our study, the incidence of methicillin resistant strains is low. These results are similar to those reported by a Moroccan study [16].

The diagnosis of HAP is based on clinical, radiological, and biological criteria. Given the lack of specificity of the clinical signs, an etiologic diagnosis by quantitative microbiological culture on respiratory samples is essential. Protected specimen brush (PSB) is the most used sample (91.42%) in the surgical ICU of our hospital. This could be motivated by the performance of blind PSB which is close to fibroscopic techniques unlike bronchoalveolar lavage that requires fibroscopy and therefore rigorous procedures. Moreover, the specificity and sensitivity of PSB are close to 80% [17].

During the study period, five of our strains were identified as MRSA based on the phenotypic study. We searched for the *mecA* gene presence in these isolates by PCR, which concluded that only two strains of the five MRSA carried the gene. Actually, in 2011, multi-susceptible MRSA strains were first described in humans and animals in the United Kingdom and Denmark [18]. These strains carried a variant of the *mecA* gene (less than 70% homology) called *mecC* encoding a PLP2c and having, like PLP2a, a low affinity for beta-lactam. Consequently, the 3 MRSA *mecA*-negative strains could possibly carry this gene. Due to variable increases in MIC with antibiotics of the beta-lactam family, the *mecC* gene resistance proves to be phenotypically difficult to detect particularly by automated testing [19]. This leads us to conclude that a routine detection of the *mecC* gene presence by PCR would be of a considerable contribution in our laboratory.

On the other hand, *S. aureus* is known for the severity of its infections mostly related to the increased production of its virulence factors. Actually, these factors help initiate colonization and interfere with the immune system allowing the bacterium to adhere to tissues, damage them, and disseminate to other organs [20].

Staphylococcus aureus is commonly present in the otolaryngeal and tracheobronchial flora. Consequently, VAP pathogenesis is usually a result of the rupture of the balance between the immune system defenses and the production of virulence factors [21, 22]. In our study, the corresponding virulence genes *SpA, FnbpA, Hla,* and *PVL* were present with the rates of 45.7%, 80%, 85.7%, and 20%, respectively. Clinically, PVL, a very potent toxin, has been associated with necrotizing community-acquired pneumonia encountered mainly in children and young adults [23]. In our case, among 7 patients infected with PVL (+) *S. aureus*, only one had a poor outcome (14.3%). A French case–control study of 133 children and adults suffering from HAP due to PVL (+) *S. aureus* did not show any worsening clinical symptoms [24]. Therefore, other cytolytic toxins may also interfere in the pathogenesis of *S. aureus* HAP. Indeed, attention has recently been focused on other cytolysins, in particular the staphylococcal Hla secreted by 80–90% of *S. aureus* strains. This toxin plays an active role in the pathogenesis

of pneumonia by activating the inflammasome "NOD-like receptor family, pyrin domain containing 3" (NLRP3), leading to severe alveolar necrosis, induction of platelets and neutrophils aggregation, and tissue disruption [25–27]. The prevalence of the *Hla* factor in our study was 85.7% and was higher in the MSSA strains than in the MRSA (93.3% versus 40%; $P = 0.014$). In this context, Tabor *et al.* reported a *Hla* prevalence of 86.9% in MSSA and 78.8% in SARM with a significant difference ($P = 0.0007$). Moreover, they showed that independently from geography, patient age, or length of hospital stay, more MSSA isolates than MRSA isolates expressed *Hla*, as well as higher levels of *Hla*. This suggests that the genetic background of MSSA allows higher *Hla* expression [28]. The gene encoding the fibronectin A binding protein was present in 80% of the *S. aureus* isolates, with a non-significant difference between MRSA and MSSA ($P = 0.744$). Our results are comparable to those published by the teams of Sharma-Kuinkel *et al.* and that of Doudoulakakis *et al.* [29, 30]. In another study conducted by Ghodousi *et al.* 100% of the *S. aureus* isolates expressed the gene encoding for the FnbpA [31]. A French study suggested that FnbpA played a major role in the colonization of the respiratory tract and insisted on the importance of this protein's regulatory mechanisms in the expression of the staphylococcal pathogenicity [32].

The *SpA* gene, encoding for one of the most important adhesins produced by *S. aureus*, was prevalent in our study with a percentage of 45.7%: 43.3% (13/30) in MSSA and 60% (3/5) in MRSA ($P = 0.489$). Libert N *et al.* showed that mice infected with a *PVL* (+) and *SpA* (−) strains had localized lesions and massive leukocyte infiltration and that infection with a *PVL* (+) and *Spa* (+) strains resulted in more severe lesions and death. *SpA* has also been reported to have a pro-inflammatory effect on pneumonia via the tumor necrosis factor. Immune cells lysis by *PVL* associated with an increased *SpA* production would therefore have a synergistic effect on the lung tissue inflammation [33, 34].

The choice of an initial empiric antimicrobial treatment targeting MRSA combined with a broad-spectrum agent is crucial. It is usually made based on the presence of certain risk factors, local epidemiology, and pneumonia severity. Nevertheless, this empirical treatment is not without risk; other than its iatrogenic risk, it is less effective on the MSSA strains and it leads to the selection of resistant mutants of GISA "glycopeptides-intermediate *S. aureus*" strains [35]. Once the susceptibility is documented, the antibiotic treatment must be narrowed and optimized. Regarding the duration of therapy, the French Society of Anesthesia & Intensive Care Medicine and the American Thoracic Society specify that the duration of antibiotic therapy for HAP should not exceed 7 days outside severe clinical cases [5, 36].

The continued progress in understanding pathogenesis mechanisms of *S. aureus* infections has enabled the

development of specific anti-virulence factor (AVA) agents that can reduce the pathogenicity of this bacterium in many clinical situations especially in HAP. These advances are interesting not only on a microbiological but also on a therapeutic level because other than the specific antimicrobial effects of AVA, they can also potentiate antibiotics efficacy prescribed in the ICU [37].

Our work has some limitations. Besides of the small sample size, it needs further elucidation using more technologies, such as DNA sequencing and appropriate in vitro methods and in vivo models, to assess the virulence factors expression during infection. In fact, the determinants of infection severity are far more complex than the presence or absence of a few virulence characteristics. We therefore could not correlate clinical outcomes with the expression levels of the screened virulence genes. Despite these limitations, to the best of our knowledge, this is the first study conducted in Tunisia that established the clinico-epidemiological profile of *S. aureus*-related VAP in the ICU, studied the bacteriological characteristics, and elucidated the virulence factors of the isolated strains. It provided also valuable insights into the presence of *Hla* and *FnbpA* genes in both MSSA and MRSA populations. The production of the genes encoding for *Hla* and *FnbpA,* respectively, seems to be independent of the production of the antibiotic resistance genes.

Conclusion

VAP remains among the most encountered and most feared infections in the ICU. *S. aureus*-related VAP in our hospital structure affected a young population, predominantly male with particular co-morbidities. Our collection of isolates is essentially associated with the production of *Hla* and *FnbpA* genes encoding for their respective virulence factors. It would be very interesting to supplement this work by other studies for a better understanding of the pathogenesis of *S. aureus* VAP. The prevalence of MRSA strains remains low in our study, even though the average length of hospitalization was 36.26 days. It seems crucial to implement adequate preventive measurements in the daily clinical practice in order to prevent these infections, improve their prognosis, and to avoid the spread of MRSA.

Declarations

References

1. Leone M, Bouadma L, Bouhemad B, Brissaud O, Dauger S, Gibot S et al (2018) Pneumonies associées aux soins de réanimation. Anesth Réanimation 4:421–441. https://doi.org/10.1016/j.anrea.2018.07.003

2. Benhamou D, Carrié AS, Lecomte F (2005) *Staphylococcus* : place et impact dans la prise en charge des pneumopathies nosocomiales. Rev Mal Respir 22:595–603. https://doi.org/10.1016/S0761-8425(05)85612-8

3. Zhu J, Zhang X, Shi G, Yi K, Tan X (2015) Atrial fibrillation is an independent risk factor for hospital-acquired pneumonia. PLoS ONE 10:e0131782. https://doi.org/10.1371/journal.pone.0131782

4. Pasquale TR, Jabrocki B, Salstrom S-J, Wiemken TL, Peyrani P, Haque NZ et al (2013) Emergence of methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of late-onset nosocomial pneumonia in intensive care patients in the USA. Int J Infect Dis 17:e398–e403. https://doi.org/10.1016/j.ijid.2012.12.013

5. Jung WJ, Kang YA, Park MS, Park SC, Leem AY, Kim EY, Chung KS, Kim YS et al (2013) Prediction of methicillin-resistant *Staphylococcus aureus* in patients with non-nosocomial pneumonia. BMC Infect Dis 13:370. https://doi.org/10.1186/1471-2334-13-370

6. Tadros M, Williams V, Coleman BL, McGeer AJ, Haider S, Lee C et al (2013) Epidemiology and outcome of pneumonia caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in Canadian hospitals. PLoS ONE 8:e75171. https://doi.org/10.1371/journal.pone.0075171

7. Wałaszek M, Kosiarska A, Gniadek A, Kołpa M, Wolak Z, Dobroś W et al (2016) The risk factors for hospital-acquired pneumonia in the intensive care unit. Przegl Epidemiol 70:15–20

8. Pisanu G, Fartoukh M, Garnier M (2018) Pneumonie associée à la ventilation mécanique. Prat En Anesth Réanimation 22:10–16. https://doi.org/10.1016/j.pratan.2018.01.005

9. Suka M, Yoshida K, Uno H, Takezawa J (2007) Incidence and outcomes of ventilator-associated pneumonia in Japanese intensive care units: the Japanese nosocomial infection surveillance system. Infect control hosp epidemiol 28:307–313. https://doi.org/10.1086/511997

10. Pujol M, Corbella X, Peña C, Pallares R, Dorca J, Verdaguer R et al (1998) Clinical and epidemiological findings in mechanically-ventilated patients with methicillin-resistant *Staphylococcus aureus* pneumonia. Eur J Clin Microbiol Infect Dis 17:622–628. https://doi.org/10.1007/BF01708344

11. Rello J, Ollendorf DA, Oster G, Vera-Llonch M, Bellm L, Redman R et al (2002) Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. Chest 122:2115–2121. https://doi.org/10.1378/chest.122.6.2115

12. Fortaleza CM, Abati PA, Batista MR, Dias A (2009) Risk factors for hospital-acquired pneumonia in nonventilated adults. Braz J Infect Dis 13:284–288. https://doi.org/10.1590/s1413-86702009000400009

13. Espersen F, Gabrielsen J (1981) Pneumonia due to *Staphylococcus aureus* during mechanical ventilation. J Infect Dis 144:19–23. https://doi.org/10.1093/infdis/144.1.19

14. Meyer E, Schwab F, Gastmeier P (2010) Nosocomial methicillin resistant *Staphylococcus aureus* pneumonia—epidemiology

and trends based on data of a network of 586 German ICUs (2005–2009). Eur J Med Res 15:514–524. https://doi.org/10.1186/2047-783x-15-12-514

15. Lentino JR, Hennein H, Krause S, Pappas S, Fuller G, Schaaff D et al (1985) A comparison of pneumonia caused by gentamicin, methicillin-resistant and gentamicin, methicillin-sensitive *Staphylococcus aureus*: epidemiologic and clinical studies. Infect Control 6:267–272. https://doi.org/10.1017/s0195941700061737

16. Shimi A, Touzani S, Elbakouri N, Bechri B, Derkaoui A, Khatouf M (2015) Les pneumopathies nosocomiales en réanimation de CHU Hassan II de Fès. Pan Afr Med J 22:285. https://doi.org/10.11604/pamj.2015.22.285.7630

17. Pham LH, Brun-Buisson C, Legrand P, Rauss A, Verra F, Brochard L et al (1991) Diagnosis of nosocomial pneumonia in mechanically ventilated patients: comparison of a plugged telescoping catheter with the protected specimen brush. Am Rev Respir Dis 143:1055–1061. https://doi.org/10.1164/ajrccm/143.5_Pt_1.1055

18. García-Álvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, Curran MD et al (2011) Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11:595–603. https://doi.org/10.1016/S1473-3099(11)70126-8

19. Marisa H, Jouy E, Madec JY, Laurent F (2012) *Staphylococcus aureus* résistant à la méticilline (SARM): un partage entre l'Homme et l'animal ? Bull Epidémiol SantéAnim Aliment 53:40–43

20. Dayan GH, Mohamed N, Scully IL, Cooper D, Begier E, Eiden J et al (2016) *Staphylococcus aureus*: the current state of disease, pathophysiology and strategies for prevention. Expert Rev Vaccines 15:1373–1392. https://doi.org/10.1080/14760584.2016.1179583

21. Parker D, Prince A (2012) Immunopathogenesis of *Staphylococcus aureus* pulmonary infection. Semin Immunopathol 34:281–297. https://doi.org/10.1007/s00281-011-0291-7

22. Ferry T, Perpoint T, Vandenesch F, Etienne J (2005) Virulence determinants in *Staphylococcus aureus* and their involvement in clinical syndromes. Curr Infect Dis Rep 7:420–428. https://doi.org/10.1007/s11908-005-0043-8

23. Rájová J, Pantůček R, Petráš P, Varbanovová I, Mašlaňová I, Beneš J (2016) Necrotizing pneumonia due to clonally diverse *Staphylococcus aureus* strains producing panton-valentine leukocidin: the Czech experience. Epidemiol Infect 144:507–515. https://doi.org/10.1017/S0950268815001521

24. Sicot N, Khanafer N, Meyssonnier V, Dumitrescu O, Tristan A, Bes M et al (2013) Methicillin resistance is not a predictor of severity in community-acquired *Staphylococcus aureus* necrotizing pneumonia–results of a prospective observational study. Clin Microbiol Infect 19:E142–E148. https://doi.org/10.1111/1469-0691.12022

25. Prevost G, Mourey L, Colin D, Monteil H, Dalla Serra M, Menestrina G (2006) Alpha-helix and beta-barrel pore-forming toxins (leucocidins, alpha-, gamma-, and delta-cytolysins) of *Staphylococcus aureus*. In: Alouf JE, Freer JH (eds) The comprehensive sourcebook of bacterial protein toxins, 3rd edn. Elsevier, Amsterdam, pp 590–607

26. Menestrina G, Dalla Serra M, Comai M, Coraiola M, Viero G, Werner S et al (2003) Ion channels and bacterial infection: the case of beta-barrel pore-forming protein toxins of *Staphylococcus aureus*. FEBS Lett 552:54–60. https://doi.org/10.1016/s0014-5793(03)00850-0

27. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28:603–661. https://doi.org/10.1128/CMR.00134-14

28. Tabor DE, Yu L, Mok H, Tkaczyk C, Sellman BR, Wu Y et al (2016) *Staphylococcus aureus* alpha-toxin is conserved among diverse hospital respiratory isolates collected from a global surveillance study and is neutralized by monoclonal antibody MEDI4893. Antimicrob Agents Chemother 60:5312–5321. https://doi.org/10.1128/AAC.00357-16

29. Sharma-Kuinkel BK, Ahn SH, Rude TH, Zhang Y, Tong SY, Ruffin F et al (2012) Presence of genes encoding panton-valentine leukocidin is not the primary determinant of outcome in patients with hospital-acquired pneumonia due to *Staphylococcus aureus*. J Clin Microbiol 50:848–856. https://doi.org/10.1128/JCM.06219-11

30. Doudoulakakis AG, Bouras D, Drougka E, Kazantzi M, Michos A, Charisiadou A et al (2016) Community-associated *Staphylococcus aureus* pneumonia among Greek children: epidemiology, molecular characteristics, treatment, and outcome. Eur J Clin Microbiol Infect Dis 35:1177–1185. https://doi.org/10.1007/s10096-016-2651-7

31. Feizabadi M, Ghodousi A, Nomanpour B, Davoudi S, Maleknejad P, Omrani M et al (2012) Application of fnbA gene as new target for the species-specific and quantitative detection of *Staphylococcus aureus* directly from lower respiratory tract specimens by real time PCR. Indian J Pathol Microbiol 55:490–495. https://doi.org/10.4103/0377-4929.107787

32. Mongodin E, Bajolet O, Cutrona J, Bonnet N, Dupuit F, Puchelle E et al (2002) Fibronectin-binding proteins of *Staphylococcus aureus* are involved in adherence to human airway epithelium. Infect Immun 70:620–630

33. Mortaza S, Zahar J-R, Kouatchet A (2010) Pneumonie à *Staphylococcus aureus* : quand faut-il l'évoquer et comment la traiter? Réanimation 19:304–307. https://doi.org/10.1016/j.reaurg.2010.03.017

34. Libert N, Batjom E, Cirodde A, de Rudnicki S, Grasser L, Borne M et al (2009) Traitements antitoxiniques et pneumopathies nécrosantes à *Staphylococcus aureus* sécréteurs de leucocidine de panton-valentine. Médecine Mal Infect 39:14–20. https://doi.org/10.1016/j.medmal.2008.10.008

35. Wongthong S, Tippayawat P, Wongwattanakul M, Poung-ngern P, Wonglakorn L, Chanawong A et al (2020) Attenuated total reflection: fourier transform infrared spectroscopy for detection of heterogeneous vancomycin-intermediate *Staphylococcus aureus*. World J Microbiol Biotechnol 36:22. https://doi.org/10.1007/s11274-019-2788-5

36. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB et al (2016) Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis 63:e61–e111. https://doi.org/10.1093/cid/ciw353

37. François B, Luyt CE, Stover CK, Brubaker JO, Chastre J, Jafri HS (2017) New strategies targeting virulence factors of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Semin Respir Crit Care Med 38:346–358. https://doi.org/10.1055/s-0037-1602715