

## New insights into pathogenicity and antimicrobial resistance in ventilator associated pneumonia

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Host susceptibility can trigger pneumonia and etiological factors can aggravate clinical outcomes. Mechanical ventilation (MV) can expose patients to pathogenic microorganisms in the lungs frequently including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The development of ventilator-associated pneumonia (VAP) remains associated mainly with host factors and the presence of different pathogenic factors that can limit treatment as well. One of the most concerning facts is treatment failure due to difficulties in detecting and managing the resistant strain. Resistant strains are frequently involved in VAP and outbreaks in intensive care units (ICU) result in increasing treatment costs and loss of lives. The mechanisms of antimicrobial resistance vary according to the pathogen. *Staphylococcus aureus* can harbor the oxacillin resistant *mecA* gene in the Staphylococcal cassette chromosome *mec* that can possess other resistance determinants. *A. baumannii* presents several types of “OXA” carbapenemases mediating the hydrolysis of the beta-lactam ring, belonging to three molecular classes classified by Ambler. *P. aeruginosa* displays intrinsic resistance to wide antimicrobial classes due to low permeability of its outer membrane, increased efflux and enzymatic antibiotic changes. In view of the above considerations, the aim of this chapter is to present the pathogenicity mechanisms of the cited agents as well as review the background of the main antimicrobial resistance mechanisms involved in VAP.

**Keywords** VAP; resistance; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; *Staphylococcus aureus*.

### 1. General Remarks

Frequently the airway passage in humans is challenged by several types of microorganisms posing a risk to the immune system in eliminating pathogens and preventing infection. However some factors contribute to adherence, establishment and growth of infection including immune system clearance as well as microorganism pathogenic factors. Therefore, determining pneumonia origin usually contributes to a better prognosis and the best treatment since the etiology can differ according to the infection source [1].

Hospital acquired pneumonia (HAP) is characterized by the development of signs and symptoms in a period from 48 to 72 hours from hospital admission up until or even after hospital discharge [2]. The infection is acquired within the hospital, and the infection source is not considered from normal flora. Ventilator associated pneumonia (VAP) is a type of HAP occurring 48 hours after introducing ventilation [3, 4]. There are risk factors that may contribute to VAP development, with advanced age being one of the most important factors, followed by previous antimicrobial usage, antimicrobial resistance in ICU, length of stay in hospitals and ICU, severity of illness, and acute respiratory distress syndrome [4 – 7].

Ventilator-associated pneumonia (VAP) is one of the most frequent types of infection [2, 8]. In surveillance performed in medical ICUs in the US, pneumonia was the second most frequent infection (27%) [9]. In a large US database of 9,080 patients admitted to an ICU and receiving mechanical ventilation for >24h, 9.3% developed VAP [10]. VAP rates are reported ranging from 4.3/1,000 ventilator days in adult ICUs [11] to 5/1,000 ventilator days in pediatric patients and 35/1,000 days in burn patients [12]. The incidence of VAP varies among different studies, depending on the definition, type of ICU, population studied and level of antibiotic exposure [13]. Bekaert et al. (2011) [14] estimated that 4.4% of the deaths in the ICU on day 30 and 5.9% on day 60 are attributable to VAP. Survival of patients is determined mainly by severity of illness at the time of diagnosis [15]. Mortality can vary from 30.5% to 50% and can reach 76% in some specific settings or when lung infection is caused by high-risk pathogens [10, 16]. Melsen et al. (2011) [8] estimated that the attributable mortality rate of VAP is 9%, ranging between 3% and 17% in subgroup analyses. Additionally, it has been demonstrated that patients with VAP had longer mean durations of mechanical ventilation (21.8 vs 10.3 days), ICU stay (20.5 vs. 11.6 days), hospitalization (32.6 vs. 19.5 days), and costs (\$99,598 vs. \$59,770) than patients without VAP [17].

### 2. Overview of VAP Etiology

Several studies have attempted to elucidate the origin and possible transmission routes for pneumonia pathogens. In this context, the pathogenesis of VAP is associated with the colonization of the upper respiratory tract (oropharynx and

trachea) with potentially pathogenic microorganisms. Among these microorganisms, colonization with Enterobacteriaceae, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are associated with VAP development [5, 18].

Medical devices can facilitate the acquisition of Enterobacteriaceae family members reaching the lungs through aspiration of enteral feeding. It is estimated that about 45% of individuals can aspirate the content of the tubes, frequently associated with abdominal distention and use of artificial feeding. The post-operative period increases the risk of pneumonia development mainly in individuals who are at risk, such as the elderly, obese, malnourished, smokers, chronic obstructive pulmonary disease (COPD) or tracheostomy, and other additional factors. The use of sedatives impairs ciliary activity in the respiratory tract, which enhances the secretion accumulation that normally would eliminate the colonizing pathogens [19].

Enteric gram-negative bacilli are common agents involved in HAP followed by gram-positive cocci and polymicrobial infections [5]. In a study, MRSA was implicated in 61% of nosocomial pneumonia according to Shorr et al. (2010) [20]. Pobo et al. (2009) [21] in a prospective study verified that 63.6% of all VAP episodes in which the etiology was determined, *S. aureus*, *Haemophilus influenza* and *Streptococcus pneumonia* were the most frequent isolated microorganism. Table 1 summarizes the main agents and isolation frequency among nosocomial pneumonia.

**Table 1** Nosocomial pneumonia etiology and frequency of pathogen isolation.

Nosocomial pneumonia etiology		%
<b>Gram negative</b>	<i>Pseudomonas aeruginosa</i>	55 to 85
	<i>Enterobacter</i>	
	<i>Acinetobacter</i>	
	Enteric gram negative bacilli ( <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Serratia marcescens</i> , <i>Proteus</i> spp.)	
<b>Gram-positive cocci</b>	<i>Staphylococcus aureus</i>	20 to 61
	<i>Streptococcus pneumoniae</i>	
<b>Polymicrobial</b>	Examples: <i>S. aureus</i> , <i>Haemophilus influenzae</i>	40 to 60
	<i>S. aureus</i> , <i>Serratia marcescens</i> ,	
	<i>P. aeruginosa</i>	

Adapted from [5, 20, 22] Rello et al. (1991), Lynch III (2001), Shorr et al. (2010).

Antimicrobial resistance occurs in microorganisms isolated in VAP. Patients over 60 years old are susceptible to nosocomial pneumonia caused by multiresistant pathogens (defined by the resistance to 2 or more different antimicrobial classes). Patients with HACAP admitted to ICUs are susceptible to acquire resistant pathogens and are prone to have treatment failure in the initial treatment leading to a poor prognosis [7].

## 2.1. *Pseudomonas* spp

### 2.1.1. Epidemiology

*Pseudomonas aeruginosa* is a nonfermentative gram-negative bacilli widely distributed in water, soil, animals and plant surfaces. Its ability to use nitrogen as a final receptor of electrons makes it possible to survive in anaerobic conditions since nitrates or arginine is available. Its use of over 50 nutrients and ability to grow at high temperatures (43° C) can also explain the survival of *Pseudomonas* strains in different environments, including soil and hospital sinks [23]. Environmental elimination remains challenging since *P. aeruginosa* possess a wide variety of intrinsic resistance mechanisms [24, 25].

As an opportunistic pathogen it is considered one of the main causes of hospital-acquired infection in immunocompromised patients [25]. *P. aeruginosa* is associated with chronic infections in cystic fibrosis patients [26], HCP, HCAP and late onset VAP [10]. VAP caused by *P. aeruginosa* is related to high morbidity and mortality in ICUs. During an outbreak *P. aeruginosa* VAP is usually associated with length of ICU stay and increased treatment costs [27]. Even with proper treatment the mortality rate attributed to the presence of *P. aeruginosa* remains among the most significant [28]. Its presence in the respiratory tract can be fatal since virulence mechanisms such as the presence of exotoxin A and type III secretion system are related to poor prognosis [29].

### 2.1.2. Virulence factors

Lung cells produce two types of surfactant proteins, A and D (SP-A and -D), which play an important role in host defense [30]. SP-A and -D binds to the bacteria acting as an opsonin facilitating bacterial removal by the alveolar macrophages [30, 31]. SP-D is essential for binding to the gram-negative pathogens such as *P. aeruginosa*, *Klebsiella*

*pneumoniae* and *Haemophilus influenzae*. SP-D binds mainly to the core oligosaccharide of the lipopolysaccharide (LPS) of the rough form but binding also to some smooth, unencapsulated strains [31].

Several virulence mechanisms have been described for *P. aeruginosa* against airway epithelia. There are three main virulence determinants: quorum sensing, type three secretion system (TTSS) and lipopolysaccharide. In quorum sensing the bacteria sense the bacterial cell density to coordinate the production of virulence factors (such as elastase and pyocyanin) by the two main signaling systems, *las* and *rhl* [32]. The TTSS has four effector proteins (or exotoxins) - Exo S, Exo T, Exo U and Exo Y. The production of elastase, pyocyanin and TTSS exotoxins in strains have been linked to lung injuries. Elastase was associated with acute lung injury and Exo U was associated with increased virulence and with a risk of bacteremia [32].

TTSS is an intriguing virulence determinant and it has been studied in a light of pulmonary infection. This is due to the ability of this system to deliver effectors into the host cells directly on the cytosol through a protein apparatus. Exo effectors have different a constitution and effects in the host cells, where the most important are the ExoU and S which have been associated with lung injury during VAP [26, 32]. Exo T and Exo S work together disrupting the cytoskeleton, impairing phagocytosis and inducing cell death. While Exo Y has not been implicated as having a toxic effect, Exo U is associated with the induction of transcription of inflammatory genes [32], rapid necrosis and damage to the cell cytoskeleton characterized as hypervirulent [26, 32, 33]. Therefore, TTSS plays an important role in *Pseudomonas* sp. virulence and directly influences patient prognosis [34].

Elastase (*lasB* gene) hydrolyses the components of extracellular matrix and intracellular tight junctions. Additionally, *lasB* has an *in vitro* degrading activity on SP-A and -D, inactivates immunoglobulin A and G, opsonin C3, cytokines, chemokines, and antibacterial peptides. A study conducted with strains that lacked *lasB* and the wild type revealed the susceptibility of  $\Delta lasB$  to SP-A-mediated opsonization thus facilitating phagocytosis [35]. The presence of elastase in guinea pig lungs revealed increased permeability acting directly on the tight junction complex of epithelium [33, 36].

Exotoxin A is a potent toxin that contributes to the severity of *P. aeruginosa* lung infection. Exotoxin A is a potent inhibitor of protein synthesis after translocation on the cytosol of vulnerable cells [37]. Quorum-sensing regulates its expression, which directly requires iron uptake caught by pyoverdine and pyochelin. The main consequence of its presence on the lungs is the apoptotic and non-apoptotic cell death through some pathways [33].

Pyocyanin inhibits the cell respiration and impairs the ciliary function in human cells, being a crucial factor for lung injury during infection [32]. Pyocyanin also causes neutrophil killing, catalase inactivation in human alveolar and bronchial epithelial cells as well as in cell free systems, and leads to transcription decreasing of the gene encoding catalase [33]. These functions lead to bacterial persistence and contribute to infection success [38]. Attenuated strains in *in vivo* infections helped explain the important role of these compounds in the host immune system [33, 38].

Mucoid *P. aeruginosa* strains synthesize the alginate, an exopolysaccharide, protecting the strain against environmental changes. Alginate as a physical barrier plays an important role in immunological response impeding the clearance, the complement activation, neutrophil chemotaxis, phagocytosis and action of free radicals released by macrophages [26]. Alginate can provide adherence to tracheal epithelial cells in a complex manner involving cilia-alginate-cilia bridges according to an experiment conducted previously [39].

Biofilms are important virulence factors that may contribute to environmental persistence and dissemination as well as to antimicrobial resistance. Planktonic cells attach to epithelial cells or medical devices and in order to establish the biofilm, exopolysaccharides (i.e. alginate), adhesins and cognate receptors are synthesized producing a complex matrix. Quorum sensing triggers the biofilm formation since they sense the bacterial load and construct a well-organized community with intimate relationships of competition or cooperation [40].

Lipid A lipopolysaccharide present in the *P. aeruginosa* cell interacts directly with toll-like receptor 4 in epithelial cells but apparently has no direct effect in lung infection during pneumonia. Pili and flagella are involved in respiratory cells attachment, trigger interleukin release and cell signaling [33].

As described previously, *P. aeruginosa* has an arsenal of virulence factors studied and well described as mandatory to poor prognosis in pneumonia. The understanding of these factors and the way they affect host cells provide an important tool that are now considered a target in antibiotic treatment.

### 2.1.3. Resistance factors

Intrinsically resistant to several antibiotic classes, *P. aeruginosa* is a potent pathogen, being difficult to treat. The main resistance factors remain in efflux pumps and diminished permeability of the outer membrane, enzymatic drug changes and target changes.

The antibiotic entrance is important to achieve the target and the cell wall remains as a barrier to this uptake. The outer membrane is capable of selecting the uptake of antibiotic molecules mainly due to the size of the porin channels that mediate the antibiotic entrance. The small hydrophilic molecules of antimicrobials, beta-lactam and quinolones, cross the outer membrane in water channels through porin protein usually associated in trimers. The most common porin is the oprF, but a lack of this porin is not associated with hydrophilic antimicrobial resistance. The oprD is responsible for amino acid catchment and its absence causes imipenem, but not meropenem, resistance. LPS plays an important role in antimicrobial entrance, since the aminoglycosides and colistin do not cross the porins, they usually

link directly in the LPS. This linkage destroys the outer membrane stability, affecting the permeability allowing the antimicrobial to reach the cytoplasm. Aminoglycosides interfere with the protein synthesis at the ribosome level while colistin is bactericidal, affecting the cytoplasmic membrane [41].

Eflux pumps play an important role in *P. aeruginosa* resistance. It is an adaptive resistance with at least 4 different mechanisms: *mexAB-oprM*, *mexXY-oprM*, *mexCD-oprJ* and *mexEF-oprN* [42]. Due to the efflux pumps *Pseudomonas* have become increasingly resistant to a broad range of antimicrobial classes. MexAB-oprM confers resistance to disinfectants, beta-lactams, fluorquinolones and meropenem [43]. MexXY-oprM up-regulation affects the extrusion of aminoglycosides, while the *mexCD-oprJ* and *mexEF-oprN* are responsible for expelling fluorquinolones and some beta-lactams [41, 43], where the *mexEF-oprN* has an additional effect on carbapenem extrusion. The genes are not highly expressed in all strains only in samples with some mutation on the regulator gene [41].

Enzymatic change of antimicrobial agents is carried out by extent spectrum metallo-beta-lactamases (ESBLs) and metallo-beta-lactamases (MBLs), which hydrolyze penicillins, the first three generations of cephalosporins and aztreonam. Their activity can be inhibited by beta-lactamases inhibitors, such as clavulanic acid [44]. ESBL are classified according to protein homology, the Ambler scheme, and according to their functional similarities, the Bush-Jacob-Mederos scheme. Several types of MBL have been described in *P. aeruginosa*, such as PER-1 OXA, OXA-ESBL, IMP and VIM, all have specific types widely distributed around the world. Cassettes are frequently found in strains susceptible only to polymyxin and usually carry a combination of IMP, VIM and OXA-ESBL types [43]. Enzymatic antimicrobial change is a relevant matter of discussion that we could address in a chapter just for ESBL and MBL.

Changes in the targets protect *P. aeruginosa* against the interference of the basal functions since the antimicrobial is no longer able to reach the site. Topoisomerase II and IV are responsible for the catenation/decatenation of, introduction/ removal of supercoils in the DNA and play other essential roles in the maintenance of DNA integrity [45]. The most common are the mutations in *gyrA* and *parC*, which result in reduced affinity of topoisomerase II and IV, respectively. These changes confer resistance to quinolones, and the *parC* mutation can confer additional resistance to carbapenems [46].

#### 2.1.4. Treatment

New antibiotics and new approaches are being studied in order to improve treatment of *P. aeruginosa* VAP. The advent of pan-resistant *P. aeruginosa* emergence in ICUs and the challenge that this pathogen represents to immunocompromised patients brings to the medical and scientific community several concerns. A combination of two or three antimicrobials showed no differences against monotherapy efficacy to treat multi drug resistant *P. aeruginosa* (MDR-PA) and other associated pathogens. However the patients that underwent combined therapy presented other benefits suggesting this modality is favorable depending on patient conditions [47].

A combination of treatments could minimize resistance emergence, and synergistic activity is more prone to lead to treatment success [48]. In order to minimize the emergence of antimicrobial resistance and improve the clinical treatment, nebulized antibiotics (amikacin and ceftazidime) to PA VAP treatment have shown to be clinical cures in treating PA with decreased susceptibility to one or both antimicrobial tested compared to intravenous treatment. Despite the efficacy of nebulization against intermediate resistant PA, there were no differences in outcomes between intravenous and nebulization treatment [49].

In order to make the right choice to treat VAP, important points must be considered. National surveillance may be accessed to provide a background of the most common resistance patterns of the geographically-distributed isolates. Surveillance cultures and susceptibility profile of the isolates may predict the possible causative agent of VAP assisting the right antimicrobial choice success. The knowledge of the patient risk factors may provide an assumption of MDR *P. aeruginosa* infection. Results of a local antibiogram may help track the resistance pattern tendency in hospitals [48]. These considerations are important and useful in making the best antimicrobial choice to ensure the treatment success. Several antibiotics have re-emerged as good options to treat *P. aeruginosa* pneumonia. Polymyxins are now being used in cases of MDR-PA with satisfactory results. There are 5 types (A – E) including polymyxin B and polymyxin E (colistin) used in the clinical practice. Polymyxin agents disrupt the membrane integrity leading to permeability changes causing cell death, and these agents also interfere in endotoxin activity [50].

Some new options such as Ceftobiprole, Tomopenem, Sitafloracin, Biapenem, Doripenem and Ticarcillin have been described to treat *P. aeruginosa*. Doripenem was found to have better results in pneumonia than imipenem and meropenem [51]. During a human-simulated exposure to *P. aeruginosa* and MRSA, Tomopenem showed good results for these pathogens as a promising option [52]. Biapenem in a murine model presented as a viable option to treat VAP [53]. All these antimicrobials can be excellent options to treat *P. aeruginosa* VAP but more studies are necessary in order to choose the adequate dosage and which will better fit specific situations.

Due to the several resistance mechanisms presented by *P. aeruginosa*, new alternatives of targets for antimicrobials seem to be promising by interfering in quorum sensing which may lead to a decrease in virulence factor expressions and biofilm formation [54, 55, 56]. This issue has been discussed in detail in a noteworthy study by Roy et al. (2011) [57].

## 2.2. *Acinetobacter* spp

### 2.2.1. Epidemiology

*Acinetobacter* species have been implicated in infections in immunocompromised patients as an opportunistic pathogen. This genus is characterized as a gram-negative coccobacillary, aerobic, non-glucose fermenting, immobile, nonspore-forming [58], extracellular [59], oxidase negative, pathogen. *Acinetobacter* spp. may change morphologically during the rapid growth phase, becoming rod-shaped, and during the stationary phase, it is characterized as coccobacilli [58]. Commonly found in environments such as tap water faucets, sinks, near urinals, medical equipment and hospital food [60].

The presence of multidrug resistant (MDR) *A. baumannii* in colonization/infection is associated with poor outcomes and increased mortality [61]. Its wide distribution allows for isolating in several sites, including sputum, urine, extremity wounds, blood, decubitus ulcer, vascular catheters as well as other sites [61]. Despite the conclusions of this study, it is necessary to clarify the role of *A. baumannii* colonization and its association with infection development. *A. baumannii* can colonize healthy individuals; however, it remains unclear whether its presence predisposes individuals to infection.

The isolation of *A. baumannii* from patients with nosocomial pneumonia rose significantly since 1986 to 2003 in multiple hospitals [62]. Ventilator associated pneumonia caused by *A. baumannii* (VAP-AB) in patients in ICU is frequent and represented 14% up to 37% of overall VAP [60, 63, 64]. There seem to be fewer studies on the role of *A. baumannii* in VAP mainly due to the higher prevalence of *Pseudomonas* and *S. aureus* VAP and/or other nosocomial pneumonia.

The risk factors for VAP-AB development were found to be previous use of ceftriaxone, ciprofloxacin [60], risk of aspiration associated with neurological problems, and previous antimicrobial use [65]. As independent risk factors for carbapenem resistant *A. baumannii* pneumonia development were found, APACHE II score (>20) at pneumonia onset, systemic illness, presence of mechanical ventilator, and commonly used antimicrobials [66].

Compared to *S. aureus* and *P. aeruginosa* pneumonia, few studies focus on *A. baumannii* infections due to low frequency in VAP and HAP. Nevertheless it has been emerging as the most life-threatening pathogen not only in pneumonia but also in several sites of hospital-acquired infections with high morbidity and mortality among hospitalized patients. The existing studies are valuable in clinical features regarding *A. baumannii* risk factors, mortality rates, clinical outcomes of infection, resistant profiles and its implications for the patient and less attention is given to the pathogen per se.

### 2.2.2. Virulence factors

Little is known about the role of the mechanisms of virulence factors of *A. baumannii* in a pneumonia model. Virulence factors such as lipopolysaccharides, siderophores, biofilm, capsule, phospholipases and outer membrane protein A (OmpA) need to be further studied in VAP pathogenesis due to the invasiveness and host damage.

The currently known *A. baumannii* virulence factor that allows pathogen survival and immune response challenge and evasion is the OmpA, an outer membrane protein involved in solute transportation and virulence. In high concentrations, OmpA is extremely immunogenic and can cause apoptosis of dendritic and epithelial cells favoring bacterial invasion into deeper tissues [67]. In addition, the adherence function of OmpA increases the binding to epithelial cells playing an important role in pneumonia initiation and pathogenesis during infection [68].

Lipopolysaccharide (LPS) is a membrane component that contributes to gram-negative pathogenesis. *A. baumannii* LPS confers resistance against the killing action of human serum [67]. In addition, the interaction of *A. baumannii* LPS with TLR 4 and MD-2 is highly toxigenic to human cells [69]. The immunogenic characteristic of LPS induces the synthesis of pro-inflammatory cytokines leading to an exaggerated response [67]. However the role of LPS in lungs needs to be clarified.

Siderophores are molecules responsible for iron binding. The host iron is present in association with other factors such as transferrin, lactoferrin and hemin hampering iron acquisition by *A. baumannii*. Due to this low availability of free iron in the host environment, bacterial species developed a strategic molecule to iron uptake. Acinetobactin is one of these molecules responsible for scavenging iron from transferrin and lactoferrin [70]. Other pathways of iron uptake like haem-uptake and ferrous iron uptake have been described [70]. Even if the siderophores have been studied, the potential virulence factors for pneumonia pathogenesis remain unclear.

Biofilm formation has been recognized as important factors for persistence on abiotic and biotic surfaces and in life-threatening infections as well. Biofilm formation follows some critical steps that require *A. baumannii* characteristics. The type IV pilus provides mobility leading to spread on surfaces followed by the chaperone-usher assembly system (CsuA/BABCDE) required for adherence on abiotic surfaces. Once attached, the extracellular matrix is synthesized, and for biofilm maturation and adhering to the eukaryotic cell, the Bap protein is required [70]. Persistence and survival in a hospital environment is possible due to the ability of biofilm formation providing a good source of hospital-acquired infections. Biofilm-forming strains can be also associated with serum resistance [71]. VAP development has been

associated with pathogens that colonize and produce biofilm in the endotracheal tube. Bacterial survival on biofilm was associated with microbial persistence as well [72].

### 2.2.3. Resistance Factors

Recently several resistance mechanisms have been characterized in *A. baumannii* isolated inside hospitals posing a real challenge to patient treatment. The resistance factors can be listed more than the virulence factors, such as AmpC cephalosporinases, beta-lactamases, serine and metallo-beta-lactamases, efflux pumps, changes in Omps and aminoglycosides-modifying enzymes [73]. Quinolone resistance mechanism is associated with parC and gyrA mutations [74, 75].

Efflux pumps are naturally found in bacterial cells expelling substances that could interfere with membrane stability. Occasionally this system is used to expel antibiotic molecules and chemical substances for example: tetracycline, aminoglycosides, cefotaxime, erythromycin, fluorquinolones, chloramphenicol, trimethoprim, ethidium bromide [76].

Carbapenems typically present resistance to most types of beta-lactamases and are frequently used in clinical treatment. However the carbapenemase enzymes hydrolyze antimicrobials of this class characterizing the resistant strains. OXA-type-beta-lactamases are the most prevalent types of carbapenemases in *A. baumannii* [77] and the genetic mechanism of these resistances have been well studied. Several OXA-like enzymes are found in *A. baumannii* and are frequently distributed geographically according to the types. Peleg et al. (2012) [78] discuss these and other characteristics of resistance factors in a worthwhile review [78].

Carbapenem-hydrolysing oxacillinase (CHDL) and metallo-beta-lactamase (MBL) are able to hydrolyze imipenem and are resistant to the inhibitory characteristic of clavulanate and tazobactam. CDHL are frequently found as resistance factors acquired in *A. baumannii*, whereas MBLs are rarely found [79].

Aminoglycosides can be changed enzymatically, or the target can be modified through 16S rRNA methylation impairing the aminoglycosides binding conferring resistance to gentamicin, amikacin and tobramycin. Glycylcyclines (e.g. tigecycline) likewise tetracycline, are expelled by efflux systems such as the AdeABC pump [79].

### 2.2.4. Treatment

The treatment has been challenging since MDR *A. baumannii* has emerged in hospital environments as important pathogens involved in diverse types of infection. Therapeutic failures are common due to the empiric antimicrobial treatment and the time frame between microbiological results and patient prognosis. Strategies such as inhaled antimicrobial agents that could be neurotoxic or nephrotoxic if administered on certain unique occasions, is a current study option. Premature babies were successfully treated with colistin (polimixin E) aerosolized as an adjuvant therapy with no adverse effects [80]. Polimixin E is a long-established antimicrobial that is now reemerging as a feasible option to treat MDR *A. baumannii* and *P. aeruginosa* [80]; however, its parenteral use may be limited for pneumonia due to the low concentration in the lungs and the severe side effects [63].

Non-antibiotic therapies have been emerging as effective options to eradicate and treat *A. baumannii* infections. Phage therapy to treat infections and remove biofilm has been studied as an option due to specificity to clinical strains. Iron chelating therapies deserve attention due to the natural control of acquiring iron and the necessity of this mechanism varies according to the strain. Antimicrobial peptides are being studied in this field and must to be further explored, as this remains a promising area. Vaccines and passive immunization, photodynamic and NO-based therapies aim to improve the treatment options and provide better results in patient morbidity and mortality [81].

The first-line treatment option for MDR *A. baumannii* infections are the carbapenems, a broad-spectrum beta-lactam. However, due to isolation of pan-resistant strains, characterized by the resistance to all clinical options, polimixins and tigecyclines are being introduced to manage these strains [59]. Due to the presence of several resistance mechanisms beta-lactams should be extensively tested before use. In summary the treatment options have been a combination of aminoglycosides and extended-spectrum penicillin, imipenem or broad-spectrum-cephalosporins as suggested before [82].

Due to the influence of prompt and efficient treatment options in patient survival, empiric treatment is a good option against *A. baumannii* infections. Patient underlying disease, pharmacokinetics, risk factors to acquire certain pathogens, drug side effects and susceptibility patterns are important points to be considered in the right choice treatment. In a previous study it was found that ciprofloxacin was considered the best monotherapy option while ciprofloxacin plus vancomycin was the best combination due to the high isolation of *A. baumannii* and *S. aureus* in that hospital [63]. Similar studies may determine the best choice of empiric therapeutic strategies according to local epidemiology and patient needs. These data may improve the mortality and morbidity rates regarding infection and empiric treatments.

### 2.3. *Staphylococcus aureus*

#### 2.3.1. Epidemiology

*Staphylococcus aureus* (*S. aureus*) is a Gram-positive coccus, typically human commensal, which frequently colonizes the nares [83] and is one of the most important causes of VAP [84, 85]. Infections caused by drug-resistant pathogens have shown to result in poorer clinical and economic outcomes compared to those due to sensitive strains [86, 87]. Because of this, many studies have tried to determine whether MRSA (methicillin-resistant *S. aureus*) are more pathogenic than MSSA (methicillin-susceptible *S. aureus*) when causing cases of VAP [86, 88 - 90].

Another characteristic observed between VAP occurrence by MSSA and MRSA was that trauma influenced the microbiology of pneumonia and it should be considered in the initial antibiotic regimen choice. Agbaht et al. (2007) [91] demonstrated that patients with trauma had a higher prevalence of MSSA, but the overall prevalence was sufficiently high to warrant *S. aureus* coverage for both groups. On the other hand, since no MRSA was isolated during the first 10 days of mechanical ventilation in trauma patients, MRSA coverage in these patients becomes necessary only 10 days after admission. Below we will discuss studies evaluating the virulence factors and mechanisms of resistance involved in VAP caused by this microorganism.

#### 2.3.2. Virulence factors

*S. aureus* has the ability to adapt to the respiratory tract environment as it can control expression of a number of virulence factors including enterotoxins, proteases, exfoliative toxins, immune-modulatory factors, hemolysins, leukocidins and biofilm [92, 93]. Moreover, it has a metabolic versatility, ability to scavenge iron, coordinate gene expression, and the horizontal acquisition of useful genetic elements that have contributed to its success as respiratory microbiota. And surface adhesins are expressed to facilitate persistence in the airways [94]. These characteristics facilitate its emergence as a respiratory pathogen [93, 94].

In community-acquired pneumonia (CAP) caused by MSSA and MRSA strains, the Panton-Valentine leukocidin (PVL) is a relevant virulence factor when present, because it is a bicomponent pore-forming leukotoxin that causes necrotizing pneumonia [95]. Patients with these MRSA PVL-positive strains have severe clinical presentation and poor clinical outcomes. In contrast, in HAP and VAP cases in which MRSA PVL-positive strains were isolated, severity of disease and clinical outcomes were not influenced compared to MRSA PVL-negative strains [96]. Additionally, alpha-hemolysin (Hla) has been identified as a critical virulence factor in animal models of pneumonia inducing lung injury. It is also a pore-forming toxin able to bind to eukaryotic cells and induce an inflammatory response, which has been widely studied [97 - 100]. But many studies indicate that Hla targets critical host defenses provided by both innate immune cells and the tissue barrier, and highlight the complexity of the host response to the toxin [101, 102].

Finally, many *S. aureus* strains are able to produce a biofilm, an extracellular matrix that protects bacteria from antibiotics and immune system of the patient. The endotracheal tube is a connection between the outside environment to the lungs, and bacteria are able to exude this exopolysaccharide and adhere together over the tube. Some parts of this biofilm can be released in the air passage, delivering bacteria deeper into the lungs and spurring growth in other areas of the tube [103].

#### 2.3.3. Mechanisms of Resistance

Since the 1960s, one of the most important resistance mechanisms found in *S. aureus* is the resistance to methicillin. The incidence of MRSA strains has increased significantly [104].

One study evaluated patients subjected to mechanical ventilation clustered within trauma and non-trauma groups. MSSA was more frequent (34.5% vs. 11.5%,  $p < 0.01$ ) in trauma, whereas MRSA was more frequent (11.5% vs. 2%,  $p < 0.01$ ) in the non-trauma group [91].

The importance of this resistance in the worst outcome of VAP remains controversial, due to confounders such as the adequacy of empirical treatment and severity of illness that influence analysis [86, 88].

#### 2.3.4. Treatment

A few studies have compared the use of vancomycin and linezolid in VAP caused by MRSA. This is because vancomycin has been the standard treatment for MRSA infections, but clinical efficacy has been limited. Chan et al. (2011) [105] performed a retrospective analysis to evaluate clinical outcomes in VAP caused by MRSA and treated with linezolid or vancomycin. Their results suggested no survival benefit but a trend towards a higher cure rate with linezolid therapy. On the other hand, in critically ill trauma patients, Hamilton et al. (2012) [106] obtained an acceptable cure rate for MRSA VAP using a high-dose of vancomycin.

In a recent meta-analysis of randomized controlled trials [107] they compared the efficacy and safety of linezolid with vancomycin, the gold-standard treatment, for MRSA-related infections (including VAP). Their results indicated a superior efficacy of linezolid compared with vancomycin for MRSA-related infection in terms of clinical treatment

success (OR 1.77) and microbiological treatment success (OR 1.78). No difference was found for incidence of drug-related side effects between linezolid and vancomycin therapies, but the linezolid therapy group had significantly fewer patients with abnormal renal function. This study provides evidence that linezolid presents significant advantages over vancomycin.

Some other antimicrobials are recommended for MRSA and MSSA infections in pneumonia, including ventilator-associated listed in Table 2.

**Table 2** Potential pathogens and recommended empirical antimicrobial therapy for the treatment of nosocomial pneumonia according to the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines

	Potential pathogens	Recommended therapy
HAP and VAP in patients with no known risk factors for MDR pathogens, early onset (until Day 4) and any disease severity	<i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i> <b>MSSA</b> Antibiotic-sensitive enteric Gram-negative bacilli	Ceftriaxone or Levofloxacin, moxifloxacin, ciprofloxacin <sup>a</sup> or Ampicillin/sulbactam or Ertapenem
HAP, VAP and HCAP in patients with late-onset disease or risk factors for MDR pathogens and all disease severity	Pathogens as above, plus: <i>Pseudomonas aeruginosa</i> ESBL-positive <i>Klebsiella pneumoniae</i> <sup>b</sup> <i>Acinetobacter</i> spp. <sup>b</sup> <b>MRSA</b> <i>Legionella pneumophila</i> <sup>b</sup>	Antipseudomonal cephalosporin (cefepime, ceftazidime) or Antipseudomonal carbapenem (imipenem or meropenem) or $\beta$ -lactam/ $\beta$ -lactamase inhibitor (piperacillin/tazobactam) plus <sup>c</sup> Antipseudomonal fluoroquinolone (ciprofloxacin or levofloxacin) or Aminoglycoside (amikacin, gentamicin or tobramycin) plus Linezolid or vancomycin <sup>d</sup>

Adapted from Welte & Pletz (2010) [108].

HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; MDR, multidrug resistant; MSSA, methicillin-susceptible *Staphylococcus aureus*; HCAP, healthcare-associated pneumonia; ESBL, extended-spectrum  $\beta$ -lactamase; MRSA, methicillin-resistant *S. aureus*.

<sup>a</sup>The frequency of penicillin-resistant *S. pneumoniae* and MDR *S. pneumoniae* is increasing; levofloxacin or moxifloxacin are preferred to ciprofloxacin. In the authors' opinion, ciprofloxacin as empirical antimicrobial therapy for early-onset HAP is insufficient owing to limited activity against pneumococci, which occur frequently in early-onset pneumonia.

<sup>b</sup>If an ESBL-positive strain, such as *K. pneumoniae*, or an *Acinetobacter* spp. is suspected, a carbapenem is a reliable choice. If *L. pneumophila* is suspected, the combination antibiotic regimen should include a macrolide (e.g. azithromycin), or a fluoroquinolone (e.g. ciprofloxacin or levofloxacin) should be used rather than an aminoglycoside.

<sup>c</sup>A combination therapy with fluoroquinolones or aminoglycosides is recommended but remains an issue of debate as clinical data are contradictory.

<sup>d</sup>If MRSA risk factors are present or there is a high incidence locally.

Quinupristin/dalfopristin was demonstrated to be inferior to vancomycin in patients with HAP/VAP and daptomycin is inactivated by surfactant and therefore is inappropriate for pneumonia treatment. Clindamycin and trimethoprim/sulfamethoxazole have been used for CA-MRSA skin infections, but their use as monotherapy for pneumonia remains unclear. Moxifloxacin has good activity against MSSA compared with other fluoroquinolones, but emergence of resistance in MRSA suggests that the fluoroquinolone class is unreliable as monotherapy [109].

Combination of daptomycin with adjunctive antimicrobials, such as rifampin, aminoglycosides or cotrimoxazole has been tested to evaluate the synergistic effect in MRSA strains [110]. And novel antimicrobials or antimicrobials previously used in skin infections have been studied for MRSA pneumonia such as ceftaroline, telavancin, ceftobiprole, oritavancin, iclaprim and dalbavancin [108].



Researchers note that continuous evaluation of the antimicrobial therapeutic options, such as new lipoglycopeptides, along with their pharmacodynamic and pharmacokinetic profiles, are necessary to create novel therapeutic protocols and reduce VAP-related mortality [111, 112].

### 3. Conclusion

New strategies of treatment are becoming available, as knowledge of bacterial biology has steadily increased. Diverse signaling systems between bacterial cells in different species have been discovered and understood. A few quorum sensing inhibitors have been developed to impair cell-to-cell communication, making possible the successful treatment of infections caused by *S. aureus*, *P. aeruginosa* and *A. baumannii*. Additionally, novel actions with biological control have been tested to control bacterial persistence of pathogens in hospital environments. These new approaches demonstrate that some feasible solutions will be available in the future to control bacterial infections as a substitute for or support treatment with antimicrobials in an era of multidrug resistant pathogens.

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